Nitrate Reduction in Agricultural Acid Sulfate Soil

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy
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Candidate's Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of the author’s knowledge it contains no material previously published or written by another person, except where due reference is made in the text.

Richard J Reilly

Date: 7 June 2011
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Abstract

A significant proportion of Australia’s sugarcane crop is grown on east-coast estuarine floodplains underlain by pyritic gel-clay subsoils. At the current study site these agricultural acid sulfate soils are typically characterised by a topsoil horizon of river alluvium, a subsoil of oxidised actual acid sulfate soil (AASS), a zone of partially oxidised AASS and a deep sulfidic horizon of pyritic potential acid sulfate soil (PASS). Addition of nitrogenous fertiliser at key points in the sugarcane cropping cycle can create soil nitrogen levels in excess of immediate soil flora/fauna and crop requirements. In high rainfall tropical and sub-tropical regions conditions are thus suitable for nitrate, a strong oxidising agent, to leach down to the sulfidic soil layers with the consequent risk of pyrite oxidation. Little information is available on the fate of nitrogenous fertilisers in these pyritic subsoils. The purpose of this field and laboratory study was to evaluate the potential for nitrate reduction to occur in the presence of pyrite in sugarcane soils in the Tweed River valley, northern NSW, Australia. The study focus was on examining the soil profile hydrology including leaching mechanisms and nitrate concentrations down the profile to the AASS/PASS interface, as well as evaluating the potential for nitrate to increase the rate of pyrite oxidation in this generally anoxic soil zone.

Following an investigative nitrogen field trial to gather initial data, a second replicated urea fertiliser treatment trial with a nil-treatment control plot and three nitrogen (N) treatments was set up on a plant-cane-block in collaboration with a Tweed region cane grower, Robert Quirk. Installed loggers recorded rainfall, air and soil temperature, soil moisture and watertable data. Separate surveys and analytical work characterised selected soil physical, morphological and geochemical aspects. Soil profile sampling on four occasions over the twelve month crop cycle was analysed for N-species, NH$_4^+$ and NO$_3^-$.

Hydraulic data analysis showed the watertable generally varying between 0.2 and 1.4 m below ground level with observed strong and rapid responses to rainfall events greater than approximately 15 mm per day. This and associated data supports the postulate that soil nitrate could move down the profile under even moderate precipitation events in these soils. Temperature, pH, redox potential and biological substrate soil data demonstrated the biogeochemical suitability of these subsoil zones to support nitrate reduction. Soil-N analysis revealed significant differences between N-trial treatments using urea fertiliser and also significant nitrogen transformation and movement within the soil profile.

Over a period of weeks, the urea fertiliser was rapidly transformed and appeared in the upper profile as elevated levels of ammonium and nitrate ions. The initial high ammonium levels quickly declined to be replaced almost completely by nitrate in the upper layers of the cane soil. Subsequently, increasing soil nitrate concentrations were evident deeper in the soil profile on
higher nitrogen treatment plots during the middle phase of the crop cycle. In no instances were significant levels of nitrate detected below the soil redoxcline (the oxic-anoxic boundary) at around 1.0 m depth, nor was nitrate pooling evident anywhere in the AASS transition zone. Laboratory experimental work was undertaken to evaluate nitrate reduction coupled with pyrite oxidation under the biogeochemical conditions existing in the AASS transition zone. Results indicated that nitrate reduction associated with pyrite oxidation does take place in pyritic gel clay from the field site.
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List of Acronyms and Abbreviations

ASS  Acid Sulfate Soil
AASS  Actual Acid Sulfate Soil
AEA  Alternative Electron Acceptor
AHD  Australian Height Datum
AVS  Acid Volatile Sulfur
CCS  Commercial Cane Sugar
CRS  Chromium Reducible Sulfur
DNRA  Dissimilatory Nitrate Reduction to Ammonia
Eh  Oxidation-reduction potential relative to a standard hydrogen electrode.
N  Nitrogen
PASS  Potential Acid Sulfate Soil
POS  Peroxide Oxidisable Sulfur
SOC  Soil Organic Carbon
SOM  Soil Organic Matter
SEM  Scanning Electron Microscopy
TAA  Total Actual Acidity
XRD  X-Ray Diffraction
<table>
<thead>
<tr>
<th>Glossary and Terms</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Actual Acid Sulfate Soil</strong></td>
<td>Pyritic soil that has partially or fully oxidised, generating soil acidity below pH 4</td>
</tr>
<tr>
<td><strong>Acid Sulfate Soil</strong></td>
<td>A generic name given to soils containing oxidisable pyrite</td>
</tr>
<tr>
<td><strong>Anisotropic</strong></td>
<td>Having a soil property that has a different value when measured in different directions</td>
</tr>
<tr>
<td><strong>Anoxic</strong></td>
<td>An environment devoid of oxygen, generally &lt;1 % oxygen saturation</td>
</tr>
<tr>
<td><strong>Australian Height Datum</strong></td>
<td>The geodetic datum for altitude measurement in Australia</td>
</tr>
<tr>
<td><strong>N-cycling</strong></td>
<td>The cycling of nitrogen compounds within the environment</td>
</tr>
<tr>
<td><strong>N-species</strong></td>
<td>Natural organic and inorganic compounds of nitrogen. Most commonly associated with inorganic nitrogen compounds, including: ammonia, ammonium, nitrate, nitrite, nitric oxide, nitrous oxide and nitrogen gas</td>
</tr>
<tr>
<td><strong>N-gaseous emissions</strong></td>
<td>Soil emissions of nitrogenous gases, commonly: ammonia, nitric oxide, nitrous oxide and nitrogen</td>
</tr>
<tr>
<td><strong>Oxic</strong></td>
<td>An environment containing oxygen, generally &gt; 28 % oxygen saturation</td>
</tr>
<tr>
<td><strong>Oxidation</strong></td>
<td>Chemical oxidation involving the loss of electrons by one substance (oxidation) in conjunction with the acceptance of these electrons by another (reduction). Although it is commonly associated with molecular oxygen, oxidation is supported by a variety of alternative electron acceptors, including nitrate, manganese(IV) and ferric iron</td>
</tr>
<tr>
<td><strong>Potential Acid Sulfate Soil</strong></td>
<td>Unoxidised pyritic soils with the potential to create acidity in excess of the soil’s natural buffering capacity</td>
</tr>
<tr>
<td><strong>Reduction</strong></td>
<td>Chemical reduction at its most basic level involves a gain of electrons and a change in oxidation state</td>
</tr>
<tr>
<td><strong>Redoxcline</strong></td>
<td>The redox gradient across the boundary between oxic and anoxic environments</td>
</tr>
<tr>
<td><strong>Sub-oxic</strong></td>
<td>An environment with limited oxygen between 1 and 28 % oxygen saturation</td>
</tr>
<tr>
<td><strong>Soil Organic Carbon</strong></td>
<td>Soil material of organic origin even when its present form is not recognisable as such</td>
</tr>
<tr>
<td><strong>Sparging</strong></td>
<td>A procedure where a gas is bubbled into a liquid. An inert gas such as nitrogen may be used to purge oxygen from a solution required to be anoxic</td>
</tr>
</tbody>
</table>
1 Introduction

Soil nitrogen is prominent among the many nutrients essential for crop growth and has probably received more study and attention than any other. Nitrogen occupies a unique position among the major nutrients because it occurs only in trace amounts in soil parent materials but is required by plants in relatively large quantities. ... Cropping systems have been much influenced by the availability of synthetic nitrogenous fertilizers and it appears that this trend will continue with the steadily increasing efficiency of chemical technology.

(Richards, 1965)

1.1 Overview

This study examines nitrogen fertiliser impacts on agricultural acid sulfate soils in northern NSW, Australia. In this first chapter a brief introduction to sugarcane agriculture, acid sulfate soils and nitrogenous soil amendment is provided in the context of agricultural management at the study field site. A section outlining the impetus for the current study is then presented, followed by the study aims and objectives. The chapter concludes with a section outlining the thesis structure.

1.2 Sugarcane Agriculture on Acid Sulfate Soils

1.2.1 Sugarcane on Coastal Floodplains

Agricultural soils support the production of food for an increasing world population with tropical and sub-tropical coastal floodplain soils widely used for this purpose due to their general fertility and the low frequency of frosts. Regular overbank flooding in these low lying river catchments brings deposits containing nutrients and silt that continually renews their fertility and generally makes them suitable for high value cropping (Tockner and Stanford, 2002).

One economically important crop, sugarcane, grows well on these soils. A major portion of Australia’s sugarcane production takes place on estuarine floodplains in Queensland (Qld) and northern NSW. Ninety-four percent (approximately 378 000 ha) of Australia’s sugarcane is grown in the state of Queensland, five percent (35 900 ha) in northern NSW (Canegrowers, 2006, NSW-Sugar, 2009). Due to the large biomass of mature sugarcane crops, substantial amounts of nutrients are required by the plant during the growth cycle.
Increasingly over recent decades these crop nutrients have been provided through soil amendment with artificial fertilisers (Thorburn et al., 2005) although supplementary methods for supplying and maintaining soil nutrients are now being implemented in the sugar industry in northern NSW. These latter methods include: cane-trash retention, green-cane harvesting, crop rotations with leguminous plants and minimising tillage operations to control organic matter loss from the soil (Thorburn et al., 2007).

The use of nitrogen-based soil amendment is well established in Australian agriculture and in sugarcane agriculture where application rates of 50 to 300 kg N per hectare per crop cycle were common (Weier, 1994). While optimal application of fertiliser may be managed to an extent, early rapid growth of the cane plant generally limits machine applied soil amendment to within a few months after planting. Supply of soil nitrogen to the growing crop may not be well synchronised with the plant’s needs and this disjunction between nitrogen supply and demand may be further complicated by the soil biology of N-cycling where soil nitrogen becomes assimilated into microorganisms (immobilised) and may subsequently be unavailable in the current crop cycle (Thorburn et al., 2005). Soil N-species may also be absorbed into clays, lost as gaseous emissions or leached below the plant root zone (Brady and Weil, 2001).

At some cane farms on the east coast of Australia, sugarcane cropping also has to contend with chemically reactive acid sulfate subsoils. These soils contain levels of pyrite (iron disulfide, FeS₂) sufficient to significantly affect soil chemistry when oxidised (Pons, 1972). The principal reaction here is oxidation of iron sulfide to produce sulfate ions that, when combined with water, result in sulfuric acid.

1.2.2 Acid Sulfate Soils

Acid sulfate soils are a global phenomenon occurring extensively on all continents (Pons et al., 1982, von Uexkull and Mutert, 1995). These soils are characterised by high levels of pyrite and a potential for acidity in excess of existing soil buffering capacity (Pons, 1972). They occur in various locations within the landscape including: inland near large sources of sulfide (e.g. sulfide ore deposits), in some inland water systems and near the coast in estuarine and brackish swamp environments.

Coastal acid sulfate soils formed largely during the Holocene period (beginning around 11 700 years before present) after the end of the last glacial maximum around 18 000 years ago (Melville et al., 1991). Subsequent periodic sea-level migration in and out of river valleys led to drowning and blockage of ancient incised channels. With rising sea-levels, sediments reworked by fluvial and marine forces were concentrated in embayments. Muds and other fine sediments became trapped behind estuarine sand barriers, gradually filling the estuary to sea-level to create low lying estuarine floodplains typical of the lower Tweed River valley today.
(Roy, 1984, Thom, 2002). Under specific conditions, these infilled sediments may also contain pyrite.

Sulfate in the sea water coupled with land sediments containing iron oxides and organic matter may react to create deposits of pyritic sediments in estuaries (Melville et al., 1991, van Oploo, 2000). Under flooded or high watertable conditions, these pyritic soils are relatively stable. When dried out through natural or anthropogenic perturbations, these soils may oxidise to release potentially environmentally damaging by-products including sulfuric acid, heavy metals (van Breemen, 1982) and carbon dioxide (Hicks et al., 1999), that can have significant short and long term effects on local, regional and global environments (DPI-NSW, 2009).

Coastal acid sulfate soils occur around most of the Australian coastal fringe but appear to be most prevalent on the eastern, northern and north-west coastlines of the continent (White and Melville, 1996). In 1999 it was estimated there were 40 000 km$^2$ of coastal sulfidic sediments containing over $10^8$ tonnes of pyrite in Australia (NWPASS, 1999). In the northern NSW coastal region these soils occur in generally Holocene age sediments of marine origin containing approximately three to five percent iron sulfide, generally in the form of pyrite (FeS$_2$), but also as iron monosulfides such as greigite (FeS$_{1.34}$) and mackinawite (FeS$_{0.94}$) (Fitzpatrick et al., 1998, Bush and Sullivan, 1999, Bush and Sullivan, 2002).

1.2.3 Agriculture in Acid Sulfate Soils

Due to their low lying position near sea-level, the estuarine floodplains soils would originally have been characterised by a relatively high watertable that naturally protected the reactive pyrite from oxidation in the subsoil. Over geological time and possibly through the influence of droughts and the groundwater-depleting evapotranspiration effects of forest vegetation, air gained access to the pyritic subsoil and induced oxidation of this material.

The post-1880s arrival of white settlers to the region, who took up land selections and initiated farming on the floodplain, possibly had a mixed impact on the pre-existing state of the acid sulfate soils in the region. Timber cutting and forest clearing (Graham, 2001) possibly helped to sustain high groundwater levels, but flood mitigation works and drainage to support farming and intensive cropping operations may have reversed these effects (Wilson, 1995, Lin et al., 2002). Extensive drainage operations were carried out in the study field site region between the 1920s and 1960s (Graham, 2001). Drainage converted prior back-swamps and wetlands into productive farming areas.

Whilst these latter developments undoubtedly led to growth and consolidation of rural settlements and local industries, it is clear these operations also resulted in unintended impacts on local soils, fisheries, the ecology of estuaries and downstream water users (Melville et al., 1991); (DPI-NSW, 2003). The expanded drainage network facilitates the export of acidity from
acid sulfate soils, rapidly transferring acidity and deoxygenated water from back-swamp areas to creeks and estuaries after rain (Kinsela and Melville, 2004a).

Floodgates and constructed drains also blocked fish movement to upstream habitat areas and provided conditions conducive to formation of poor quality water with high acidity, low dissolved oxygen and high concentrations of heavy metals (Sammut, 1998, DPI-NSW, 2009). Intensive cropping operations also potentially impact on acid sulfate soil geochemistry. These operations include: laser-graded of fields, deep crop tillage, soil amendment with fertiliser, watertable drawdown by crops and compaction by machinery (Reilly, 2001).

During the 1990s the regional sugar milling group, NSW Sugar Milling Cooperative, proactively embarked on integrated management programs to map the extent of these soils in their region and also to develop best practice management guidelines to minimise future adverse impacts (NSW-Sugar, 2000b). These initiatives coincided with other changes in sugarcane management in the region including: (i) a shift from burnt cane to green-cane harvesting, and (ii) greater use of legume-based break-crop rotations to rest the soil and to provide nitrogen inputs for the following crop.

Although much is known about soil-amended nitrogen on conventional soils (Bartholomew and Clark, 1965, Richards, 1965) and also increasingly about the need to balance the production benefits of fertilisers with their crop yield return (Thorburn et al., 2007), much less is known about the impacts of fertiliser-derived nitrogen on acid sulfate soils. This particularly applies to its interactions with residual soil acidity and oxidisable sulfur species.

1.3 Impetus for this study

Estuarine soils in northern NSW are used extensively for sugarcane cropping (NSW-Sugar, 2009) and this agriculture involves application of nitrogen fertilisers to enhance crop viability and yields (Meier et al., 2003). Although there appears to be a growing acceptance and practice of natural soil amendment through green manure crops and trash retention in sugarcane farming (Thorburn et al., 2004) and in agriculture generally (Oikada, 2008), the use of artificial fertilisers in intensive cropping systems generally has been steadily increasing and this trend is expected to continue for some decades yet (Bumb and Baanante, 1996, Heffer and Prud’homme, 2006).

With respect to sugarcane agriculture in the ASS regions, these trends give fourfold cause for concern. The first relates to the chemically reactive nature of the widely used urea fertiliser and its products. One of these products, nitrate, is a powerful oxidant that can be highly reactive under specific conditions in soils.
From a biogeochemical oxidation-reduction perspective, nitrate may be used as an alternative electron acceptor (AEA) in place of oxygen when soil oxygen concentrations are low.

The second issue has to do with the potential lack of synchrony between the supply of nitrogen fertiliser and the growing crop requirements. When large amounts of N-fertiliser are added to the crop at planting, it transforms through mineralisation into plant-available ionic forms such as ammonium and nitrate. However, the sugarcane plant may not be able to immediately use all of this available nitrogen. The surplus may subsequently be vulnerable to ‘loss’ through a variety of physical mechanisms and microorganism assimilatory (biosynthesising) and dissimilatory (energy producing) processes, including: (i) leaching downwards or sideways in the soil profile (Prasertsak et al., 2002), (ii) adsorption to clays and humic substances, (iii) immobilisation by microorganisms, (iv) nitrate reduction leading to N-gaseous emissions. Under specific conditions in cane-block soils this biochemical reduction sequence may be interrupted, resulting in emission of nitrogen oxides, including nitrous oxide (N₂O) a potent greenhouse gas (Weier, 1999).

The third issue pertains to the fate of soil nitrogen in these Australian sub-tropical sugarcane soils and particularly that of nitrate in excess of immediate plant requirements. There appears to be little published information on nitrogen concentrations in regional sugarcane soils at depths greater than 30 cm, the greatest depth for historical soil sampling in the industry (Reilly, 2001). Apart from two studies led by Rasiah (Rasiah and Armour, 2001, Rasiah et al., 2003) evaluating nitrate retention at anion exchange sites in deep soil profiles under sugarcane in tropical Queensland, little other work appears to have been carried out. Consequently many questions remain unanswered as to the fate of applied nitrogen during the sugarcane crop cycle on these soils.

The fourth point relates to the existence of pyrite in the study sugarcane subsoils. Pyrite oxidation has historically been viewed largely within a framework of oxic transformation of sulfide to sulfate using oxygen and ferric iron (Fe³⁺) as electron acceptors (Pons, 1972, Nordstrom, 1982b). Here, sulfide is the electron donor in a close-coupled redox transformation where one substance is chemically oxidised and one is reduced. These are part of a broad range of natural bio-catalysed reactions initiated by soil microorganisms with the objective of obtaining energy for their own survival and growth (Madigan and Martinko, 2006). However, depending on the soil geochemical conditions, many other electron acceptors exist in most soils and from a thermodynamic perspective offer favourable alternate pathways for the oxidation of pyrite. Nitrate (NO₃⁻) is one such alternative electron acceptor (AEA).
Chapter 1: Introduction

There is a substantial body of work, mainly from Europe, linking nitrate reduction with pyrite (i.e. sulfide) oxidation in aquifers (Kolle et al., 1983, Engesgaard and Kipp, 1992, Pauwels et al., 1998, Schippers and Jørgensen, 2001, Schippers and Jørgensen, 2002a, Haaijer et al., 2007, Schwientek et al., 2008).

Apart from reference to a potential association between nitrate reduction and pyrite oxidation in the 2000 acid sulfate soil study by van Oploo (van Oploo, 2000), no other work appears to have been carried out to date examining potential biogeochemical interactions between soil nitrate and iron sulfide in agricultural acid sulfate soils.

The physicochemical conditions of saturated pyritic subsoils at the study field site coupled with the agricultural addition of urea to these soils appear to provide all the necessary conditions for redox reactions of this type to occur. This provides considerable impetus for the current study to investigate and evaluate these potential transformations.

1.4 Study Aims and Objectives

The underlying aim of this research was to examine ammonium and nitrate products of applied near-surface fertiliser nitrogen in a sugarcane agricultural ASS system and to evaluate nitrate’s potential impact on increasing the rate of pyrite oxidation in this soil. Rather than taking a reductionist approach to soil geo- and bio-chemistry, the approach here was to examine and characterise the soil broadly from a perspective of oxidation-reduction (redox) theory, with a particular emphasis on redox chemistry theory and oxic / anoxic states within the soil profile.

The novel nature of this approach means that much background information that could support this study was not available and this dictated study objectives. The initial gaps in understanding of acid sulfate soil redox reactions were perceived to lie in (i) understanding the primary physicochemical characteristics of the study soil from a soil redox chemistry perspective, associated with (ii) knowing the soil nitrogen concentrations over a sugarcane cycle, and (iii) understanding the impact of soil nitrate on pyrite in a controlled environment.

Specific objectives here are to:

(i) Critically review the literature on ASS and nitrogen in these soils with a particular emphasis on soil redox reactions and those soil properties that mediate them

(ii) To characterise selected geochemical, physical, hydrological, and biogeochemical functioning properties of the field site soils that support informed interpretation of field trial and laboratory experiment results.

(iii) To study changes in the soil profile distribution of NO$_3^-$ and NH$_4^+$ over the course of a cane crop cycle in the field

(iv) To study nitrate induced anaerobic oxidation of ASS in the laboratory
Due to the extensive work carried out by Durr (Durr, 2009) to identify and describe soil microorganisms in these ASS, and van Oploo (van Oploo, 2000), in comprehensively characterising the soil geochemistry of these regional acid sulfate soils, the intention here was to draw on this existing work but not to duplicate it here. The approach here is to seek clarity of ASS biogeochemical functioning, not through analysis of microorganisms and their activity, but through seeking to understand, from a redox chemistry perspective, what types of reactions may be possible in the study soils.

1.5 Thesis Structure and Outline

This study comprises four main parts: (i) a review of relevant literature, (ii) a field component evaluating soil ecology and geochemical properties including soil water dynamics, (iii) a field-study evaluation of in-ground nitrogen species within the context of a urea fertiliser field trial on sugarcane, and (iv) a laboratory experimental evaluation of the effect of soil solution nitrate on pyritic gel-clay subsoil. This thesis comprises ten chapters plus appendices followed by references. It is structured as follows:

Chapter 1: Introduction - introduces the study topic and sets out the aims and objectives and structure of the thesis

Chapter 2: Nitrogen in Agricultural ASS - reviews the literature on the theory and current research relating to acid sulfate soils, sugarcane, soil physical properties, hydrology, soil microbiology, biogeochemical cycles and agriculture on acid sulfate soils in northern NSW

Chapter 3: Field Study Site - describes the study region where the field work was conducted

Chapter 4: Selected Soil Properties - in two separate sections this chapter describes and presents results of: (i) cane soil acidity and redox potential characterisation, and (ii) cane-block soil organic carbon testing

Chapter 5: Mineralogy and Clay Characterisation - in three sections this chapter evaluates cane-block soil mineralogy, clay type and PASS gel-clay micromorphology

Chapter 6: Thermal and Hydrological Properties - this chapter presents results of cane-block soil ecology evaluation with respect to two parameters: (i) soil temperature and (ii) cane-block soil - water relations

Chapter 7: Cane-block Soil Nitrogen - presents aims, methods and procedures and results of the field study and discusses these in the context of the field study objectives

Chapter 8: Nitrate-coupled Pyrite Oxidation - presents the methods, procedures and results of laboratory experimental work evaluating the impact of nitrate-N on PASS gel-
clay under suboxic conditions. Results are discussed in the context of the study objectives.

**Chapter 9: General Discussion** - Summarises the study results and discusses the implications of the combined results of the field and laboratory studies for sugarcane agricultural management in northern NSW. Methodological issues raised during the course of this study are highlighted and briefly discussed.

**Chapter 10: Conclusion** - Summarises the key findings of the current research in the context of the study aims and objectives. Suggestions are made for future research. Finally, recommendations are made for the management of specific sugarcane agricultural issues in the study region.
2 Nitrogen in Agricultural ASS

2.1 Overview

Given the biogeochemical basis of many inorganic nitrogen transformations in sugarcane agricultural acid sulfate soils, this review of literature is necessarily broad-ranging. It spans many disciplines including: acid sulfate soils, sugarcane cropping, soil physics, geomorphology and mineralogy, microbiology and biogeochemistry, geochemical cycles and agricultural management. This chapter begins with a review of acid sulfate soils used for sugarcane agriculture and gives an overview of the physiology and horticulture of sugarcane. Soil properties are then reviewed, including soil physical, hydrological and chemical aspects pertinent to the current study soils. Microorganism and microbiological functioning in soils is examined to evaluate the effect of soil chemical and biochemical processes in the context of sugarcane cropping. The final section reviews key aspects of agricultural sugarcane soil management in the context of the study site estuarine floodplain.

2.2 Soils as a Resource

The term ‘fertility’ when applied to soil has no clear definition (Patzel et al., 2000). Anecdotal and even much scientific knowledge supports the idea that high fertility is often associated with soil properties such as high soil organic matter content, microflora and fauna activity and structural stability (Watt et al., 2006). However, crop yields and farmer evaluation of conservation practices enhancing these properties have often been highly variable (Kirkegaard et al., 1994, Lyon et al., 2004), cited in (Watt et al., 2006). This suggests that biogeochemical processes within the soil cropping environment may be influential but still not fully understood. Increased use of artificial fertiliser on many crop trials have often not resulted in any increase in soil organic matter (SOM). This has been observed even with substantial additions of organic matter in the form of manure crops, soil amendments and crop residue incorporation. Some studies even show SOM decline after years of synthetic fertiliser use (Khan et al., 2007).

Over the last century in Australia, agricultural crop production has relied increasingly on broad acre monoculture, intensive tillage and use of fertiliser. These practices have not always sustained soil properties important for long term health of the soil. These key properties include soil organic matter, soil biodiversity and soil structure (Hamblin, 1991, Lee and Pankhurst, 1992, Abbott and Robson, 1994, Fraser, 1994, Gupta, 1994, Haynes, 1994, Whalley et al., 1995, Reeves, 1997). These trends appear to be widespread across all cropping regions in Australia.
2.3 Acid Sulfate Soils

Acid sulfate soils were identified in the Netherlands by Linnaeus in the 18th century (Pons, 1972). They were subsequently recognised in Northern Europe and described with terms such as *cat clay* (Dutch *Kattekleigrond*) or *Gifterde* (German for poison earth) in Germany (Pons, 1972; Benzler, 1973). These soils were first formally recognised in Australia in 1917 near Albany, Western Australia (Woodward 1917, cited in Angeloni et al., 2004). In eastern Australia, ASS was observed on the Macleay River floodplain by Walker in 1963 who, nine years later, warned of its potential to create environmental damage (Walker, 1972). Despite this early awareness, subsequent land development in the coastal zones was carried out without due regard for the potential problems from this pyritic soil (Atkinson et al., 1995; Angeloni et al., 2004). It was only after a 1987 significant fish kill event in the Tweed estuary that concerted action was taken to investigate and manage these problem soils (Graham, 2001).

In the mid 1900s the investigation of sulfide oxidation by many workers (Colmer and Hinkle, 1947; Silverman et al., 1961; Lorenz, 1962; Hart, 1963; Lorenz and Tarpley, 1963; Silverman et al., 1963) did much to frame and inform early understanding of mineral pyrite. Specific knowledge about pyrite in soils was then boosted by the work of others (Pons, 1964; Berner, 1970; Kawalec, 1971; Benzler, 1973; Pons, 1972; Brinkman and Pons, 1973; van Breemen, 1972; van Dam and Pons, 1973). In Australia the early awareness of these soils (Woodward, 1917; Voisey, 1934; Walker, 1963; Walker, 1972) did not translate into effective assessment of ASS problems until the early 1990s. ASS have since been intensively investigated and reported in the work of many researchers (Veness and Thompson, 1990; Melville et al., 1991; Melville et al., 1993; White et al., 1993; Wilson, 1995; Sammut et al., 1996; White et al., 1997a; Sammut and Lines-Kelly, 2000; Wilson et al., 1999; van Oploo, 2000; Lin et al., 2002; Macdonald et al., 2002).

2.3.1 ASS Classification

Early Australian soil classification systems did not satisfactorily include descriptions for ASS (Stace et al., 1968; Northcote, 1979). The most recent soil classification scheme does have an ASS category: *Hydrosols* (Isbell, 1996); (Isbell, 2002), but this does not adequately describe the ASS heterogeneity found in Australian landscapes (van Oploo, 2000). A table of classification and soil categories relevant to ASS is shown in Table 2-1:
Table 2-1: Acid Sulfate Soil Classification

<table>
<thead>
<tr>
<th>Classification System</th>
<th>ASS Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Soil Groups (Stace et al., 1968)</td>
<td>Humic Gleys</td>
</tr>
<tr>
<td>U.S. Soil Taxonomy (USDA, 1999)</td>
<td>Typic Sulfaquept</td>
</tr>
<tr>
<td>Australian Soil Classification System (Isbell, 2002)</td>
<td>Sulfidic or sulfuric Hydrosol</td>
</tr>
<tr>
<td>World Reference Base for Soil Resources (FAO/ISRIC/ISSS, 2006)</td>
<td>Thionic Fluvisol</td>
</tr>
</tbody>
</table>

In the *Australian Soil Classification*, Ray Isbell (2002) adopted the USDA Soil Taxonomy (USDA, 1975) approach for the definition of acid sulfate soil materials for rigorous soil classification purposes. The *Australian Soil Classification* (Isbell, 1996); (Isbell, 2002) divides acid sulfate soil materials into sulfidic and sulfuric along similar lines to that currently used in the USDA Soil Taxonomy soil classification. In Australia the two acid sulfate soil taxonomic classes, sulfidic and sulfuric, are commonly associated with the terms: *potential acid sulfate soil* (PASS) and *actual acid sulfate soil* (AASS) materials respectively. These latter terms along with their abbreviations will be specifically used throughout this study. The term *acid sulfate soil* (ASS) will be used to generically describe all these soils.

### 2.3.2 Acid Sulfate Soil Description

Potential acid sulfate soils are pyritic sediments generally found below the watertable. The iron disulfide (FeS₂) is relatively chemically stable whilst continually submerged. Whilst PASS are often found as pH neutral blue-grey or grey to black saturated clays (Dent, 1986a), they may also include muds, sands and sandy clays interspersed with layers of silts, sands, gravels and fragmented shells. This reflects their complex formation in varying high and low energy estuarine environments (Diemont *et al.*, 1992, Wilson, 2005). When exposed to oxygen these sulfidic sediments have a significant potential to produce acidity through chemical and biocatalysed processes. These involve mineral transformations in the soil which van Bremen referred to as *ripening* (van Breemen, 1972). Soil ripening is the process whereby unconsolidated sediments are gradually transformed into soil through dewatering, consolidation, biogeochemical transformations and soil structure development (Dent, 1986a, Vermeulen *et al.*, 2005). Through processes of oxidation, biotic activity, irreversible drying, shrinking, cracking and the effects of vegetation interaction, the sediment properties shift irreversibly towards those of dryland soil. This is often characterised by development of pedality and profile differentiation (Pons, 1972). Pyrite oxidation produced acidity that exceeds the soil acid-buffering capacity invariably produces a ripened soil horizon with very low pH properties.
2.3.3 Acid Sulfate Soil Identification and Analysis

In the 1990s in Australia, definitive methods for ASS identification were not well known or used. The most popular field method was a qualitative procedure using hydrogen peroxide (a strong oxidant) to evaluate by observation the presence of reduced mineral sulfide species. Soil organic matter in the sample interfered with the test but this response could generally be differentiated from that of pyrite by the absence of hydrogen sulfide odour. Towards the latter part of the 1990s, published information was emerging which allowed more rigorous quantitative testing based on specific laboratory procedures. These included methods analysing for the percentage of oxidisable sulfide (e.g. Peroxide Oxidisable Sulfur (POS) (Sullivan et al., 1999); Acid Volatile Sulfur (AVS) and Chromium Reducible Sulfur (CRS) (Lin et al., 1996) and potential ASS acidity (e.g. Total Actual Acidity (TAA) (Lin et al., 2000).

In Australia the AVS and CRS methods emerged as the most reliable indicators of iron monosulfide and disulfide content respectively in acid sulfate soils. Several other general methodologies exist for analysis of these components, including those reported by: (Sugino, 1983); (Hsieh and Yang, 1989, Hsieh and Shieh, 1997, Sullivan et al., 1999, Lancaster, 2005, Burton et al., 2008).

The Hsieh and Shieh method used in this study nominated iodometric titration to quantify sample sulfide. Rather than describe this procedure, their paper simply referenced the APHA method cited below. However, there are several widely cited and used ‘standard’ iodometric methods, including: Vogel (Vogel, 1961), APHA (Franson, 1985), and USEPA (EPA, 1996). Review and testing of these procedures reveals some ambiguity, inaccuracy, and contradiction in the described methods. A contributing factor here could be the fact that the APHA iodometric method was historically used to analyse for dissolved soluble sulfide in ground and waste waters (Franson, 1985), where the concentrations would be low given the relatively low solubility of hydrogen sulfide in water (Anon., 2009). This same method appears to have been later used in the analysis of far higher concentrations of sulfide in insoluble zinc sulfide form (as in the (Hsieh and Yang, 1989) digestion / diffusion method), and appears to be not accurate and precise enough for these purposes.

Acting on reports of poor experimental results, in 1999 Pawlak and Pawlak reviewed iodometric titration methods. They highlighted the sometimes poor methodology within and between these procedures which had led to reported highly variable recoveries (e.g. 40 % to 200 %) when iodometrically analysing for sulfide (Pawlak and Pawlak, 1999). The Pawlak paper largely attributed this to poor analysis technique as well as inadequate control of acidity during the procedure. This led to hydrogen sulfide loss and inadvertent side reactions producing sulfate. The former results in under-reporting while the latter product induces greater consumption of iodine leading to significant over-reporting of sulfide in the soil sample. After
extensive testing, Pawlak and Pawlak highlighted several key problems and offered modifications to techniques to resolve these. These are described below.

The first potential problem identified relates to timing of the acidification of the zinc sulfide precipitate in the trap solution. If this is done in the highly alkaline trapping solution by addition of concentrated hydrochloric acid (HCl), then hydrogen sulfide release (from the zinc sulfide) is likely to occur and be lost to the analysis. The zinc sulfate precipitate must therefore not be acidified with HCl before being transferred into the acidified iodine solution.

The second issue relates to the high alkalinity of the zinc acetate solution and the neutralising effect of this when and how it is introduced into the acidified iodine solution, potentially creating conditions for a sulfate side reaction to occur. A procedure modification involves use of glacial acetic acid to decrease the alkalinity of the zinc sulfide trap solution from pH 13 to between pH 5 and 6 to maintain the acid integrity of the iodine solution when the trap solution is added to it. Acetic acid is used here as zinc sulfide remains insoluble only in this acid.

2.3.4 Acid Sulfate Soil Genesis

Acid sulfate soils of both actual and potential type exhibit a wide range of profile forms reflecting their morphogenesis.

At the peak of the last glaciation, sea-levels up to 130 m below present day levels (Fairbanks, 1989) allowed coastal river valleys to cut down into the adjacent continental shelf. With onset of the post-glacial warming phase, rising sea levels slowly inundated these coastal valleys, setting up conditions for many to be in-filled with sediments including clays, sands, sandy clays and muds (Pons and van Bremen, 1982a, Wilson, 2005). Sedimentation keeping pace with slowly rising seas led to the accumulation of thick layers of sulfidic deposits in broad low lying estuarine swamps which often reached many tens of kilometres inland from the coast (Dent, 1986a).

Cessation of sea-level rise allowed fluvial processes to dominate in some low energy estuaries such as the study site, leading to the accumulated pyritic sediments being covered with flood overbank deposits. This infill and consolidation of the land surface allowed growth and subsequent decay of vegetation leading to peat accumulation in some back-swamp areas supplemented with intermittent freshwater sediments (Diemont et al., 1992).

2.3.5 Pyrite Formation

Drawing on much previous work, Kaplan et al. determined the basic underlying mechanisms of sedimentary pyrite formation in 1963 (Kaplan et al., 1963). They suggested that seawater derived sulfate-reduction to hydrogen sulfide (H₂S) was converted to hydrotroilite (FeS nH₂O) and them to pyrite (FeS₂). Berner (1970) reviewed pyrite forming mechanisms, summarising
the processes as sulfate reduction, reaction of the resulting hydrogen sulfide with iron minerals and transformation of black iron monosulfides to pyrite. He asserted the two principal sources of H$_2$S were bacterial reduction of sulfate and decomposition of organic sulfur compounds (Berner, 1970). Rickard (1975) in reviewing pyrite formation found support for pathways also involving elemental sulfur, polysulfides and thiosulfate (Rickard, 1975).

Iron disulfide (pyrite) accumulates in brackish, waterlogged sediments which have a ready supply of easily decomposed organic matter. Anaerobic bacteria oxidising this organic matter also reduce dissolved sulfate ions to sulfides and iron III oxides to iron II (Ritsema et al., 1992). Pyrite, a relatively stable end product of these transformations, requires essential conditions for its formation including:

- An anaerobic environment
- Reducing conditions of waterlogged organic-matter-rich sediments allowing sulfide reduction to take place
- A source of dissolved sulfate (generally from seawater)
- Organic matter, which provides the energy requirements of the sulfate-reducing bacteria, viz.: $\text{SO}_4^{2-} + 2\text{CH}_2\text{O} \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^-$
- A source of iron - usually of sediments containing iron oxides and iron hydroxides. In an anaerobic environment, this is reduced to Fe$^{2+}$ which is relatively soluble in estuarine ecology pH ranges
- Removal of the HCO$_3^-$ product. This is typically achieved by tidal flushing in estuarine embayments which also renews the supply of sulfate ions
- Time

(van Breemen, 1972, Pons, 1972, Dent, 1986a)

Pons (1964) noted the sedimentary origin of individual pyrite crystals and small frambooids within the clay matrix, highlighting the difference between this particulate pyrite and that formed in situ within the remains of plant remnants. The interspersed pyrite was interpreted by van Dam and Pons (1973) as reflecting pyrite formation from sediment detritus likely to be evenly and finely dispersed within the sediment (Pons, 1964). In 1972 Rickard reviewed pyrite formation and concluded that pyrite formation occurs through the sulfur oxidation of metastable ferrous sulfides such as mackinawite and greigite. These ferrous sulfides are produced through the reaction between detrital iron minerals: mainly ferric oxyhydroxides, and bacteriogenic sulfide (Rickard, 1972).

In 1975 Rickard proposed two separate pyrite formation pathways, one leading to spherical frambooidal forms made up of ball shaped clusters of single pyrite particles, with the other pathway resulting in particulate form (Rickard, 1975). Discrete pyrite crystals of 1.5 μm diameter may form by reaction between ferric and (hydrogen) sulfide ions in anoxic aqueous solutions in the pH range 3.6 – 5.7. Intermediate products of elemental sulfur and FeS (or Fe(HS)$^-$) have been identified as precursors for this pyrite formation (Wei and Osseo-Asare, 1996), while others have suggested that the precursor could be a polysulfide form (Roberts 1969, cited in (Perry and Pederson, 1993), or a soluble monosulfide complex such as Fe(SH)$^+$, [Fe$_2$(SH)]$^{3+}$, or [Fe(SH)S$_2$]$^-$. (Wilkin and Barnes, 1997). Thus, under suitable conditions in
reducing environments, particulate pyrite could potentially form and be relatively evenly distributed through the accumulating sediment.

Wilkins and Barnes commented in 1997 that framboïds are the dominant pyrite form in modern anoxic environments and that the formation mechanism(s) of these remain unresolved. These researchers reviewed the literature to propose a four-stage formation process involving: (i) nucleation and growth of initial iron monosulfide microcrystals, (ii) reaction of the microcrystals to greigite (Fe$_3$S$_4$), (iii) aggregation of uniformly sized greigite microcrystals (i.e. framboid growth), and (iv) replacement of greigite framboids by pyrite (Wilkin and Barnes, 1997).

In scanning electron microscopy (SEM) studies of ASS soil from eastern Australia Bush and Sullivan (2002) reported pyrite distributed variously in the soil matrix. At sites of former organic matter accumulations (e.g. root channels, small shells and plant remains) pyrite was most abundant, occurring as framboïds varying in size from 3 to 20 μm and made up of individual mainly octahedral crystals of 0.5 to 3 μm diameter. They found dense and loose irregular shaped clusters frequently associated within root remnants. Framboïds and irregular-shaped pyrite clusters were also frequent within macropores of the clay matrix, at times filling whole void spaces in the clay up to 500 μm in diameter. Pyritehedral (dodecahedral) crystal morphologies predominated within the clay matrix at many sites (Bush and Sullivan, 2002).

Once formed, the stability of pyrite in subsoils is conditional upon it being kept in a reduced state in an anaerobic environment. As this anoxic state is typically maintained when the sulfidic material lies below the existing anoxic groundwater surface, any long term natural or anthropogenic disturbance (such as draining or cropping that lowers the groundwater) may initiate or increase the rate of pyrite oxidation by allowing oxygen into the profile sulfidic horizon (Nordstrom, 1982a).

2.3.6 ASS Oxidation

The iron sulfide species in the PASS zone of acid sulfate soils are considered to be most stable when retained under reducing conditions (i.e. below high groundwater levels) and with minimal physical disturbance of subsoil horizons which may allow contact with air. However multiple factors can mediate and change these conditions. As coastal acid sulfate soils often occupy land which is desirable and of high value from residential, industrial, agricultural and recreational perspectives, these soils are at risk of significant disturbance with a consequent substantial cost to the natural and built environment, including impacts on major infrastructure and economic resources such as fishing and tourism (NWPASS, 2000).
It is rarely stated in the scientific literature that pyrite is relatively stable under two widely different conditions: (i) when the pure mineral form is in moisture free environments at room temperature and (ii) in reducing environments. Although dry mineral pyrite oxidation kinetics are reportedly very slow at ambient temperatures, it would appear that high temperature and moisture regimes significantly increase this rate (Chen et al., 2006) with heat alone initiating rapid oxidation at elevated temperatures greater than 450 °C (Ferrow and Sjoberg, 2005). This crystalline form at ambient temperature is then stable enough to be incorporated into jewellery (Adin, 2009) and is also available as a chemical reagent without special storage conditions being nominated (Chem-Supply, 2009a).

Iron sulfides in soils oxidise when exposed to oxidants (electron acceptors). The most common and active terminal electron acceptor is molecular oxygen (O₂). Other oxidants or alternative electron acceptors (Mountfort and Bryant, 1982, Haggblom et al., 1993) include nitrate (NO₃⁻), manganese(IV) (Mn⁴⁺) and iron(III) (Fe⁷⁺) (Nordstrom, 1982a, Kolle et al., 1983). Iron sulfides have two main forms, iron monosulfides (FeS) and iron disulfide (FeS₂). Iron monosulfides are reported to be the initial iron sulfide minerals formed under reducing conditions in estuarine sediments and have been found to be non-exclusive precursors in the rapid formation of pyrite (Smith and Melville, 2004). Iron monosulfide (FeS) oxidises abiotically with oxygen to produce ferrous sulfate (ionised in solution) and water (Equation 2-1).

\[
\text{Equation 2-1: Molecular oxygen oxidation of iron monosulfide}
\]

\[
\text{FeS} + 3\text{O}_2 + 4\text{H}^+ \rightarrow \text{Fe}^{2+} + \text{SO}_4^{2-} + 2\text{H}_2\text{O} \quad \text{[abiotic]}
\]

Pyrite oxidation by molecular oxygen has several stages and pathways, including: (i) oxidation of FeS₂ to ferrous ions and sulfate, (ii) oxidation of the ferrous ions to ferric ions at low pH (<3.6), and (iii) ferric ion oxidation of FeS₂ to produce ferrous sulfate and protons (van Breemen 1972). The combined process of reactions is depicted in Equation 2-2.

\[
\text{Equation 2-2: Pyrite oxidation – overall reaction (van Breemen, 1972)}
\]

\[
\text{FeS}_2 + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+
\]

Pyrite oxidation is reviewed in more detail in later sections within Soil Biogeochemistry 2.7. As sugarcane is grown at the study site on substantial tracts of land containing ASS, sugarcane agriculture is described in the following sections.

### 2.4 Sugarcane

Sugarcane agriculture occupies a significant portion of the lower Tweed River valley estuarine floodplain. In Australia most of the sugar consumed domestically comes from sugarcane (ABS, 2007). Although varieties of sugarcane have been extensively developed for
their high sugar content and disease resistance qualities, this plant originally existed in the environment as a naturally occurring grass species. Sugarcane (*Saccharum* spp.) belongs to the extensive *Poaceae* (or *Gramineae*) family of mainly herbaceous grasses which includes some woody plants such as reeds and bamboo as well as other widely cultivated crops such as oats, barley, wheat, rice and sorghum (King *et al*., 1953).

Sugarcane grows on a variety of soils but prefers fertile frost-free lowland soils in subtropical and tropical climates with rainfall in excess of 1 000 mm per year (Barnes, 1964). Frost-free areas are preferred as frosts can damage the growing tips of the sugarcane plant (King *et al*., 1953). High rainfall regions increase the risk of flooding but sugarcane plants are reportedly able to survive and even thrive in short-term flooding conditions (Glaz *et al*., 2004).

### 2.4.1 Sugarcane Physiology

Sugarcane varieties exhibit differences in morphology but also have many similarities. All are tall growing, multi-stemmed grasses with a root system comprising shallow superficial roots, stabilising buttress roots and deep rope roots (Barnes, 1964). Commercial sugarcane is propagated asexually by cuttings, each containing one or more buds. When planted, these buds develop to a primary stem which in turn is able to bud off other stems (van Dillewijn, 1952). Whilst cane varieties naturally vary in growth characteristics, water and nutrient availability substantially effects yield (Robertson *et al*., 1996a) and this is largely mediated by the root system.

Roots provide many critical functions for a plant, including provision of structural support in the soil medium, facilitating nutrient acquisition from the soil profile and providing the means for water intake by the plant. Much has been written on the physiology of sugarcane (Blackburn, 1984, Alexander, 1973, Barnes, 1964, van Dillewijn, 1952) but less on its root physiology, function and growth.

Sugarcane root systems generally comprise three functional groups in the one plant. These are: (i) highly branched superficial roots for nutrient uptake, (ii) downward oriented structural buttress roots, and (iii) deeply penetrating agglomerated vertical roots known as rope roots (Smith *et al*., 2005) (Figure 2-1).

The biomass density of sugarcane root systems generally shows an exponential decline with depth from 0.3 m to around 1.3 m (Smith *et al*., 2005:172). Blackburn pointed out that
sugarcane root cluster shape and depth are highly variable by cane variety and soil structure and type, but that roughly 50 per cent by weight of the roots occur in the top 0.2 m of soil and 85 per cent in the top 0.6 m (Blackburn, 1984). If it is to survive and produce viable yields, sugarcane must acquire energy and nutrients to build tissue to support and sustain itself. Whilst nutrients are obtained largely from the soil, its energy requirements are met through the process of photosynthesis which provides the sugars and other organic compound building blocks and energy for cell growth and activity (Starr and Taggart, 1987).

2.5 Soil Properties

In agricultural topsoils air and water occupy approximately one half the soil volume (in the pore space), with mineral matter accounting for roughly the other half. Organic matter may vary from 0.5 to greater than 10%, with the living fraction generally occupying less than 1% of the total soil volume (Alexander, 1967). Despite its low concentration, organic matter plays a crucial role in the structuring of soil particles into large units.

2.5.1 Soil Structure and Mineralogy

In general, three broad categories of soil structure are recognised: single grained, massive and aggregated (Hillel, 2004). At the individual clay platelet scale (0.2 to 2 μm) it is the electrochemical forces that tend to be the dominant mechanism holding particles together to create characteristic microstructures, the unique assemblage of individual particles and pores unique to clay samples (van Olphen, 1977).

This arrangement of clay platelets is influenced largely by electrostatic forces of attraction and repulsion from charged surfaces on the platelet due to ionic exchange. The characteristics of these charges, however, may vary depending on soil salt concentrations and acidity. Some clay minerals achieve permanent charge through isomorphic substitution of the clay-interlayer aluminium ions with lower charged soil cations, resulting in a permanent negatively charged plate surface and positively charged edges. Other clay particles such as iron oxides have pH dependent charges. These attract cations due to surface negative charge at high pH, but attract anions due to surface positive charge at low pH (Van Olphen, 1951).

Adequate soil structure for plant growth depends on the presence of porous (>75 μm diameter pores) water stable soil aggregates (1 to 10 mm diameter) in a soil matrix having pore space between aggregates large enough to allow rapid infiltration and drainage (Tisdall and Oades, 1982). Although a fertile agricultural soil may have significant aggregation and allow ready movement of air and water through the soil matrix, the composition and physical size of some individual peds may still support increasingly anoxic environments towards their centres after wetting. This creates apparently anomalous situations where anaerobic biochemical processes may still occur alongside aerobic transformations in normally oxic soil environments.
(Leffelaar, 1993) and includes situations where an oxic transformation may be closely coupled with the anoxic one.

Soil structure development in acid sulfate soil horizons is a process of pyrite oxidation accompanied by either acid-buffering, transformation, or leaching of the acid products. Although Brinkman and Pons (1973) acknowledged unoxidised potential acid sulfate soil as being stable when kept in a reduced state, they described the soil ripening as a 'transient' process and suggested this involved periods ranging from several years up to one hundred years (Brinkman and Pons, 1973).

**Soil Mineralogy**

The type and form of minerals in soils significantly impacts on soil biogeochemistry in determining what mineral species are available as reactants. Minerals, organic matter, and microorganisms form a composite system in the terrestrial environment. Interactions between these components are critically important to ecosystem functioning (Huang et al., 2002). Silicates make up more than 90% of the weight of the Earth's crust and as a consequence of weathering and erosion, silicate minerals tend to predominate in soils. Various size particles are evident ranging from sand to micron-sized clay micelles (Chernicoff and Venkataramanan, 1995) (Table 2-2).

**Clays**

Clays form part of a wide range of argillaceous rocks including clays, shales, mudstones and marls (calcareous mudstone). Clays have been historically defined by two factors, that of mineralogy and particle size. Clay minerals generally result from the hydrolysis of alkaline and plagioclase feldspar minerals (Chernicoff and Venkataramanan, 1995). Definitions vary for clay particle size (Brady and Weil, 2001) but <2 μm is a commonly accepted size range. Silt generally refers to mineral soil particles in the 2 to 50 μm range.

<table>
<thead>
<tr>
<th>Particle size type</th>
<th>Size range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>2.0 mm to 0.05 mm</td>
</tr>
<tr>
<td>Silt</td>
<td>0.05 mm to 0.002 mm [50 to 2 μm]</td>
</tr>
<tr>
<td>Clay</td>
<td>Less than 0.002 mm [&lt;2 μm]</td>
</tr>
</tbody>
</table>

Table 2-2: Defined mineral particle sizes in soil (adapted from Baver, 1948, Troech and Thomson, 1993)

Clay particles possess relatively large surface areas by clay volume and comprise specific but often variable mineralogy (Goldberg et al., 2000). This variability highlights the fact that particle size alone does not make a substance a clay mineral (Monash, 2003). Clays may therefore contain a mixture of many different materials, a greater or lesser proportion of which may be clay minerals. The clay sized fraction in soil is dominated by layer silicates (Goldberg et al., 2000) and these clay minerals result primarily from the hydrolysis of alkali and
plagioclase feldspars, producing the commonly recognised clay forms such as *kaolinite*, *illite*, *smectite* and *chlorite* (Chemicoff and Venkatakrishnan, 1995).

Clay minerals are usually classified as colloids due to their size and may be divided into two main groups, structured and amorphous (non-crystalline). The structured types may be further subdivided according to the layered arrangement of their tetrahedral and octahedral sheets into two principal types: the 1:1 and 2:1 minerals. In the 2:1 clay minerals there are expanding and non-expanding categories (Hillel, 2004). The most common mineral of the 1:1 type is *kaolinite* which is structured around a pair of silica-alumina sheets exhibiting little swelling capability and low ion exchange capacity, as water and ions cannot penetrate between the sheets.

Smectite clays, by comparison, are hydrated aluminosilicate 2:1 clay minerals of the expanding type that also includes *montmorillonite*, *vermiculite* and *beidellite*. These clays exhibit pronounced shrink-swelling properties, a large chemically active surface area and generally a high ion exchange capacity (Odom, 1984, Hillel, 1998).

An intermediate variety, *illite*, is a hydrous mica mineral which has a 2:1 silica:alumina ratio but is a non-expanding type of clay. Another mineral similar to illite is *chlorite*, a 2:2 type clay in which magnesium rather than aluminium ions predominate in octahedral sheets (Hillel, 1998).

Other constituents of the clay sized fraction in soils include *allophone*, consisting of non-crystalline mineral colloids of loosely structured alumina and silica components and a group of hydrous iron and aluminium oxides known as *sesquioxides* (e.g. limonite, goethite). Gibbsite is also frequently encountered aluminium oxide colloid in soils (Hillel, 2004).

Clay particle surfaces are predominately negatively charged and this charge is balanced by neutralising cations. Cations in this double layer may be replaced or exchanged. Due to the laminar shape and the layer silicate arrangement of most clay particles there is a tendency for the cations to be replaced at the edges, resulting in uneven charge distribution on the clay platelet or micelle. Positive charges are associated with the micelle edge and negative charges with the face (Hillel, 2004).

### 2.5.2 ASS Clay Micromorphology

Clay is variously structured at atomic, molecular, platelet and aggregate scales and these structures interact with other soil constituents to produce observed variations in physical, hydraulic and biogeochemical properties (Nelson et al., 1999b). Investigation of ASS morphology by Bush and Sullivan in 1997 used epoxy substitution and freeze drying techniques of previously liquid-nitrogen frozen samples to show new micro-morphological features of pyrite forms in ASS (Bush and Sullivan, 1997, Bush and Sullivan, 1999). Although this work did much to reveal significant new information on the geomorphology, mineralogy and chemical nature of iron sulfides in northern NSW soils, understanding of the PASS gel-clay
platelet microstructure still lay largely in the realm of informed conjecture. This was aided by indirect information such as the observed high porosity of this material (van Oploo, 2000, White et al., 2001). Apart from a somewhat indistinct electron micrograph image in Dent (Dent, 1986a) and the two Bush and Sullivan (1997, 1999) pyrite morphology papers, prior to 2003 little other work appears to have been done to characterise microstructure features in PASS pyritic gel-clays. Additionally, there were few techniques available for any such examination of moist gel-clay given the reactive and physically fragile nature of these soils. On drying, pyritic gel-clays shrink and distort markedly, resulting in significant changes in morphology and chemistry (Pons, 1972, van Breemen, 1972). Procedures developed and used in other fields, however, offer fruitful avenues of investigation.

2.5.2.a Soil micromorphology evaluation

Following early SEM development work beginning in the mid 1930s, Ingles (1968) expanded SEM instrumental techniques to examine clay structures. It was Ingles who coined the term ‘card-house’ to describe the observed open-stacked structure of some flocculated clays examined (Ingles, 1968, Bennet and Hulbert, 1986). The use of freezing techniques in biological sample preparation appears to have been pioneered by Heuser (Heuser et al., 1977) and Chandler (Chandler and Heuser, 1979). This was further developed by Heuser and others to include freeze-etching to remove sample surface embedded water prior to microscopy imaging (Heuser and Salpeter, 1979, Roof et al., 1980, McCann et al., 1990).

Here the term microstructure refers to the micron-scale micelle arrangement within clays. Whilst it is appealing to broadly categorise this microstructure into two exclusive structural groups: that of the edge-to-face card-house open structure or the face-on-face laminar structure, the reality encountered in practice is more complex (Hillel, 2004). In clay mixtures, the varying physical and electrochemical characteristics of the different 1:1, 2:1 and 2:2 type clays seem to suggest that a mixed structure varying by the proportion of each clay type may be more likely.

Traditional procedures used to preserve the structure of moist and often fragile environmental samples during structure analysis include: (i) solute dehydration and replacement with hardening materials such as gels (Echlin, 1992); wax or resins (Ringose-Voase, 1991, Tippkotter and Ritz, 1998) or cryoprotectants (Echlin, 1992), and (ii) use of cryogenic methods to freeze the sample in a manner that preserves its original structure (Echlin, 1992). Cryogenic techniques involve rapid cooling of the moist sample in either liquid-hydrocarbons (e.g. propane), or liquid nitrogen or liquid helium to quickly freeze the sample water into an amorphous or microcrystalline form, thereby minimising distortion of the sample structural matrix. Sublimation etching, involving transformation of the ice-phase water directly to the gas phase is then used to remove ice from the surface of the sample. While liquid helium allows lower absolute temperatures (and potentially faster freezing) to be attained (Table 2-3), rapid
freezing and sublimation etching may be adequately carried out with liquid nitrogen using specific techniques.

Table 2-3: Liquid gas specifications (Air-Products, 2009a, Air-Products, 2009b)

<table>
<thead>
<tr>
<th>Gas</th>
<th>Boiling Point at 1 atm.</th>
<th>Temperature (°C)</th>
<th>Temperature (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helium</td>
<td></td>
<td>-268.9</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Freezing Point at 1 atm.</td>
<td>-272.2</td>
<td>0</td>
</tr>
<tr>
<td>Nitrogen</td>
<td></td>
<td>-195.8</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>Freezing Point at 1 atm.</td>
<td>-210.0</td>
<td>6</td>
</tr>
</tbody>
</table>

The successful application of cryogenic methods involves freezing the water or solute in the sample in such a way so as to avoid structural damage resulting from slow expansion of the water on freezing, as well as through large ice-crystal formation that potentially distorts and destroys the natural structure within the sample. Many forms of solid water exist. Echlin (1992) identifies at least nine different stable crystalline forms, one metastable crystalline form and one or two metastable amorphous (non-crystalline) forms.

Hexagonal ice (I_h) is generally considered to be the only ice form occurring in natural environments and this comprises ice crystals of hexagonal plates together with hexagonal prisms and needles (Echlin, 1992). The other solid water form of interest here is non-crystalline amorphous ice. Non-crystalline ice may be formed in several ways, but the method most relevant to the current study involves the rapid cooling of minute (µm³) quantities of the moist sample (Echlin, 1992). This volume equates to gram quantities of moist gel clay.

Echlin reviewed and studied ice crystal formation in cooled hydrated samples under conditions of moderate (1 to 100 K per second), fast (1000 K per second) and ultra-rapid (10⁴ to 10⁵ K per second) cooling. He found that under fast and ultra-rapid cooling any ice-crystal formation was likely to be limited to the microcrystalline ice form (i.e. nm scale) along with formation of the non-crystalline form, amorphous ice (Echlin, 1992). The theory and practice suggested that more rapid cooling led to production of fewer and smaller ice crystals. Thus the key to minimal crystal formation in environmental samples appears to be (i) small sample size, (ii) a very low temperature cryogen, and (iii) rapid conduction of heat away from the sample.

Although there are cryogens with lower absolute temperatures and higher coefficients of thermal conductivity (Echlin, 1992), liquid nitrogen may be used as a cryogen when prepared in specific ways. Liquid nitrogen slush at 63 K (−210 °C), prepared by evaporating liquid nitrogen in a open container under a vacuum of 10 to 15 kPa, can achieve cooling rates of 1.2 x 10³ K per second in small environmental samples (Echlin, 1992). This method then appears suitable for the rapid freezing of small moist samples with consequent minimal ice crystal formation.

Examination of the micron-scale structural detail of frozen moist environmental samples effectively takes place only in the absence of the solid water and the primary method of removal of this involves freeze-drying, or sublimation etching. Under specific temperatures and
pressures, solid water may evaporate or sublime directly from the frozen solid-state phase into the gas phase.

This procedure requires apparatus that allows drying to occur between 173 K and 193 K (i.e. between -100° and -80 °C) and that also has the capability of maintaining a state of high vacuum to increase the rate of solid water removal and to minimise re-crystallisation. Echlin (1992) stated that freeze-drying carried out at pressures between 1.3 mPa and 130 μPa (10⁻³ to 10⁻⁶ Torr) and at temperatures between 173 and 193 K enables etching rates in pure ice of between 77 and 2 nm depth per second. This equates to an average etching depth of around 9 μm in four minutes and 16 μm in seven minutes.

Although the Echlin work focussed largely on biological sample examination, use of the information and techniques markedly assisted development of methods used in this study.

This enabled investigation of gel-clay particle size and type, pore-space characterisation and primary mineralogy. Given that clay particles are defined as mineral platelets less than 2 μm (Hillel, 1982), one of the few instrumental methods available for viewing and imaging this material is a scanning electron microscope. Evaluation of the study PASS gel-clays including microstructure analysis is described in chapter 5.

2.6 Soil Hydrology

The presence of adequate water in soil is crucial not only to plant growth but also for biogeochemical functioning. Chemically, water acts as a transport agent for inorganic and organic substances. The high specific-heat of water also creates a moderating influence on diurnal and seasonal temperature cycles in the soil profile (Troech and Thomson, 1993, Topp and Ferre, 2002). The following sub-sections outline the essential properties of water relating to the current study.

Precipitation, Infiltration and Runoff

Precipitation includes condensation (dew and frost), rain, mist, hail and snow (Fetter, 2001). Given the field site location in a sub-tropical near-coast region, the only precipitation generally relevant to the current study is rainfall and this is the focus in this section of the review of literature.

Some water is intercepted by vegetation during rain precipitation and this amount is generally greatest at the beginning of the rainfall event when plant surfaces are dry. Rainfall reaching the ground surface may infiltrate pervious soil, but its capacity to do this is finite. Soil infiltration rate is mediated by soil type, surface compaction, soil texture, porosity and pre-existing soil moisture content (Li et al., 2001, Fetter, 2001). If precipitation continues to be greater than the infiltration rate then runoff will occur. This may be accompanied by interflow or throughflow (lateral flow within the soil profile) if slope and soil horizon conditions are conducive to this (Hillel, 1971, Fetter, 2001).
Hydraulic Conductivity

All soils possess porosity, the characteristic of having open spaces within the soil matrix able to be occupied by water or water vapour. If these spaces are connected, then the soil will have the property of permeability or hydraulic conductivity, the ability for water to flow through the matrix. This depends on soil particle size and shape as well as pore size distribution and connectedness (Hillel, 1998). High permeability in soil helps prevent runoff during intensive rainfall events as most of the water infiltrates the soil profile. However, this may be mediated by relatively impermeable layers higher or lower in the profile or by the proximity of a shallow watertable (Troech and Thomson, 1993). One readily accepted measure of water flow in soil is saturated hydraulic conductivity \( K_s \) (Hillel, 1998).

This reflects the speed of flow in different soil materials. In micron size clay material, \( K_s \) varies between \( 10^{-9} \) and \( 10^{-6} \) cm sec\(^{-1} \), which equates to 1 mm and 0.001 mm per day (Fetter, 2001). Hydraulic parameters for some typical soil materials are nominated by Boeker and von Grondelle (1995) and Fetter (Fetter, 2001) in Table 2-4 and 2.5 respectively. Of note here is the extremely slow water movement (<1 mm day\(^{-1} \)) and the high capillary-rise of two to four metres in clay materials.

Table 2-4: Saturated hydraulic conductivity \( K_s \), particle diameter \( d \), and capillary rise \( h_c \) for some common soil materials (after Boeker and von Grondelle, 1995)

<table>
<thead>
<tr>
<th>Material</th>
<th>( K_s ) (m/sec)</th>
<th>( K_s ) (mm/day)</th>
<th>( d ) (mm)</th>
<th>( h_c ) (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td>( 10^{-10} ) – ( 10^{-8} )</td>
<td>0.0086 – 0.86</td>
<td>&lt;0.002</td>
<td>2 - 4</td>
</tr>
<tr>
<td>Silt</td>
<td>( 10^{-8} ) – ( 10^{-6} )</td>
<td>0.86 – 86</td>
<td>0.002 – 0.05</td>
<td>0.7 – 1.5</td>
</tr>
<tr>
<td>Sand</td>
<td>( 10^{-5} ) – ( 10^{-3} )</td>
<td>860 – 86 000</td>
<td>0.05 – 2</td>
<td>0.12 – 0.35</td>
</tr>
<tr>
<td>Gravel</td>
<td>( 10^{-2} ) – ( 10^{-1} )</td>
<td>860 000 – 8 600 000</td>
<td>&gt; 2</td>
<td>nil</td>
</tr>
</tbody>
</table>

Table 2-5: Saturated hydraulic conductivity for unconsolidated sediments (after Fetter, 2001)

<table>
<thead>
<tr>
<th>Material</th>
<th>( K_s ) (cm/sec)</th>
<th>( K_s ) (mm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td>( 10^{-9} ) – ( 10^{-6} )</td>
<td>0.00086 – 0.86</td>
</tr>
<tr>
<td>Silt, sandy silts, clayey sands, till</td>
<td>( 10^{-6} ) – ( 10^{-4} )</td>
<td>0.86 – 86</td>
</tr>
<tr>
<td>Silty sands, fine sands</td>
<td>( 10^{-5} ) – ( 10^{-3} )</td>
<td>8.6 – 860</td>
</tr>
<tr>
<td>Well sorted sands, glacial outwash</td>
<td>( 10^{-3} ) – ( 10^{-1} )</td>
<td>860 – 86 000</td>
</tr>
<tr>
<td>Well sorted gravel</td>
<td>( 10^{-2} ) – 1</td>
<td>8 600 – 860 000</td>
</tr>
</tbody>
</table>

With regard to \( K_s \) values for acid sulfate soils, White (White et al., 1993) reported hydraulic conductivity in an acid sulfate soil profile using the auger-hole method in the McLeods Creek study site locale (Table 2-6). Wilson carried out an extensive study of soil hydrology at McLeods Creek (the current study site) in 1995 but apart from a comment on the “rapid response” of groundwater to significant rainfall events, no determination of hydraulic conductivity values was made (Wilson, 1995).
Table 2-6: Soil saturated hydraulic conductivity, McLeods Creek, NSW (adapted from White et al., 1993)

<table>
<thead>
<tr>
<th>Soil Horizon</th>
<th>Saturated Hydraulic Conductivity (K_s mm/hr)</th>
<th>Saturated Hydraulic Conductivity (K_s mm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidised Actual Acid Sulfate Soil (AASS)</td>
<td>30.8</td>
<td>739</td>
</tr>
<tr>
<td>Transition zone: Jarosite-blue estuarine clay</td>
<td>28.6</td>
<td>686</td>
</tr>
<tr>
<td>Partially oxidised Acid Sulfate Soil (pyritic gel-clay)</td>
<td>1.1</td>
<td>26</td>
</tr>
</tbody>
</table>

Comparing K_s values in the two tables (Table 2-4 and 2-6) it can be seen that, despite its fine-grained clay origin, the AASS soil horizon has relatively high saturated hydraulic conductivity, measured between 686 and 739 mm per day.

This approaches the hydraulic conductivity quoted for sand in Table 2-4 and is in the range for silty sands in Table 2-5. By comparison, the partially oxidised pyritic gel-clay at the top of the PASS sulfidic soil horizon has a much lower K_s value at 1.1 mm per hour or around 26 mm per day. In old continuously used cane soils, the presence of decomposed long rope roots can impart vertical anisotropic properties to the soil (Fetter 2001), postulated here to result in greatly increased vertical hydraulic conductivity.

Downward infiltration into initially unsaturated soil occurs generally under the combined influence of gravity and suction gradients. As water penetrates deeper and the wetted part of the profile lengthens, the suction gradient (the difference in the pressure head between the saturated surface and the unwetted zone) decreases over an ever increasing distance. As this trend continues, the suction gradient diminishes to zero leaving the gravity component to move the water downward (Hillel, 2004). Johnston et al. (2009) reviewed literature on ASS hydraulic conductivity of ASS and noted that hydraulic conductivity (K_s) is a notoriously heterogeneous soil property. K_s of sulfuric horizons can greatly influence rates of seepage from adjacent drains, particularly during periods when the general watertable is relatively lower than the drain. Johnston et al. (2009) concluded that K_s in sulfuric horizons is highly variable in the range < 0.5 m day\(^{-1}\) to >500 m day\(^{-1}\) and thus can have very high values in these soils.

Infiltration rates in soils may be significantly influenced by preferential flow pathways, including cavities, sand and clay lenses and fissures caused by shrinkage and cracking in clay or through the action of burrowing soil animals or decaying roots (Hillel, 1998). Being in a relatively high rainfall sub-tropical zone, the study field site soils rarely dry out and have few soil macrofauna, but are extensively and deeply penetrated by sugarcane roots to depths of over 1.8 m as shown by current study soil profile descriptions in Appendix 12 and noted by van Oploo (van Oploo, 2000). Many of these root channels are old with the original roots degraded and mineralised and the potential exists in this way for sugarcane cropping over many decades to significantly alter cane soil infiltration properties. These anisotropic properties may promote water flow more in vertical rather than horizontal directions (Fetter, 2001).
Drainage infrastructure also impacts on infiltration rates and control of groundwater movement both in terms of its rate and levels. At the study site and throughout the region generally, mole and field drains are used to control water saturation in the upper soil profile where the cane plant sets most of its roots (Reilly, 2003a). Field drains 0.4 to 0.7 m deep are excavated at the sides of the cane-block and drain into headland drains mostly at each end of the block. Mole drains are formed within high clay subsoils with a special tractor-drawn implement that forms an unlined channel running laterally at an angle across the cane-block. These are approximately 0.4 to 0.6 m below the soil surface and connect with the field drains (DPI-VIC, 2008, Quirk, 2009). Mole drains are intended to drain high groundwater levels in the upper soil profile, thereby promoting soil aeration in the topsoils above them and allowing cropping to take place.

2.6.2 Groundwater

The earth layer that contains the three-phase system of solids, liquids and gases is generally known as the zone of aeration, the unsaturated zone, or the vadose zone (Hillel, 1982). Below this lies the saturated or groundwater zone. The position of the watertable often follows the general shape of the topography, but in estuarine floodplains the groundwater surface is often level in unconfined situations with relatively porous solum material (Fetter, 2001).

Fetter (2004) summarised five observations with respect to groundwater:

- In the absence of groundwater flow, the watertable will be flat
- A sloping watertable indicates groundwater is flowing
- Groundwater discharge zones are in topographical low spots
- The watertable has the same general shape as the surface topography
- Groundwater generally flows away from topographical highs and toward topographical lows

Depending on the particle size of the soil material, above the soil watertable sits a zone of saturated soil grading upwards to unsaturated material. This is the capillary fringe zone.

2.6.3 Capillary Rise

Loss of water occurs not only from near surface soil layers but may also come from deeper soil layers, including the watertable, through capillary rise, the movement of moisture through the minute pores between soil particles that act as capillaries. This water movement takes place as a consequence of surface tension which occurs when evaporation occurs at the soil surface. This moisture loss exerts a surface tension negative pressure on the moisture deeper in the profile (Bell, 2004).

The degree to which the moisture rises in the soil profile is primarily a function of soil particle and pore size. This includes any channels or tunnels made by roots or soil fauna. In theory, capillary action can lift water more than seven metres above the ground watertable in the
case of silt and clay if soil pores are small enough (e.g. <10 μm) (Pitty, 1979). In practice, capillary rise greater than one to four metres is seldom exceeded (Trocch and Thomson, 1993, Boeker and van Grondelle, 1995). Given the role moisture plays in biogeochemical reactions, the dynamic properties of the capillary fringe can have a significant impact on soil geochemical and microbiological processes (Berkowitz et al., 2004).

2.6.4 Soil Moisture

Soil moisture including groundwater is a dynamic characteristic of most soil types. This moisture is impacted upon by many environmental factors, including soil type, soil organic matter content mineralogy, precipitation, relative humidity, temperature, pressure, wind speed, insolation, cloud cover, ground cover, vegetation type and coverage, landscape aspect and land management practices such as tillage and mulching (Hillel, 1971). Moisture in soil may exist in various states or phases simultaneously but it is the liquid phase that is the most important to plants (Kramer and Boyer, 1998), cited in (Hillel, 1998) and biogeochemical cycling (Lohse et al., 2009).

Soil texture and soil organic matter may have considerable effect on the available waterholding capacity (Khaleel et al., 1981). Fine-textured soils have a large total volume of pore space between the particles relative to courser textured soils. Soil organic matter levels impact on hydraulic conductivity in many complex ways. Whilst high SOM may improve soil texture and thus potentially increase $K_s$, water is also highly adsorbed to organic matter which will slow infiltration until the soil has reached saturation point (Nemes et al., 2005).

Soil moisture may be measured using either direct or indirect methods with the direct procedures involving removal and direct measurement of water in the soil matrix. Gravimetric and volumetric methods physically measuring the weight or volume of water in a known quantity of soil are typical of the direct methods. Indirect methods measure some soil physical or chemical property related to its water content and use this to estimate soil moisture. Soil properties typically used by these indirect procedures include: the dialectic constant; electrical conductivity; heat capacity and magnetic properties (Topp and Ferre, 2002). One of these instruments used in the current study, a Capacitance Probe, uses the soil profile as the dialectic medium of a capacitor in a capacitive-inductive resonant circuit.

Capacitance devices emerged in the 1930s and have since developed into stable and robust instruments possessing properties of fast response times; ease of use; and safety and accuracy provided good electrode-soil contact is maintained at all times (Starr and Paltineanu, 2002). Cylindrical paired metal-ring capacitance probes placed inside a tightly fitting plastic tube installed with no air gaps to the surrounding soil offer the functionality of being able to measure volumetric soil moisture at multiple discrete depths in the soil profile. There are three procedural phases in the use of these electrode ring probes: (i) sensor normalisation, (ii)
calibration, and (iii) field measurement (Starr and Paltineanu, 2002). Normalisation is a factory procedure to ensure that the calibration equation covers all sensors and allows sensors to be interchanged or replaced with no loss of data continuity. Calibration adapts the probe to the specific soil type due to the varying electromagnetic properties of different soil minerals. The standard reference for calibrating a soil water measuring device is the thermo-gravimetric method (Topp and Ferre, 2002) using soil samples from the same soil depth as the relevant sensor.

2.6.5 Evapotranspiration

Moisture loss from soil occurs via three primary pathways: (i) throughflow or leaching, either downwards or sideways in the soil profile; (ii) evaporation from the soil surface; and (iii) transpiration of moisture through stomatal openings in plant leaves (Fetter, 2001). Although it is relatively simple to measure total losses in soil water content, it is less simple to apportion this reduction between evaporative loss from the soil surface, loss through transpiration at the leaf surface of plants (Brady and Weil, 2001) and losses through leaching. The main point here is that in cropping systems water is continually being gained and lost from the soil and, without extensive scientific measuring equipment, quantification of the above components can often only be estimated or inferred.

2.6.5.a Transpiration

Plants maintaining cellular processes throughout their whole structure require moisture, metabolites and nutrients. Whereas a large proportion of the nutrients are retained by the plant, one of its outstanding characteristic is that moisture is continually being lost from the plant through transpiration, mainly through the leaf stomata (Russell, 1961). This loss occurs incidentally when the plant leaf stomata open to begin their photosynthesis cycle (Barry and Chorley, 1968). Of the water moving into the leaf, more than 90 % is lost in transpiration and only around 2 % is used in photosynthesis (Starr and Taggart, 1987). The water lost from the leaves through transpiration creates a negative pressure gradient that extends unbroken to the roots and the total effect is that of a pump bringing water into the plant (Russell, 1977).

Factors impacting on this solute flow are: sunlight intensity, air temperature, atmospheric CO₂ concentration, soil moisture availability, groundwater depth, wind speed and relative humidity. Whilst there is some evidence of moisture loss control by the stomata (Inman-Bamber and Smith, 2005), the bulk of current evidence appears to support a root-driven process through chemical signals in the plant water (Saliendra and Meinzer, 1989).

Living plant tissue may contain more than 90 % water yet transpiration rates are such that this water can be lost from the plant in minutes unless it is replaced from soil water reserves via its roots (Huck, 1984). In the case of high density large plants such as a sugarcane crop, this
pumping action may draw down the volume of soil water to the point of lowering the ground watertable. Due to transpiration activity being greatest during sunlight hours and lowest at night, Jackson (Jackson, 1973) observed watertable diurnal height change cycles during these periods, although evaporation could also be a factor here. Lower soil evaporation rates have been observed after sugarcane canopy closure (Bristow and Popham, 2002).

2.6.5.b Evaporation

Under suitable conditions of temperature and humidity, soil moisture passes into the atmosphere as water vapour through evaporation. The mass of water in grams per cubic metre of air is termed the absolute humidity and evaporation will continue until the air becomes saturated with moisture, but the water holding capacity varies by air temperature. On cooling, an air mass can no longer hold the same amount of moisture resulting in condensation, often seen as dew (Fetter, 2001).

Evaporation from soil occurs when liquid water is transformed into the gaseous state. This only occurs when moisture is available within the soil profile and is also dependent on the relative humidity of the local atmosphere being less than at the evaporating surface (Pidwirny, 2008). The amount and rate of moisture vapour loss at the soil surface is a function of soil moisture content in the surface layers, soil moisture at depth, capillary rise characteristics of the soil structure, groundcover, soil and air temperatures, wind speed and vapour pressure. Soil surface evaporation may be estimated using a pan evaporimeter (Bloemen, 1978) or mathematically estimated from other climate data using experimentally derived formulae (Cooke et al., 2008).

2.7 Soil Biogeochemistry

Soil organisms, particularly soil flora, play a highly significant part in primary geochemical cycling within many soil profiles including the acid sulfate soils studied here. As the microbiology of acid sulfate soils was the subject of concurrent research work by an ANU Fenner School colleague, Mira Durr, no microbiological fieldwork was consequently carried out within the current research. Mira Durr’s work is described and cited in relevant sections of this literature review. However, as it was considered important to this study to understand potential microbially mediated reactions in ASS, from a functional perspective, a review of biogeochemistry principles is conducted here.

The living biomass component in soil may be described within two broad groupings: (i) a large assemblage of Eukaryote organisms (e.g. animal, plant, fungi and protists), and (ii) Prokaryote organisms (e.g. Bacteria and Archaea) (Chatton, 1938). Of interest here is a sub-grouping of both of the above, the microorganisms, that inhabit the micron scale soil pore space yet possess the ability to significantly affect soil chemistry and soil chemical cycles.
Microorganisms include small unicellular organisms such as Bacteria, Archaea, and a few fungi species including multicellular fungi and protists (algae, protozoa, slime molds and water molds (Madinian and Hartman, 2006).

Microorganisms operate in varying habitats using a wide range of substrates for respiration (energy acquisition) and nutrition. Figure 2-2 summarises some of these naming conventions. For example, a microorganism using an inorganic chemical substrate for energy and carbon dioxide as its only carbon source in an anoxic habitat is termed a chemolithoautotrophic obligate anaerobe.

Microorganisms fall into two broad functional categories based on the respiration substrate: (i) oxygen, and (ii) alternative electron acceptors. In oxic environments molecular oxygen primarily serves as the terminal electron acceptor in energy-gaining chemical processes whereas, in its absence, alternative electron acceptors such as nitrate, iron(III) and manganese(IV) may be used by microorganisms. Within these two oxic/anoxic categories, microorganisms may further be defined by electron donor pathways and their source of carbon. Apart from carbon, which forms a ubiquitous part of all organic matter, microorganisms require other nutritive substances from their environment.

The two primary functions in microorganisms are energy acquisition (respiration) and the assimilation and biosynthesis of nutrients. The energy is used to acquire and transform nutritive substances for cellular maintenance and growth (biosynthesis). Energy acquisition takes place in a biogeochemical context of electron transfer within coupled oxidation-reduction reactions. Here, one substance donates electrons and the other is the terminal electron acceptor.
The word ‘terminal’ is important here as the stripped electrons enter an electron transport chain within the microorganism which generates cellular energy units before the electron is passed on to the final external electron acceptor (Madigan and Martinko, 2006).

The term ‘nutrients’ is often used in a more general sense to include all metabolic substances (Videla, 1997) including those for both energy acquisition (respiration or dissimilation) and cellular biosynthesis (assimilation). In this review the term is applied only to the latter sense and these include acquisition of the essential elements of hydrogen, carbon, nitrogen and phosphorus with small amounts of potassium, magnesium, sodium, calcium and iron. Essential trace minerals include: manganese, cobalt, molybdenum, nickel, zinc and selenium (Madigan and Martinko, 2006).

Microbial physiological functioning (including assimilation, respiration, growth, replication and dormancy) is dependent on and responds to external conditions of temperature, light, moisture and organic and inorganic chemical properties including metabolic substrates (Alexander, 1967). Of the many properties of soils, oxygen concentration; temperature; moisture; substrate availability and pH are noted as being of primary importance in supporting growth of microbial populations and functional activity (Madigan and Martinko, 2006).

### 2.7.1.a Temperature

From a soil perspective, heat energy derives from two primary sources: (i) solar radiation, and (ii) the Earth’s core. Solar radiation arrives at the Earth’s atmosphere with a practically constant flux of nearly 1.4 Joules sec\(^{-1}\) m\(^2\) (1.353 kW m\(^2\)) of which about half is visible light (Hillel, 2004). Within the lithosphere, temperature increases with depth at the rate of 25 – 30 °C per kilometre (Fridleifsson et al., 2008). This indicates an internal sources of heat from radioactive mineral decay (approximately 80 % of the total) and residual heat from the time of planetary formation (20 %) (Stuwe, 2007; Turcotte and Schubert, 2002).

From a microorganism perspective, particular microbial species have preferred a temperature range within which metabolic activity is optimised. Activity decline observed at both the lower and upper extremes of this range (Blackman, 1905). Mediating this somewhat is reported microorganism adaptation where similar bacterial species may acclimatise over time to quite disparate environmental conditions (Young et al., 2008). Madigan & Martinko (2006) recognise four broad temperature categories for microorganisms within which optimal activity occurs (Table 2-8).

<table>
<thead>
<tr>
<th>Microorganism category</th>
<th>Temperature range (optimal temperature) °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychrophile</td>
<td>-5 to 12 (4)</td>
</tr>
<tr>
<td>Mesophile</td>
<td>8 to 47 (35)</td>
</tr>
<tr>
<td>Thermophile</td>
<td>41 – 68 (60)</td>
</tr>
<tr>
<td>Hyperthermophile</td>
<td>66 - 113 (88 - 106)</td>
</tr>
</tbody>
</table>

*Table 2-7: Relation of temperature to microbial growth rates (from Madigan and Martinko, 2006)*
Temperature is one of the most important factors affecting soil microbial function and enzyme activity (Madigan and Martinko, 2006) including soil organic matter decomposition (Kirshbaum, 1995, McInerney and Bolger, 2000). Increasing soil temperature with depth is reported to increasingly activate the breakdown of soil organic material into biological substrates such as acetate, methane and hydrogen (Parkes et al., 2007). Temperature mediated non-linear enzyme activity response has been observed in temperate soils where greater nitrous oxide (N₂O) emission occurred due to inferred low-temperature impacts on N₂O reductase enzyme to a greater extent than for other denitrifying stage enzymes (Holtan-Hartwig et al., 2002).

Raised temperatures increase the concentration of respiratory adenosine triphosphate (ATP) energy units in the microbial soil biomass (Contin et al., 2000) and increases carbon mineralisation in both topsoils and subsoils. Subsurface soils are more sensitive to temperature rise than surface soils (Jin et al., 2008). Decreasing temperature not only impacts on specific soil processes such as mineralisation of nitrogen-rich residues, it can change the process balance from, say, net mineralisation to net immobilisation, potentially locking up soil nitrogen from crop plants during cold periods (Cookson et al., 2002).

2.7.1.b Acidity in ASS

Soil pH has been termed a master variable in the way it can affect the availability and mobility of plant nutrients and toxins (Brady and Weil, 2001). A summary of soil acidity in acid sulfate soil systems appeared in a 2004 conference paper (Macdonald et al., 2004) and the following three paragraphs are derived from this account.

There are two acid pools within soil and water systems: mineral acidity and total potential mineral acidity. Total actual acidity (TAA), which is analogous to mineral acidity, is defined by Konsten as the total amount of freely available soil acidity (Konsten et al., 1988).

TAA is comprised of soluble acidity, exchangeable acidity, acidity from protonated variably charged particles and acidity carried by basic sulfate compounds (Lin et al. 2000). In ASS systems, the major mineral acid is sulfuric acid (H⁺ in Equation 2-3 ) generated from pyrite oxidation and is measured by the total actual acidity method (McElnea, 2002b). TAA has been defined by (Stumm and Morgan, 1996) as:

\[ \text{Mineral acidity or TAA} = [H^+] - [HCO_3^-] - 2[CO_3^{2-}] - [OH^-] \]

Total potential mineral acidity (TPA) is the second acid pool in waters and soils and is composed of dissolved metal and organic acid species and protons, defined by (Morel and Hering, 1993) and (Stumm and Morgan, 1996) (Equation 2-4) as:
Chapter 2: Nitrogen in Agricultural ASS

Equation 2-4: Total potential mineral acidity (TPA)

\[
TPA = 2[\text{Fe}^{2+}] + 3[\text{Fe}^{3+}] + [\text{Fe(OH)}_2^+] + 2[\text{FeOH}^{2+}] - [\text{Fe(OH)}_3^-] + 3[\text{FeSO}_4^-] + 3[\text{Al}^{3+}] - [\text{Al(OH)}_3^-] + 2[\text{Al(OH)}^{2+}] + [\text{Al(OH)}_2^+] + 3[\text{H}_2\text{SO}_4^-] + 2[\text{Mn}^{2+}] + [\text{HSO}_4^-] - [\text{HCO}_3^-] - 2[\text{CO}_3^{2-}] - [\text{OH}] + [\text{H}^+] + [\text{HA}]
\]

where [HA] represents organic acids

Sulfide oxidation supplies a significant source of Fe$^{2+}$ and H$^+$ to the soil profile and further mineral weathering of aluminosilicates and oxide minerals releases other metals such as Al, Si, and Mn and heavy metals such as Cr (Evangelou, 1995). Mineral compounds, including jarosite, ferrhydrite, schwertmannite, goethite, hematite and oxyhydroxides, represent a significant store of acidity within the actual acid sulfate soils. Until recently this acidity store has been overlooked. However their importance is now being recognised and methods are being developed to quantify and examine these (McElnea et al., 2002a); (McElnea, 2002b). The potential mineral acidity or TPA, as defined by Equation 2-4, is the acidity that is generated as a result of the oxidation and hydrolysis of dissolved metal ions and organic acids in water. During inundation of actual acid sulfate soil, Fe and Al minerals can be dissolved and ions of these minerals are released into the soil solution. These dissolved metal species now existing in interstitial waters could be transported to the soil surface or to the adjacent drains where oxidation, hydrolysis and precipitation reactions will occur, generating acidity. Typical reactions are defined in Equation 2-5 to Equation 2-8.

Equation 2-5: Proton activity from dissolved metals [Mn]

\[
\text{Mn}^{2+} + 1/4 \text{O}_2 + 3/2 \text{H}_2\text{O} \rightarrow \text{MnOOH}_3^- + 2\text{H}^+
\]

Equation 2-6: Proton activity from dissolved metals [Al]

\[
\text{Al}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Al(OH)}_3_{(s)} + 3\text{H}^+
\]

Equation 2-7: Proton activity from dissolved metals [Fe(III)]

\[
\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{FeOOH}_3^- + 3\text{H}^+
\]

Equation 2-8: Proton activity from dissolved metals [Fe(II)]

\[
\text{Fe}^{2+} + 1/4\text{O}_2 + 3/2\text{H}_2\text{O} \rightarrow \text{FeOOH}_3^- + 2\text{H}^+
\]

Acidity in soil has critical and wide-ranging influences on soil habitat and biogeochemical processes (Alexander, 1980b, Alexander, 1980a, Coleman, 1983) as well as nutrient availability (Arnon et al., 1942). Plant nutrients such as calcium, magnesium and phosphorus, along with trace elements such as molybdenum (Cregan, 1998) are less available below pH 5. Some plant root phytotoxins such as aluminium, manganese, iron and other heavier metals are also progressively released as the soil becomes more acid (Brady and Weil, 2001).

Many soil biogeochemical transformations are sensitive to soil acidity, including parts of the nitrogen cycle such as: nitrification: the transformation of ammonium to nitrate (Alexander, 1965, McFee, 1983) and denitrification: the transformation of nitrate to gaseous nitrogen species (Simek et al., 2002). Although mediated by soil factors such as moisture and
temperature as well as by the reported ability of microorganisms to adapt to changing soil
conditions (Bramley and White, 1989), nitrification has been observed to occur optimally in the
pH 5.8 to 8.5 range (Lawrence, 1983, Bramley and White, 1989, Tyson et al., 2007) (Table 2-9). Nitrification rates at acidity levels below pH 5 are generally acknowledged to be minimal
(Gerardi, 2002). However, under specific conditions of bacterial aggregation into bio-films, De
Boer presented some evidence for nitrification occurring down to pH 4 (De Boer et al., 1991).

Table 2-8: Characteristics of autotrophic nitrifying bacteria (Lawrence, 1983)

<table>
<thead>
<tr>
<th>Genus</th>
<th>Substrate</th>
<th>Temperature range for growth (°C)</th>
<th>pH range for growth</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrosomonas</td>
<td>NH₄</td>
<td>5 – 30</td>
<td>5.8 – 8.5</td>
<td>Soils, freshwater, seawater</td>
</tr>
<tr>
<td>Nitrospira</td>
<td>NH₄</td>
<td>15 – 30</td>
<td>6.5 – 8.5</td>
<td>Soils</td>
</tr>
<tr>
<td>Nitrosococcus</td>
<td>NH₄</td>
<td>2 – 30</td>
<td>6.8 – 8.0</td>
<td>Soils, seawater</td>
</tr>
<tr>
<td>Nitrosolobus</td>
<td>NH₄</td>
<td>15 – 30</td>
<td>6.0 – 8.2</td>
<td>Soils</td>
</tr>
<tr>
<td>Nitrobacter</td>
<td>NO₂</td>
<td>5 – 40</td>
<td>6.5 &lt; 7.7 – 8.5</td>
<td>Soils, freshwater, seawater</td>
</tr>
</tbody>
</table>

Denitrifying microorganisms tend to have a broader pH range of activity from pH 3 to 8 (Yamulki et al., 1997) but functional aspects of the full reduction sequence from nitrate (NO₃⁻) to nitrite (NO₂⁻) to nitric oxide (NO) to nitrous oxide (N₂O) to gaseous nitrogen (N₂) may be interrupted by soil pH conditions. Hynes and Knowles reported sharp decreases in denitrification below pH 6.5, with maximum NO₂⁻ and subsequently N₂O being produced in the range: pH 4.7 to 5.8 (Hynes and Knowles, 1984). Above pH 5.8, N₂O production virtually ceased (Weier and Gilliam, 1986) with N₂ mainly produced between pH 7 and 8 (Yamulki et al., 1997).

Waring and Gilliam also observed low nitrate loss below pH 4 relative to loss in the range pH 4 to 6 (Waring and Gilliam, 1983). However in pH <4 soil experiments with ¹⁵NO₃⁻ – N, results showed that 16 to 20 % loss of NO₃⁻ - N was due to reduction to ammonium ions and organic nitrogen, but only 1 to 2 % for soils with pH > 4.0. Denitrification was substantial in the soils with pH <4.0 and of a similar level to that for moderately acidic soils (pH 4.4 to 5.4). In the neutral soils (pH 6.9 to 7.3) denitrification was much higher but much of the increase appeared due to the higher level of soluble C (Waring and Gilliam, 1983).

2.7.1.c Soil Oxygen

The efficacy of an agricultural soil depends as much on the quantity and properties of its pore space as it does on its mineral content. Soil pores not filled with water contain gases and these gases constitute the soil atmosphere. Whilst microorganisms may be found in either anoxic or oxic environments the majority of known organisms are thought to respire oxygen. Some facultative species are able to exist in both environments (Stanier et al., 1977). New analytical techniques in microbiology are revealing an increasing number of anaerobic
Eukaryotes, Archaea and Bacteria, with Schmitz (Schmitz et al., 2006) reporting up to 200 genera of obligate anaerobes. Durr also reported many microorganisms within the Bacteria and Archaea domains within often anoxic pyritic subsoils (Durr, 2009). These anaerobic species also exist in saturated anoxic systems using a variety of alternative electron acceptors for respiration (Thauer and Shima, 2006). Most natural environments have transitions between oxic and anoxic zones and microbes functionally adapt to different habitats of specific oxygen tension according to their physiology and functional needs (Fenchel and Finlay, 2008).

Oxygen occurs in soils in two states or phases: (i) as part of the soil atmosphere, and (ii) dissolved in the soil solution. It is the combination of these two phases that determines the oxic status of the soil. The solubility of oxygen in water is low. Under equilibrium conditions at 20 °C and 1.013 bar, pure water will contain around 9 mg L⁻¹ of dissolved oxygen (DO) (Schmitz et al., 2006).

There appears to be no standard quantitative classification system for oxygen saturation in soils. Munsun et al. described the level at which aquatic organisms experience stress as less than 5 mg DO L⁻¹ and reporting they cannot survive below oxygen levels of 2 – 3 mg DO L⁻¹ (Munsun, 2009). Under the altitude and temperature conditions in the study laboratory, 5 mg DO L⁻¹ equates to 48 % DO saturation and the lower limit of 3 mg DO L⁻¹ or 28 % DO saturation. Quastel (Quastel, 1965) nominates 3 μM oxygen (0.05 mg O₂ L⁻¹) as the point at which soils generally become anaerobic. By comparison, Jorgenson accepted a maximum value of less than 1 mg L⁻¹ (9 % oxygen saturation) as being anoxic (Jorgenson et al., 2009). A three part oxygen saturation classification adopted for this study is shown in Table 2-10:

<table>
<thead>
<tr>
<th>Oxygen Saturation Class</th>
<th>DO (ppm, or mg/L)</th>
<th>Oxygen saturation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxic</td>
<td>&gt; 3 ppm</td>
<td>&gt; 28 % DO</td>
</tr>
<tr>
<td>Sub-oxic</td>
<td>0.1 ppm to 3 ppm</td>
<td>1 % to 28 %</td>
</tr>
<tr>
<td>Anoxic</td>
<td>&lt; 0.1 ppm</td>
<td>&lt; 1 % DO</td>
</tr>
</tbody>
</table>

Even in normally highly aerobic zones in the soil profile (such as topsoil) it is possible for zones of anoxia to occur at the centre of soil aggregates, particularly those that are moist (Sextone et al., 1985). Microbial processes at the centre of soil aggregates, even as small as 4 mm in diameter, may quickly deplete oxygen in these regions allowing a switch to anaerobic processes (Zausig et al., 1993). This can result in aerobic and anaerobic reactions occurring simultaneously in the same soil zone. The concept of geochemical gradients (Fenchel and Finlay, 2008) also holds for soil water where a gradual profile transition occurs between a saturated aquatic microhabitat to a fully unsaturated aerated system in which water, if still present, is tightly adsorbed to mineral surfaces (Young et al., 2008).
2.7.1.d Biological soil moisture dynamics

From fossil evidence it is known that microorganisms evolved on earth when the volatile atmosphere was still anoxic and made up largely of methane, carbon dioxide, nitrogen and ammonia (Canfield et al., 2005c). There is thus ample evolutionary precedence for the existence today of anaerobes using inorganic chemical sources of energy and able to thrive in some extreme oxygen-free or low-oxygen environments. It was not until the emergence of *Cyanobacteria*, microorganisms capable of using sunlight as an energy source and producing oxygen as a by-product, that evolutionary development of aerobic *prokaryotes* and multicellular *eukaryotes* was supported (Madigan and Martinko, 2006). This view is somewhat mediated by those of Towe (Towe, 1990) suggesting that aerobic bacteria could have been present during the latter part of the anoxic phase of Earth’s formation to maintain a homeostatic anaerobic environment for the dominant *chemoautotrophs* of the time.

Soil water changes are intimately associated with soil pore geometry, including their size and connectedness. Microorganisms, being essentially aquatic, live in thin films within these soil pores (Young et al., 2008). Although pore size is often reported as being crucial for moisture retention, pore shape has also been identified as a key factor (Or et al., 2007). Pore size and connections directly affect the rate of diffusion processes bringing nutrients and substrates to microbial habitats (Young et al., 2008).

Low water availability can inhibit microbial activity by lowering intracellular water potential and thus reducing hydration and activity of enzymes. In solid matrices, low water content may also reduce microbial activity by restricting substrate supply.

As pores drain and water films coating surfaces become thinner, diffusion path lengths become more convoluted and the rate of substrate diffusion to microbial cells declines (Stark and Firestone, 1995). Conversely, progressive soil pore filling increasingly limits oxygen diffusion (Or et al., 2007). Franzluebbers reported that 50% water-filled pore space (WFPS) is the optimum for maximum microbial activity (Franzluebbers, 1999).

(i) Salinity

High concentrations of dissolved salts (salinity) in soils may adversely affect plant growth (Brady and Weil, 2001) as well as microorganism activity (Gour et al., 1990, Omar et al., 1994). The term salinity refers to the total dissolved concentration of major inorganic ions (i.e. Na, Ca, Mg, K, HCO₃, SO₄, and Cl) in ground and surface waters. Whilst individual cation and anion concentrations may be reported either on a chemical equivalent basis in mmol/L, or on a mass basis in mg/L, for analytical convenience an *electrical conductivity* (EC) index of salinity is used. This property is measured using a calibrated EC meter. Readings are normally expressed in units of deciSiemen per metre (dS m⁻¹) at a standard temperature of 25 °C.
Although dependent on specific ionic composition, an approximate relation between EC and total salt concentration is 1 dS/m = 10 mmol L\(^{-1}\) = 700 mg L\(^{-1}\) (Rhoades et al., 1992). Freestanding and underground saline waters may be categorised by the level of dissolved salts. A common classification is described by (Rhoades et al., 1992) (Table 2-11). Not only is the total dissolved concentration of ions important, but also the balance of specific ions relative to the total. In addition to total salt concentration, sodium and pH can adversely affect soil properties for irrigation and cropping.

**Table 2-10: Classification of saline waters (adapted from Rhoades et al., 1992)**

<table>
<thead>
<tr>
<th>Water Class</th>
<th>Electrical Conductivity (dS/m)</th>
<th>Salt concentration (mg L(^{-1}))</th>
<th>Type of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-saline</td>
<td>&lt; 0.7</td>
<td>&lt; 500</td>
<td>Drinking and irrigation water</td>
</tr>
<tr>
<td>Slightly saline</td>
<td>0.7 – 2</td>
<td>500 – 1 500</td>
<td>Irrigation water</td>
</tr>
<tr>
<td>Moderately saline</td>
<td>2 – 10</td>
<td>1 500 – 7 000</td>
<td>Primary drainage water and groundwater</td>
</tr>
<tr>
<td>Highly saline</td>
<td>10 – 25</td>
<td>7 000 – 15 000</td>
<td>Secondary drainage water and groundwater</td>
</tr>
<tr>
<td>Very highly saline</td>
<td>25 – 45</td>
<td>15 000 – 35 000</td>
<td>Very saline groundwater</td>
</tr>
<tr>
<td>Brine</td>
<td>&gt; 45</td>
<td>&gt; 45 000</td>
<td>Seawater</td>
</tr>
</tbody>
</table>

At high sodium concentration relative to divalent cations in the soil solution, clay particles in soils tend to swell and disperse. Aggregates may fall apart or slake, particularly under conditions of low total salt concentration and high pH. Whether from clay dispersion or from slaking or swelling, the permeability of the soil may be markedly reduced and the surface more crusted and compacted (FAO, 1992).

The ability of the soil to transmit water, therefore, can be significantly reduced by excessive sodicity. As high total salt concentration tends to increase a soil’s stability with respect to aggregation and permeability, a distinction is made here between saline soils and sodic soils. With sodicity, it is the proportion of adsorbed exchangeable sodium relative to the cation exchange capacity (often expressed as the exchangeable sodium percentage: ESP) that is important, rather than the absolute amount of exchangeable sodium. Also important here is the total salt concentration of the infiltrating and percolating water and the soil pH (FAO, 1992).

### 2.7.1.e Microorganism and soil interactions

Soils may best be described from a microorganism perspective as a juxtaposed multitude of micro-environments and habitats characterised by a variety of physical and chemical conditions. The spatial pattern and size of solid particles results in an often discontinuous and complex arrangement of variable-sized pore spaces intermittently filled with soil solution and/or soil gases, including air. It is in this environment that soil microorganisms live (Chenu and Stotzky, 2002).

This ecosystem is dominated by solid soil particles, some of which have large surface areas, shown in Table 2-12. Specific surface area is defined as the total surface area per unit mass of a
given substance (Uvarov et al., 1982). The colloidal fraction of soil is usually defined as particles in the 0.002 to 2 μm diameter range. This fraction exhibits permanent charge (in the case of most clays) or variable charge (in the case of oxyhydroxides and organic matter), that contributes largely to the property of cation exchange capacity (CEC). This CEC reflects the capability to attract and hold chemical compounds and plant nutrients (see Table 2-12).

Table 2-11: Specific Surface Area and Cation Exchange Capacity of common soil constituents (after Chenu and Stotzky, 2002)

<table>
<thead>
<tr>
<th>Soil Constituent</th>
<th>Specific Surface Area (m² g⁻¹)</th>
<th>CEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>External</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>11 - 26</td>
<td>11 - 26</td>
</tr>
<tr>
<td>Illite</td>
<td>24 - 93</td>
<td>101</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>800</td>
<td>14 - 33</td>
</tr>
<tr>
<td>Goethite</td>
<td>17 - 81</td>
<td>17 - 81</td>
</tr>
<tr>
<td>Allophane</td>
<td>638 - 897</td>
<td>292 - 582</td>
</tr>
<tr>
<td>Ferrhydrite</td>
<td>225 - 340</td>
<td></td>
</tr>
<tr>
<td>Humic Acid</td>
<td>121</td>
<td>4.9</td>
</tr>
<tr>
<td>Particulate Organic Matter</td>
<td>(&gt; 50 μm)</td>
<td>-</td>
</tr>
<tr>
<td>Coarse dense OM fraction</td>
<td>(&gt; 50 μm)</td>
<td>-</td>
</tr>
<tr>
<td>Medium OM fraction</td>
<td>(0.2 – 2 μm)</td>
<td>-</td>
</tr>
<tr>
<td>Colloid OM fraction</td>
<td>(&lt; 0.2 μm)</td>
<td>-</td>
</tr>
</tbody>
</table>

Microbial communities respond uniquely to soil variables with differing spatial patterns and the distribution of those communities is likely to be highly variable and complex. Pore size and architecture, mineral variation, substrate availability and chemical variability all create microsite patchiness that determines population densities and species mix (Madigan and Martinko, 2006, Young et al., 2008). The unsaturated soil vadose zone comprises an extremely heterogeneous dynamic aquatic and chemical environment that supports this variability. Experimental and theoretical evidence indicates that, rather than being free swimming, surface attachment is the primary mode of survival for bacteria. Being embedded in biosynthesised extracellular polymeric substances helps them cope with variable and unpredictable hydration conditions near the earth surface (Or et al., 2007). This supports survival in hostile or otherwise unsuitable habitats through formation of biofilm communities (O'Toole et al., 2000). Some researchers go so far as to suggest that these communities, with their intercellular signalling and quorum sensing abilities, are an analogue of multicellular higher organisms (Webb et al., 2003).

2.7.2 Microbial Function

In a broader functional sense microorganisms have no purpose other than to survive and replicate. However, in the biosphere microorganisms have variously evolved symbiotic, independent, inter-dependent and complementary existences involving consumption and
production of substances that are now a crucial part of many global biogeochemical cycles. Together these processes are referred to as metabolism and they may be categorised as either catabolic or anabolic reactions. **Catabolism**, or **respiration**, involves the energy releasing breakdown of molecules into smaller units, whereas **anabolism**, or **assimilation**, is a nutrient-gaining energy consuming process whereby molecules are synthesised from smaller units (Paustian, 2000b, Madigan and Martinko, 2006). Microorganisms are also capable of combining these two functions for greater metabolic efficiency (Aziza and Amrane, 2007).

From this metabolic perspective it is obvious that a critical function of microorganism life is respiration involving the acquisition of energy. This supports the biological ‘work’ of cellular growth and repair, replication, acquisition of nutrients and the obtaining of more energy. It is the acquisition of energy that drives the above-mentioned global cycles including those for carbon, sulfur, nitrogen, iron and phosphorus (Paustian, 2000b).

Paustian (2000b) classified microbial respiration by the type of catabolic reactions involved. These are:

**Photosynthesis**: Using ATP energy units stored directly by chlorophyll in primary producer organisms, electrons are stripped from water and transferred to an electron transport chain creating organic compounds (mainly sugars) from carbon dioxide.

The principal reactions are the *Calvin Cycle* used by plants, algae and cyanobacteria and the *Reverse Krebs Cycle* adopted by many phototrophic bacteria.

**Aerobic respiration**: Organic compounds donate electrons with oxygen being the terminal electron acceptor. The organic substrates are often completely oxidised to water and carbon dioxide. Large amounts of energy are typically harvested by microorganism in these oxic processes.

**Anaerobic respiration**: Organic and inorganic substances supply electrons into the cellular electron transport chain with specific inorganic molecules (e.g. nitrate, iron (II), sulfate and carbonate) being the final *electron acceptors*. Anoxic respiration typically extracts more energy than fermentation processes, but is less efficient than aerobic respiration.

**Fermentation**: An energy yielding transformation whereby organic molecules serve as both electron donors and electron accepters. The molecule being metabolized does not have all its potential energy extracted from it, i.e. it is not completely oxidized. Energy yields are low as the full potential of the substrate is not used. Fermentation begins when alternative electron acceptors are depleted or become unavailable in anoxic environments (Paustian, 2000c).
2.7.2.a Assimilation of nutrients

Another primary function within microorganisms is anabolism. This is the acquisition of nutrients that serve as the building blocks of synthesis, the creation of new organic substances required by the organism for growth, maintenance and replication. These substances principally consist of: carbon, hydrogen, oxygen and nitrogen. Other macronutrients include phosphorus, sulfur, potassium, magnesium, calcium and sodium, obtainable from both organic and inorganic sources. In addition, trace elements and growth factors (e.g. vitamins, amino acids) are also required for effective microorganism functioning and growth. Macronutrients are required in relatively large quantities for synthesis of the cellular building blocks of proteins and carbohydrates, whereas micronutrients are often needed in much smaller amounts. Trace elements essential for healthy organism functioning include: chromium, copper, iron, manganese, molybdenum, nickel, selenium, tungsten, vanadium and zinc (Shaw, 1960, Madigan and Martinko, 2006).

2.7.2.b Respiration or dissimilation

Respiration in a restricted sense refers to the oxidation of organic compounds in which oxygen acts as the terminal electron acceptor (Delwiche, 1967). In a more general sense the term describes all the energy-gaining reactions biocatalysed by organisms. These include both fermentation and cellular oxidation-reduction processes.

Although there appears to be a restricted use of the term dissimilation to specifically refer to the dissipation of reducing power in cellular redox balancing (Moreno-Vivian et al., 1999), here the term dissimilation is used in its broader sense as a synonym for respiration.

Of the two different forms of respiration, fermentation and aerobic or anaerobic cellular respiration, both use a similar first stage: glycolysis. Glycolysis involves the breakdown of glucose into pyruvic acid. The difference between the two forms is that fermentation stops at this step, whereas cellular respiration includes another three stages. Although oxygen may be present during glycolysis, it is not used in fermentation reactions. Cellular respiration may involve either aerobic oxidation using molecular oxygen as an electron donor, or anaerobic oxidation with inorganic alternative electron acceptors such as nitrate, manganese (IV), ferric iron, sulfate and carbon dioxide. Under anaerobic conditions, soil organic matter may alternatively act as an electron donor during reduction of the above mentioned AEsAs. These are used sequentially in the above-listed order due to the diminishing energy return from nitrate through to carbon dioxide (Ponnamparuma, 1972, Patrick and Reddy, 1978, Lovley and Phillips, 1986, Ehrlich, 1987, Postma et al., 1991, Madigan and Martinko, 2006).

Given the significant difference in energy returns from fermentation (i.e. 2 ATP equivalents) relative to cellular respiration (approximately 34 ATP equivalents), cellular respiration will preferentially occur when suitable electron donors and acceptors are available. If electron
acceptors are not available, then cellular respiration ceases and fermentation pathways take over although it may be more correct to say that cellular respiration does not proceed beyond the first glycolysis stage (Madigan and Martinko, 2006).

Many transformations at different scales may take place within the soil profile. These include: physical changes, chemical transformations, and biocatalysed reactions. Whilst both abiotic and biotic redox transformations occur in acid sulfate soils, the known microorganism association with pyrite oxidation (Nordstrom, 1982b) and with nitrate reduction (Straub et al., 1996, Haaijer et al., 2006) determines that biocatalysis be reviewed in the following sections.

(i) Cellular Redox Processes

As electrons are transferred from electron donors to electron acceptors and do not freely exist in soil solutions, reduction reactions must be coupled to oxidation reactions in order to complete redox transformations in soil (Helmke, 2000). Many important microbial respiratory reactions in soils are coupled to the oxidation of organic carbon using electron acceptors such as oxygen, nitrate and sulfate (Canfield et al., 2005e).

The free energy gain associated with the oxidation of electron donors, such as hydrogen, acetate and other organic compounds, varies by electron acceptor. However, the greatest free energy gain is consistently associated with molecular oxygen respiration and the lowest gain with carbon dioxide in methanogenesis. Therefore on a strictly energetic basis, oxic respiration is the most favourable process of organic carbon mineralisation, with anoxic respiration the next most favourable, while fermentation (e.g. methanogenesis) yields the lowest energy return (Canfield et al., 2005e).

(ii) Electron donors

Electron donors may be organic or inorganic substances. Soil organic matter comprises a complex mix of organically derived substances at various stages of decomposition and transformation. Microbial decomposition (oxidation) of SOM creates a series of products which may subsequently be used by many microorganisms to gain energy and nutrition. These SOM products include acetate, hydrogen, propionate, butyrate, ethanol, methanol and trimethylamine, glucose, glycerol, tryptone, lactate, pyruvate, long-chain fatty acids, sugars, amino acids, organic acids, alcohols, aromatic compounds, humic compounds and dissolved organic carbon (DOC) (Lovley and Phillips, 1986, Lovley and Phillips, 1988, Lovley, 1993b, Achtnich et al., 1995, Lovley et al., 1996, Devlin et al., 2000, Coates et al., 2002).

Inorganic electron donors in Fe(III) and Mn(IV) reduction include elemental sulfur, thiosulfate, aqueous ferrous iron and the sulfide in pyrite (Lovley, 1993b). Ferrous iron may donate electrons in nitrate reduction to produce ferric iron (Straub et al., 1996, Straub and Buchholz-Cleven, 1998). Although there have been some conflicting reports, pyritic sulfide oxidation has long been associated with nitrate reduction in aquifers and soils (Kolle et al.,
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1983, Strebel et al., 1985, Postma et al., 1991, Straub et al., 1996, Ottley et al., 1997, Pauwels et al., 1998, Massmann et al., 2003, Rivett et al., 2008, Schwientek et al., 2008, Jorgenson et al., 2009, Zhang et al., 2009). Part of the contention is about whether metal monosulfides and/or disulfides will react with nitrate, and specifically on whether pyrite is oxidised by nitrate. Support for the view that only elemental sulfur, thiosulfate or iron monosulfide (FeS) oxidation that couple with nitrate reduction are presented by (Devlin et al., 2000, Schippers and Jorgensen, 2001, Schippers and Jorgensen, 2002a, Haaijer et al., 2007).

(iii) Electron acceptors

Achtnich et al. (1995) cited others (Ponnampерuma, 1972, Patrick and Reddy, 1978) describing the degradation of soil organic matter as one of the most common bio-catalysed redox reactions in soil. SOM has been associated with the coupled sequential reduction of oxygen \((O_2)\), nitrate ions \((NO_3^-)\), manganese(IV) ions \((Mn^{4+})\), ferric ions \((Fe^{3+})\), sulfate ions \((SO_4^{2-})\) and carbon dioxide \((CO_2)\). Thermodynamic theory predicts that electron acceptors with the highest redox potential will be reduced first (Sorensen, 1982, Achtnich et al., 1995).

Some microorganisms are able to use humic substances as electron acceptors in the terrestrial anaerobic oxidation of organic compounds and hydrogen. Humic substances can act as both electron donor and acceptor in the reduction of metals species in the environment. Microorganisms may first use humic substances as an electron acceptor, and then the humic substance (abiotically) reduces the metal by becoming an electron donor. Microbial humic reduction also enhances the ability of microorganisms to reduce other less accessible electron acceptors such as insoluble Fe(III) oxides (Lovley et al., 1996). The typically large specific surface area of Fe-bearing solids in natural systems and the ability of these surfaces to interact chemically with reductants and oxidants (i.e. through surface complexation or ligand exchange) facilitate electron transfer as well as dissolution and precipitation of iron compounds. Complex formation reactions of Fe(III) and Fe(II) with organic and inorganic ligands to form soluble and solid complexes makes it possible for electron cycling of Fe(III) - Fe(II) transformations to occur over the entire Eh range within the stability of water (i.e.- 500 mV to + 1 100 mV) (Stumm and Sulzberger, 1992).

(iv) Thermodynamics and kinetics

In any complex and heterogeneous medium such as soil, many chemical transformations can potentially take place. One important contribution of thermodynamic theory is to assist evaluation of which processes are energetically favourable and thus most likely to occur. Thermodynamics assumes that the system being analysed is in equilibrium but most soil systems almost never meet this criterion. Therefore, prediction in a natural system first requires consideration of the thermodynamics of processes to determine which reactions are possible,
followed by evaluation of the kinetics of the reaction to evaluate which may be important within the studied context (Suarez, 1998).

Classical thermodynamics refers to the state of a system in energetic terms and processes that support the shift from one state to another (Walz, 1979, Canfield et al., 2005e). One way of evaluating thermodynamic characteristics of electron exchange in a specific redox couple system is to express it in terms of electric potential electromagnetic force \( E^0 \) under standard conditions of 1 atmosphere and 25 °C, or \( E \) under non-standard conditions (Equation 2-9) where:

\[
E^0 = E^0_{\text{Reduction}} - E^0_{\text{Oxidation}}
\]

The Nernst Equation (Equation 2-10) (Cheng et al., 2007) allows calculation of the non-standard electrical potential from standard electrical potentials, but also using reactant-product concentrations and reaction stoichiometric coefficients within the reaction quotient \( Q \) term (Equation 2-11).

\[
E = E^0 - \frac{(RT \, nF)}{nF} \ln Q,
\]

Equation 2-11: Nernst equation reaction quotient

\[
Q = C^cD^d / A^aB^b
\]

Where:

- uppercase letters are concentrations, and
- lowercase letters are the stoichiometric coefficients for the reaction:

\[
aA + bB \rightarrow cC + dD
\]

This Nernst equation reaction quotient \( Q \) highlights the importance of reactant concentration in determining the final redox-transformation electrical potential and therefore also the likelihood of a particular redox coupling occurring (Canfield et al., 2005e). The electrode potential \( E \) is related to another energy term, Gibbs free energy \( G \) in Equation 2-12 (Canfield et al., 2005e):

\[
\Delta G = -nF \, \Delta E
\]

Where:

- \( \Delta G \) = change in Gibbs free energy
- \( n \) = number of electrons transferred
- \( F \) = Faradays constant (946,485 J/V mol)
- \( \Delta E \) = emf change under non-standard conditions (volts)

Gibbs Free Energy \( G \) is the energy associated with a chemical reaction that can be used to do work. The free energy of a system is the sum of its enthalpy \( H \) plus the product of the temperature in Kelvin and the entropy \( S \) of the system. In microbiological respiratory terms, the Gibbs free energy value is the net energy microbes gain by bio-catalytically transforming
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various substrates within the soil (Canfield et al., 2005e). In anthropomorphic terms, these are
dynamic and competitive processes where substrate availability and concentration is constantly
changing within an environment where microbial type, numbers and enzyme availability
determines which has access to those reactants possessing the most favourable properties.

Comparative reporting of Gibbs free energy values for various substances allows assessment
of energy gains under the different available reaction pathways (Table 2-13).

<table>
<thead>
<tr>
<th>Overall reactions</th>
<th>Process</th>
<th>Δ G (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g. CH₂O + O₂ ⇌ CO₂ + H₂O</td>
<td>Oxidation</td>
<td>-470</td>
</tr>
<tr>
<td></td>
<td>Denitrification</td>
<td>-448</td>
</tr>
<tr>
<td></td>
<td>Mn(IV) reduction</td>
<td>-349</td>
</tr>
<tr>
<td></td>
<td>Fe(III) reduction</td>
<td>-114</td>
</tr>
<tr>
<td></td>
<td>Sulfate reduction</td>
<td>-77</td>
</tr>
<tr>
<td></td>
<td>Methanogenesis</td>
<td>-48</td>
</tr>
</tbody>
</table>

Table 2-13 shows that the respiratory energy gain during oxidative catalysis of organic
matter (i.e. using oxygen as the electron acceptor) is higher than for the same compound
oxidised by other alternative electron acceptor. More negative Gibbs free energy values denote
greater dissimilatory energy gain for microorganisms.

This transformation hierarchy is also reflected in the redox potential (measured in mV) of
various alternative electron acceptor half reactions (Madigan and Martinko, 2006) (Table 2-14).
Of particular note in this table are the standard redox potentials for nitrate reduction (+400 mV)
and ferric iron reduction (+750 mV). This table also highlights the potential for redox reaction
sequencing of available electron acceptors from oxygen through to carbonate and hydrogen
respiration as each is depleted. A system tending toward dominance of, say, iron respiration
will display redox potentials approaching + 750 mV. Conversely, a natural system driven into
anoxia should theoretically sequentially pass through a series of stages where the various
electron acceptors are depleted as the redox potential declines into negative redox potentials.

Bailey (1971) experimentally found that saturated silty clay loams declined from Eh +400
mV to -300 mV on incubation, but rapidly increased to Eh +200 mV on addition of calcium
nitrate solution. On completion of nitrate reduction the incubation solution again declined to –
300 mV where methane was produced (Bailey and Beauchamp, 1971).

Natural environments are mixed redox systems so a platinum electrode measurement will
likely give a net redox potential reading resulting from many different reactions, some or most
of which may not be at equilibrium. Therefore platinum electrode readings of mixed systems
may only be informative when interpreted generally rather than specifically.
Table 2-13: AEA oxidation state and redox potential (after Ohlsson, 1979, Madigan and Martinko, 2006, Engler and Patrick, 1973)

<table>
<thead>
<tr>
<th>Oxidation State</th>
<th>$E'_v$ (mV)</th>
<th>Transformation</th>
<th>Respiration Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxic</td>
<td>+820</td>
<td>$O_2 \Leftrightarrow H_2O$</td>
<td><strong>Aerobic respiration</strong>: obligate and facultative aerobes</td>
</tr>
<tr>
<td></td>
<td>+750</td>
<td>$Fe^{3+} \Leftrightarrow Fe^{2+}$</td>
<td><strong>Iron(II) respiration</strong>: facultative anaerobes and obligate anaerobes</td>
</tr>
<tr>
<td></td>
<td>+400</td>
<td>$NO_3^- \Leftrightarrow NO_2^-, NO_2, N_2$</td>
<td><strong>Nitrate respiration</strong> (nitrate reduction / denitrification): facultative anaerobes</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Fumarate $\Leftrightarrow$ Succinate</td>
<td><strong>Fumarate respiration</strong>: facultative anaerobes, fermentative bacteria</td>
</tr>
<tr>
<td></td>
<td>-100</td>
<td>$S^{2-} \Leftrightarrow S^0$</td>
<td><strong>Sulfide oxidation</strong></td>
</tr>
<tr>
<td></td>
<td>-180</td>
<td>$Fe(OH)_3 \Leftrightarrow Fe^{2+}$</td>
<td><strong>Iron(III) respiration</strong> (Iron reduction)</td>
</tr>
<tr>
<td></td>
<td>-220</td>
<td>$SO_4^{2-} \Leftrightarrow HS^-$</td>
<td><strong>Sulfate respiration</strong> (sulfate reduction): obligate anaerobes</td>
</tr>
<tr>
<td></td>
<td>-250</td>
<td>$CO_2 \Leftrightarrow CH_4$</td>
<td><strong>Carbonate respiration</strong>: methanogenic <em>Archaea</em>, obligate anaerobes</td>
</tr>
<tr>
<td></td>
<td>-270</td>
<td>$S^0 \Leftrightarrow HS^-$</td>
<td><strong>Sulfur respiration</strong>: facultative anaerobes and obligate anaerobes</td>
</tr>
<tr>
<td></td>
<td>-300</td>
<td>$CO_2 \Leftrightarrow CH_3 - COO^-$</td>
<td><strong>Carbonate respiration</strong>: homoacetogenic bacteria, obligate anaerobes</td>
</tr>
<tr>
<td>Anoxic</td>
<td>-413</td>
<td>$2H^+ \Leftrightarrow H_2$</td>
<td><strong>Hydrogen respiration</strong></td>
</tr>
</tbody>
</table>

2.7.3 Biogeochemical Cycles

Although the concept of separate earth systems or spheres was first postulated early in the 20th century by the Russian geochemist, Vernadsky (Vernadsky, 1926), it was not until the 1970s with the publication of Lovelock’s work (Lovelock and Margulis, 1974) that broader assimilation, refinement and acceptance of these concepts occurred within science and academia. Inherent in these ideas is the concept of interacting physical, chemical and biological systems that mediate, create and cycle components in the global environment (Christopherson, 1997). Given their relevance to the current study, four of these cycles are described and reviewed here: (i) the carbon cycle, (ii) the sulfur cycle, (iii) the iron and manganese cycles and (iv) the nitrogen cycle. Whilst they are described separately here, these cycles are not discrete systems and are, in practice, highly interactive and interdependent.

2.7.3.a Carbon cycle

Given that carbon is one of the major building blocks of life along with hydrogen and oxygen, it is hardly surprising that the geochemical cycling of carbon plays such a significant role in global biosystems (Brady and Weil, 2001). Carbon is not plentiful in the earth’s crust (<0.1 %) but after oxygen it is the second most abundant element in the human body (17.5 %).
Combined with hydrogen and oxygen, it occurs in all animal and plant tissue as well as their derivative decomposition products such as petroleum and coal.

(i) *Biochemistry of Soil Organic Matter*

Organic carbon is a vital source of energy and nutrients for soil biota and the microorganisms decompose and recycle organic matter to obtain these. In these processes, organic substances are released back into the soil where they promote better soil aggregation and provide nutrients for soil flora and fauna, including plants. Specific organisms decompose different components and types of organic material and each species of microorganism has its own optimal environmental conditions (Gupta and Germida, 1988).

In agriculture, retention of crop residues increases the populations of microflora (Gupta and Roper, 1992) with this effect being compounded when combined with no-tillage practices (Gupta, 1994). The type of tillage favours different functional groups, with no-tillage supporting the microflora fungal group and conventional tillage promoting increase of bacteria, protozoa, bacterivorous nematodes and enchytraeid worms (Rovira, 1994). This suggests that tillage practices may result in more bio-reactive soils that potentially produce greater gaseous products from carbon and nitrogen compounds. Farming practices impact on organic matter as well as the activities of soil organisms, with intensive cultivation over time generally markedly decreasing the quantity and variety of soil biota (Gupta and Germida, 1988, Rovira, 1994). Cultivation aerares the soil and initially increases the bacterial oxidation of organic matter in the soil, but this drops off quickly as populations decline through lack of food supply (Pankhurst* et al.*, 1994). The main end product of organic matter oxidation is carbon dioxide.

Microorganisms must assimilate carbon for growth. A comprehensive study by Kramer and Gleisner found evidence of bacteria using variable carbon sources depending on substrate availability. They also observed an unusual preference by Gram-negative bacteria for plant-derived material in contrast to the SOM derived carbon favoured by Gram-positive microorganisms. Other minor unaccounted for consumption was attributed to carbon dioxide and methane (Kramer and Gleixner, 2006).

(ii) *Carbon dioxide*

Carbon in the form of carbon dioxide is an active product and component in the primary energy cycling processes in the global environment, that of *photosynthesis* and *metabolic respiration*. Respiration involves the coupled redox transformations of inorganic and organic substances to produce energy for organisms and carbon dioxide is one of the main end products of this. Both chemotrophic and phototrophic microorganisms also assimilate CO₂ as a source of carbon (Madigan and Martinko, 2006). Under anaerobic conditions where other electron acceptors are absent or depleted, carbon dioxide is reduced by fermentation during methane production (Lovley and Chapelle, 1995). The degradation of complex organic matter to
methane and CO₂ is widespread in reduced environments with limited access to alternative electron acceptors such as nitrate, manganese, oxidised iron (FeIII), or sulfate (Conrad, 2007). The low energy yield of methanogenic degradation relative to alternative oxidative processes is thought to be the reason why this reaction is last in the reported sequence of soil electron acceptors (Schink, 1997).

(iii) Soil organic matter chemical activity

Soil organic matter is highly complex chemically and its sequential oxidation sustains many bio-catalysed soil transformations. Its ability to be easily oxidised allows it to act as a general preferred electron donor in soils, and also to serve as an electron acceptor in some instances. Dissolved organic matter reportedly acts as an electron acceptor under anaerobic conditions (Heitmann et al., 2007).

In aerobic soils, SOM acts as a preferred electron donor due to its advantageous dissimilative energy return and its assimilative source of carbon. In saturated anoxic soils organic matter may be oxidised with the aid of many common soil substances, but generally in the order: NO₃⁻ → Mn⁴⁺ → Fe³⁺ → SO₄²⁻ → CO₂ due to the respective diminishing dissimilatory energy gains (Ponnampерума, 1972, Patrick and Reddy, 1978, Lovley and Phillips, 1986, Achtnich et al., 1995).

Many of the functions of microorganisms are facilitated by enzymes, both intracellular and extracellular. Intracellular enzymes are used for anabolic biosynthesis processes, whereas extracellular enzymes are mostly used in dissimilatory energy-gaining processes through the oxidative degradation of organic and inorganic substances in the immediate external environment of the microorganism (Young et al., 2008). Among many other functions, extracellular enzymes oxidise SOM and are produced specifically by organisms for this purpose. Production of these enzymes, however, is vulnerable to microorganism ‘cheaters’ who don’t produce them but use them and their by-products (Allison, 2005). Extracellular enzymes originate in soils from animal, plant and microbial sources and are present in particulate cell debris, dead cells, in viable but non-proliferating cells and in soil solution or bound to organic and inorganic soil constituents (Ladd, 1978). This indicates that some biocatalysed soil reactions may occur without the presence of viable microbial populations.

Dissimilatory organic matter oxidation degrades proteins, lipids, nucleic acids and polysaccharides to produce smaller molecules such as amino acids, fatty acids, nucleotides and monosaccharides. These products may be used in biosynthesis or broken down further in energy releasing reactions producing waste products such as organic acids (e.g. acetic, lactic), urea ammonia and carbon dioxide (Madigan and Martinko, 2006).

Soil organic matter has long been recognised for its complexing properties, particularly with metal ions, but also with organic micro-pollutants in soils. The water solubility of organic carbon is high and it is the low molecular weight organic acids that show strong metal
complexing capability, although it appears only a small mobile fraction is responsible for this (Dudal et al., 2005). Metal complexation with organic matter is reported to variously assist oxidation-reduction processes in soils by facilitating electron transfer (Stumm and Sulzberger, 1992, Luther et al., 1992, Marschner and Kalbitz, 2003). This complexation may also lead to unreactive iron formation in the presence of dissolved organic matter in acid bogs (Koenings and Hooper, 1976), suggesting that some soil iron may be able to be electrochemically protected through SOM complexation. Along with iron, sulfur is another biogeochemically active substance, particularly at oxic-anoxic interfaces where it can be chemically associated with iron.

2.7.3.b Sulfur cycle

Sulfur is the fourteenth most abundant element in the Earth’s crust. The original pool of sulfur resided largely within igneous rocks as pyrite (FeS₂). (Middleburg, 2000). Sulfur is also abundant in all organisms, appearing in organic compounds such as amino acids, enzyme cofactors, peptides, antibiotics and carbohydrates. Within organic compounds sulfur may have catalytic, structural or regulatory functions. Although assimilatory sulfate reduction processes are very common in prokaryotes, plants and fungi, dissimilatory pathways where sulfur species are used for energy creation are restricted to Bacteria and Archaea (Bruser et al., 2000).

Global sulfur cycling is complicated by anthropogenic inorganic sulfur species production and distribution through processes such as coal burning, motor vehicle use, fertiliser soil amendment and pesticide use. The surface horizons of all but a few arid-region soils contain the bulk of land-based sulfur, with a significant portion of this being bound up in soil organic matter. Inorganic sulfur species tend to be associated with ore deposits and horizons deeper within the crust (Brady and Weil, 2001) but sulfates and elemental sulfur may also occur higher in the solum as a result of biogeochemical transformations in this zone. Sulfur compounds often occur in oxidised acid sulfate soils and include elemental sulfur, gypsum and jarosite although many other sulfur forms may also be present (Fanning et al., 2002).

In local soil-plant-atmosphere interactions, sulfur species go through cycles of oxidation and reduction in soils and reduction in plants. Gains and losses occur in several ways. Sulfate ions are slowly released through weathering of sulfur-containing minerals. These sulfate ions are prone to either leaching via erosion or water movement and uptake by plants and microorganisms via assimilatory (Rhodes, 2009) or dissimilatory processes (Bruser et al., 2000). Decay of plant and animal residues may release sulfur dioxide (SO₂) (Troech and Thomson, 1993) but it appears more likely to be absorbed into, rather than emitted from, the soil (Brown, 1982).

Geochemical and biochemical processes taking place in soils, sediments and water are closely involved in the cycling of sulfur between the hydrosphere and the biosphere via the atmosphere. These processes determine the rate at which sulfur is immobilised into insoluble
forms such as pyrite and organic sulfur, or mobilised as soluble sulfate, volatile hydrogen sulfide (H₂S) and organic sulfides (Canfield et al., 2005d). From a biological perspective inorganic sulfur compounds serve nutritional needs in microbial assimilation, but also are involved in energy creation by serving as electron donors or acceptors in dissimilatory redox processes (Bruser et al., 2000).

Sulfur occurs in a variety of forms and valence states ranging from -2 (e.g. sulfide) through to +6 (e.g. sulfate) (Table 2-15). Sulfate is the most stable form, with weathering and leaching of rocks and sediments being the primary sources of stocks in the geosphere (Sievert et al., 2007).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical formula</th>
<th>Sulfur oxidation state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfide</td>
<td>HS⁻</td>
<td>-2</td>
</tr>
<tr>
<td>polysulfides</td>
<td>&quot;S(S)₅S&quot;</td>
<td>-1 (terminal S) / 0 (inner S)</td>
</tr>
<tr>
<td>thiosulfate</td>
<td>S₂O₅²⁻</td>
<td>-1 (sulfane) / +3 sulfone S</td>
</tr>
<tr>
<td>polythionates</td>
<td>&quot;O₂S(S)₅S₅O⁺&quot;</td>
<td>0 (inner S) / +5 (sulfone S)</td>
</tr>
<tr>
<td>elemental sulfur</td>
<td>S₅ rings</td>
<td>0</td>
</tr>
<tr>
<td>tetrathionate</td>
<td>S₄O₆²⁻</td>
<td>+2.5</td>
</tr>
<tr>
<td>bisulfate</td>
<td>HSO₃⁻</td>
<td>+4</td>
</tr>
<tr>
<td>sulfate</td>
<td>SO₄²⁻</td>
<td>+6</td>
</tr>
</tbody>
</table>

The biochemical oxidation and reduction of sulfur compounds constitute the biological sulfur cycle. In natural habitats that grade from oxic to anoxic, as well as those subject to alternating flooding-drying cycles, S-species may cycle locally between oxidised and reduced states. *Syntrophic* communities of sulfur oxidising and reducing bacteria may form and become mutually dependent on each other for nutritional and respiratory substrates (Bruser et al., 2000). An example of this is where hydrogen sulfide produced by some bacteria is re-oxidised by others within the same local ecosystem (Ingvorsen and Jorgensen, 1982).

Primary processes of the biochemical sulfur cycle are shown in Figure 2-3. Of note here is sulfate reduction to sulfide and *vice versa* through a serious of sequential bio-catalysed reactions. During sulfide oxidation processes in acidic environments such as acid sulfate soils, one of the intermediate products, sulfite (a weak base), may undergo acid hydrolysis in several steps. The reaction product is sulfurous acid and results in the evolution of sulfur dioxide, a greenhouse gas. Apart from oxygen, the three main inorganic chemical oxidants for sulfide are: nitrate, manganese(IV) and iron(III) (Luther et al., 1992, Schippers and Jorgensen, 2001, Canfield et al., 2005d).

(i) Metal sulfide oxidation
As seen in Figure 2-3, different sulfur compounds may be used as both electron donors (sulfate/sulfur oxidation) and electron acceptors (sulfur reduction) depending on their oxidation state (Sievert et al., 2007). Sulfur species oxidation occurs aerobically with oxygen as the oxidant, or anaerobically with nitrate, manganese(IV) or ferric iron as terminal electron acceptors. Anaerobic photosynthesis involving sulfur is also possible (Sievert et al., 2007).

![Figure 2-3: Biochemical sulfur cycle (after Canfield et al., 2005d)](image)

Sulfide oxidation reactions are significant oxidation pathways in the environment with reactions between dissolved sulfide (H₂S) and poorly crystalline iron (Fe) and manganese (Mn) known to be ubiquitous and fast (Canfield et al., 2005d). Non-biological oxidation of insoluble sulfidic materials such as pyrite is thought to occur only with oxygen as the electron donor, but some researchers have reported direct dissolution by bacteria (Silverman et al., 1963, Duncan et al., 1967). A diverse range of microorganisms carry out different reactions in the sulfur cycle with most previously thought to belong to the bacterial domain (Sievert et al., 2007) although the work of Durr showed that sulfur-metabolising Archaea are widespread in acid sulfate soils (Durr, 2009).

Although Figure 2-3 shows only hydrogen sulfide as the most reduced S-species, sulfide can exist in several different hydrogen bonded forms such as H₂S at pH < 6 and HS⁻ appearing in the pH range 6 to 10. The dissociated dianion S²⁻ only exists at pH > 10 (Tang et al., 2009). Transition metal (M) sulfide forms are covalently bonded and exist in the environment in monosulfide (MS) and disulfide (MS₂) forms. Most of the monosulfide compounds are acid-soluble (such as FeS) and are more reactive in moist environments than the acid-insoluble forms.
such as pyrite (FeS₂), molybdenum disulfide (MoS₂) and tungsten disulfide (WS₂) (Schippers and Sand, 1999). The focus here is on the disulfide forms and specifically that of naturally occurring pyrite in euhedral (particulate) or framboidal (multi-particle sphere) form in soils (Lowson, 1982).

There has been much discussion and contention in the literature since at least the early 1960s (Silverman et al., 1961, van Breemen, 1972, Duncan and Drummond, 1973, Schippers and Sand, 1999) concerning the type and number of oxidation pathways for iron disulfide (pyrite). These discussions have largely occurred in the context of aerobic reactions and the primary issues are twofold. The first concerns the question of whether direct chemical or direct biotic processes occur in pyrite oxidation. The second issue has to do with clarifying if only direct mechanisms are involved, or whether indirect chemical or bio-catalysed reactions also occur. This delineation here may not encompass the full range of actual transformations possible. In 1972, van Breemen postulated many different oxic reactions associated with pyrite dissolution including:

(i) oxidation of pyrite to ferrous ions and elemental sulfur
(ii) oxidation of ferrous ions to ferric ions
(iii) oxidation of pyrite by ferric ions to produce ferrous ions plus elemental sulfur (at pH <3.5)
(iv) oxidation of elemental sulfur (by oxygen) to sulfate ions plus acidity
(v) oxidation of elemental sulfur (by ferric iron) to produce sulfate and ferrous ions plus acidity
(vi) reaction of ferric ions with water at pH > 4 to produce ferric hydroxide

It was recognised by van Breemen (1972) that iron(II) oxidising microorganisms may indirectly increase ferric iron oxidation of pyrite by regenerating ferric from ferrous ions. These ferric ions are reported to become more soluble (i.e. reactive) only at pH <3.5 (van Breemen, 1972), but this is an oversimplification. The solubility of Fe³⁺ ions has a log-linear relationship with pH, at least in the range: pH 3.1 to 7.3 as determined by Byrne and Luo (Byrne and Luo, 2000). This indicates that some ferric ions may always be available in solution at pH above 3.5 to 7.3, but an ever decreasing amount of them as pH increases. This could be interpreted to mean that ferric iron would or could take little part in the oxidation of pyrite above pH 3.5. However, Byrne and Luo (2000) stated that equilibration between Fe³⁺ ions and its source iron precipitates is almost instantaneous. This suggests that even though ferric ions are at low concentrations, they could be replenished relatively quickly if lost through being involved in soil reactions.

The pyrite oxidation direct chemical pathway is generally more simply reported as involving three separate steps or processes. For example, Lowson (Lowson, 1982) proposed reactions within a purely chemical framework:
1. The [slow] oxidation of pyrite by molecular oxygen to produce sulfate and ferrous ions (Equation 2-13)
2. The [slow] oxidation of $\text{Fe}^{2+}$ to $\text{Fe}^{3+}$ by molecular oxygen (Equation 2-14)
3. The [fast] oxidation of pyrite by ferric ions (Equation 2-15)

*Equation 2-13: Molecular oxygen oxidation of sulfide in pyrite – first stage (slow)*

\[
\text{FeS}_2 + 2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{SO}_4^{2-} + \text{S}^0
\]

*Equation 2-14: Molecular oxygen oxidation of iron(II) to iron(III) – second stage (slow)*

\[
\text{Fe}^{2+} + 0.25 \text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + 0.5 \text{H}_2\text{O}
\]

*Equation 2-15: Iron(III) oxidation of pyrite – third stage (fast)*

\[
\text{FeS}_2 + \text{Fe}^{3+} \rightarrow \text{Fe}^{2+} + 2\text{S}^0
\]

Equation 2-13 to Equation 2-15 are simplified versions of the reactions as they do not show the activity of water and proton consumption/generation which, in the complete pyrite oxidation reaction, shows net proton creation (Jorgenson *et al.*, 2009). The full reactions for the third stage are described by Schippers and Sand (1999) as the *thiosulfate mechanism* for aerobic oxidation of acid-insoluble sulfides such as pyrite (Equation 2-16) and show 16 moles of acidity being generated for every mole of pyrite oxidized (Schippers and Sand, 1999).

*Equation 2-16: Iron(III) oxidation of pyrite (thiosulfate mechanism)*

\[
\text{FeS}_2 + 6\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{S}_2\text{O}_3^{2-} + 7\text{Fe}^{2+} + 6\text{H}^+
\]

\[
\text{S}_2\text{O}_3^{2-} + 8\text{Fe}^{2+} + 3\text{H}_2\text{O} \rightarrow 2\text{SO}_4^{2-} + 8\text{Fe}^{2+} + 10\text{H}^+
\]

Within the chemical model, others (Silverman *et al.*, 1961, Silverman *et al.*, 1963, van Bremen, 1972, Duncan and Drummond, 1973, Dent, 1986a, Schippers and Sand, 1999) also acknowledge an *indirect* role for aerobic microorganisms (e.g. *Acidithiobacillus* [formerly *Thiobacillus*] ferrooxidans) (Kelly and Wood, 2000) in regenerating Fe(III) from Fe(II) ions as per the Lowson (1982) stage 2 equation above (Equation 2-14). Equation 2-17 shows this iron regeneration half reaction.

*Equation 2-17: Bio-catalysed ferric iron regeneration from ferrous ions*

\[
\text{Fe}^{2+} \xrightarrow{\text{bacteria}} \text{Fe}^{3+} + \text{e}^-
\]

In addition, *Acidithiobacillus thiooxidans* can oxidise sulfur in aerobic low pH environments (pH 2 to 3.5) where carbon dioxide and other nutrients such as N and P are available (Brierley, 1978), generating further acidity (Equation 2-18).

*Equation 2-18: Oxidation of elemental sulfur by molecular oxygen*

\[
\text{S}^0 + 3/2\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2\text{H}^+
\]

The discussion in the literature centres on whether or not there are *direct mechanisms* for the bio-catalysed dissolution of pyrite. Duncan *et al.* (Duncan *et al.*, 1967), investigating the
role of *T. ferrooxidans* in chalcopyrite (CuFeS₂) and pyrite (FeS₂) oxidation, stated in their introduction:

*The mode of action of these organisms is not clear, although several possibilities exist. These possibilities include oxidation of the minerals by acidic ferric sulfate with the bacteria oxidizing the resultant ferrous iron to complete a cyclical process; direct action on the ferrous iron moiety of the sulfide mineral; direct action on the sulfide portion of the mineral; or a combined action involving two or more of these alternatives (Duncan et al., 1967):397.*

In summarising previous research relating to pyrite oxidation Duncan and Drummond cited Clark (1966) in finding that oxygen and ferric iron were potential oxidising agents. They also reported that the role of bacteria was to aid pyrite oxidation by regenerating ferric from ferrous ions (Silverman et al., 1961, Silverman et al., 1963, Clark, 1966, Duncan and Drummond, 1973). Conflicting experimental results began to emerge. Lorenz and Tarpley (Lorenz and Tarpley, 1963) found that ferric iron alone had no appreciable effect on pyrite oxidation whereas Silverman (Silverman, 1967) demonstrated that both chemical and biological oxidation was occurring. Duncan and Drummond (1973) experimentally concluded that dissolution of pyrite crystals was occurring solely in the presence of *Thiobacillus ferrooxidans*.

In 1978 Brierley stated that despite much evidence in support of the sole microbial mechanism being ferrous iron oxidation to ferric ions, direct microbial attack on pyrite could not yet be ruled out (Brierley, 1978). In a doctoral dissertation, Arkesteijn (Arkesteijn, 1980) provided evidence for the direct aerobic oxidation of the pyrite sulfide moiety by *T. ferrooxidans*. There is thus strong if not unequivocal support for the direct biocatalysis mechanism of pyrite dissolution, with indications that this could be through the sulfide moiety. However, the main focus here is anaerobic pyritic oxidation. Under anoxic conditions, particularly in soils, the mechanisms for pyrite oxidation appear even less clear.

With iron(II) and sulfide moieties in pyrite as the two potential electron donors, the challenge is to find thermodynamically suitable alternative electron acceptors for anaerobic pyrite oxidation. A second issue concerns clarifying whether these potential reactions are purely chemical or are bio-catalysed. Potential AEAs in decreasing standard energy yield order are: nitrate, nitrite, manganese(IV) oxide, iron(III) oxide, sulfate and carbon dioxide. Upon depletion of each preferred AEA, the next would successively be sought out by microbes for dissipilatory processes (Reddy and DeLaune, 2008). As nitrogen species and manganese and iron will be dealt with in later sections, the anaerobic electron acceptor sulfate is of interest here.

Biological sulfate reduction is a major part of the global sulfur cycle. Sulfate reduction is restricted to relatively few groups of prokaryotes, of which all but one are Eubacteria. The exceptions are the archaeal sulfate reducers in the order Archaeoglobales (Klenk et al., 1997).
From an available free energy perspective sulfate is generally acknowledged as being below nitrate and ferric iron (Achnich et al., 1995).

There is substantial theoretical support for the sequential use of preferred electron acceptors in saturated soil systems in the order: nitrate > manganese(IV) > iron(III) > sulfate >> carbon dioxide (Ponnampерuma, 1972, Peters and Conrad, 1996). However, there is also some experimental support for concurrent electron-acceptor reduction processes occurring whereby, say, iron(III) and sulfate reduction may occur together, but with a proportional preference (in this example) for the Fe(III) higher energy dissimilatory pathway (Sorensen, 1982).

Microorganisms associated with sulfate reduction belong to the delta-Proteobacteria orders (i.e. Desulfo bacteriales, Desulfovibrionales and Syntrophobacterales), but functionally this may also be carried out by members of the phylum Thermosulfobacteria, the phylum Nitrospirae, the family Petococcaceae (e.g. Thermodesulfovibrio and Desulfofotomaculum) (Dalsgaard and Bak, 1994, Tang et al., 2009). Additionally, sulfate reduction may be carried out by a genus of hyperthermophilic Archaea: Archaeoglobus (Klenk et al., 1997). Some sulfate reducing species such as Desulfovibrio desulfuricans are also capable of nitrate reduction, but this occurs only when sulfate is absent or at low concentrations (<4 μM) (Dalsgaard and Bak, 1994). Whilst hydrogen sulfide is often the end product of the anaerobic reduction of sulfate, black ferrous monosulfide and iron disulfide (pyrite) may be produced under favourable conditions when iron is available (Hurtgen et al., 1999). Therefore, although sulfate may act as an electron acceptor oxidant, indications are that it is more likely to produce iron sulfides than oxidise them.

2.7.3.c The iron and manganese cycles

Iron is the most abundant global element and is also the most frequently used transition metal in the biosphere. As it is a component of numerous cellular compounds and an essential part of many physiological functions, iron is an essential micronutrient for all eukaryote and most prokaryote organisms (Madigan and Martinko, 2006). Although iron is ubiquitous in all parts of the geosphere in particulate Fe(III) or Fe(II) iron-bearing-mineral form or as dissolved ions (Kappler and Straub, 2005), many iron minerals are not readily bio-available. Iron (oxy)hydroxides are an important exception to this (Raiswell, 2006). Iron oxides are common in soils, where some may derive from oxidation of metal sulfides. Rivett et al. (2008) report findings where the final products of coal derived pyrite oxidation were iron oxyhydroxides in the form of lepidocrocite (γ-FeOOH) and goethite (α-FeOOH) from an intermediate hydrated ferrous sulfate form (Rivett et al., 2008).

At a 50-fold lower crustal abundance than iron, manganese is the second most abundant redox-active metals. There are many similarities between iron and manganese in that the oxidation and reduction of these elements is largely promoted by microbial catalysis, but abiotic transformations are important and often compete with biological processes. Due to the general
very low solubility of oxidised species of manganese and iron, their global cycles are heavily influenced by physical processes (Canfield et al., 2005a).

Manganese (IV) compounds are insoluble and therefore not a diffusible oxidant in aqueous or saturated environments (Straub et al., 1996). Under circum-neutral pH oxic weathering, iron and manganese are re-precipitated as oxides and hydroxides that are then transported by water and wind (Canfield et al., 2005a). Buried in anoxic sediments, manganese and iron may be re-mobilised through microbial dissimilatory respiration as well as abiotic reduction, but intensive redox cycling of both elements occurs at oxic-anoxic interfaces (redoxcline) in many sediments (Canfield et al., 1993).

Dissimilatory iron cycling between the ferrous Fe\textsuperscript{2+} and ferric Fe\textsuperscript{3+} oxidation states can impact significantly on the biogeochemistry of sediments and saturated soils. A 1986 study (Lovley and Phillips, 1986) demonstrated that Fe(III) reduction has the potential to be a major pathway for organic matter decomposition in anaerobic sediments. As much as 90% of the electron flow in methanogenic tidal river sediments was diverted from methane production to Fe(III) reduction when amorphous Fe(III) oxyhydroxide was added. The results also indicate that in addition to outcompeting methanogenic food chains, there are indications that amorphous Fe(III) can also compete with sulfate for electron donors in anaerobic sediments and as such is an important pathway for electron flow in soil redox processes.

In non-sulfidogenic anaerobic soil systems, microbial assisted oxidation of organic matter by ferric iron is ubiquitous (Lovley, 1993a) with the end products being ferrous ions (Fe\textsuperscript{2+}) and CO\textsubscript{2}. In acidic environments, elemental sulfur (S\textsuperscript{0}) may provide electrons for ferric iron reduction to ferrous iron, with the sulfur being oxidised to sulfate (Lovley, 1993a). Similarly, T. ferrooxidans contains several enzymes capable of reducing Fe(III) to Fe(II) for energy under very acid conditions where the Fe(III) is soluble, thus enabling the use of reduced sulfur compounds (such as iron sulfide) as electron donors (Lovley, 1993a).

Common oxidants and reductants for manganese and iron in saturated environments are shown in Figure 2-4.

![Figure 2-4: Oxidants and reductants for Mn and Fe in saturated environments (after Canfield et al., 2005a)](image-url)
In this figure depicting reactions that may proceed in either direction, thin lines indicate abiotic reactions, thick lines indicate microbially catalysed reactions and the stippled line shows a reaction not yet experimentally documented. Iron(II) is thus capable of being bio-oxidised to iron(III) by O₂, NO₃⁻, NO₂⁻, chemically oxidised by MnO₄⁻ and phototrophically oxidised by CO₂.

The most environmentally important abiotic reductant of ferric iron is hydrogen sulfide which also reduces manganese(IV) oxide to Mn(II). The reduction potentials of the Fe²⁺/Fe³⁺ and Mn²⁺/Mn³⁺ couples depend strongly on the speciation of both the reduced and oxidized metal species. The standard potential for the Fe³⁺/Fe²⁺ couple is approximately +750 mV, but this potential is only relevant at low pH, where Fe³⁺ is soluble (Canfield et al., 2005a).

The work of Straub et al. shows substantial evidence of soluble and solid-phase Fe(II) oxidation coupled to nitrate reduction, thereby potentially supporting tight coupling between nitrogen and iron redox cycles in anoxic sedimentary environments (Straub et al., 1996, Straub and Buchholz-Cleven, 1998, Straub et al., 2001, Straub et al., 2004). This is supported by Weber et al. who observed that addition of nitrate following cessation of Fe(III) reduction resulted in immediate and rapid oxidation of both aqueous and solid phase Fe(II) and production of substantial quantities of ammonium ions (Weber et al., 2006). This latter reaction of nitrate reduction to ammonia reaction shows an alternative bio-catalysed nitrate reduction pathway to that producing the more typical N₂. The Weber (2006) results indicate microorganisms capable of both Fe(III) reduction and nitrate-dependent Fe(II) oxidation were responsible for the Fe(II) oxidation activity in their experiment. These microbes were subsequently identified as *Geobacter* species. In terms of electron acceptor free energy, it is noted that iron(III) reduction is inhibited by addition of nitrate to reaction vessels, confirming nitrate as a preferred AEA relative to ferric ions (Sorensen, 1982).

(i)  Pyrite oxidation

The contention previously discussed with respect to oxic pyrite oxidation also involves the anaerobic reactivity of acid-insoluble iron disulfide (pyrite). Whereas the initial aerobic dissolution of pyrite is now broadly accepted as being induced by molecular oxygen that oxidises the sulfide to sulfate leaving the Fe²⁺ ions free to be bio-oxidised to Fe³⁺ by oxygen (Lowson, 1982, Amend et al., 2004), under anoxic conditions little or no molecular oxygen is available to initiate the oxidation of either sulfide or Fe²⁺ ions. The primary question therefore is whether alternative electron acceptors (such as Mn(IV), NO₃⁻, or Fe(III)) can couple with anaerobic pyrite oxidation.

In reviewing and discussing the theory of pyrite oxidation processes, Schwientek (Schwientek et al., 2008) commented that anoxic pyrite oxidation at neutral pH is still not well understood. In acidic environments, the solubility of ferric iron below pH 3.6 allows it to be
more readily available for chemical dissolution of the crystalline pyrite and it does appear to be well accepted that soluble Fe$^{3+}$ ions chemically oxidises pyrite (Schippers and Sand, 1999). However, unless a supply of ferric ions is continuously available (e.g. through regeneration of Fe$^{3+}$ from Fe$^{2+}$) this reaction pathway should theoretically rapidly cease. Aerobic conditions allow for molecular O$_2$ oxidation of ferrous to ferric iron, but in anoxic environments, another pathway must be supported.

Little is known about electron transfer mechanisms between NO$_3^-$ and FeS$_2$ and this has led to the nitrate-coupled pyrite oxidation pathway being evaluated by several groups (Schippers, 2002b, Haaijer et al., 2007). Schippers and Jorgenson (2001; 2002a) could not link nitrate reduction directly with pyrite oxidation in anoxic marine sediment experiments running for up to a year (Schippers and Jorgensen, 2001, Schippers and Jorgensen, 2002a). They strongly asserted that O$_2$ and MnO$_2$ were chemical oxidants of pyrite under their experimental conditions, but that NO$_3^-$ or amorphous Fe(III) were not associated with FeS and FeS$_2$ oxidation. It is noted, however, that the Schippers and Jorgenson experiments involved incubations in carbonate buffered solutions at pH 8, thereby limiting not only ferric iron involvement in the reactions but potentially also many microorganism species.

Schippers and Sand (1999) proposed two indirect but associated pathways for oxic pyrite oxidation, the thiosulfate and polythionate mechanisms. They cited earlier work by Tributsch (1981) and Crundwell (1988) in postulating that, due to the valence bands of FeS$_2$ and MoS$_2$ being derived only from the metal orbitals, the metal disulfide valence bands do not contribute to the chemical bond between the metal and sulfur moiety in the crystal (Crundwell, 1988, Tributsch and Bennett, 1981), cited in (Schippers and Jorgensen, 2001). The implications were that acid-insoluble metal disulfides may be oxidised only by an oxidant (by the same indirect thiosulfate mechanism shown in Equation 2-16, whereas all other acid soluble monosulfides are also vulnerable to dissolution by proton attack (via the polythionate mechanism). This strongly indicates that pyrite may be oxidised only by a chemical oxidant, and then only through the sulfide moiety.

Manganese reduction

Metal monosulfides may also be oxidised chemically by manganese dioxide (Aller and Rude, 1988), or by nitrate-reducing microorganisms (such as Thiobacillus denitrificans (Beller et al., 2006) and Beggiatoa strains (Preisler et al., 2007) using nitrate as a terminal electron acceptor (Schippers and Jorgensen, 2002a). In tests using pH 8 bicarbonate-buffered marine sediments, Schippers (2002a) is quite adamant that although AEAAs such as nitrate and manganese(IV) may directly oxidise iron monosulfides (FeS), only oxygen, iron(III) and manganese(IV) have any oxidative effect on pyrite (FeS$_2$). It is noted that this direct manganese oxidation pathway is incorrect.
Ferric iron does not begin to become fully soluble and therefore significantly available as Fe$^{3+}$ ions (e.g. from iron(III) oxides / oxyhydroxides) until acidity declines below pH 4 (Straub et al., 2001). Therefore in acidic saturated systems such as estuarine floodplain ASS the ferric iron pyrite oxidation pathway becomes feasible. In their 2002a paper, Schippers and Jorgenson acknowledged this point and concluded that NO$_3^-$ could operate as an electron acceptor in these acidic environments in conjunction with 'Fe$^{3+}$-oxidising and NO$_3^-$-reducing bacteria (Schippers and Jorgensen, 2002a).

It is suggested here that in highly acid (i.e. pH <3.6) systems such as the study site sub-oxic subsoils the potential for pyrite oxidation by ferric ions is high. These ferric ions could be solubilised directly from iron oxides/oxyhydroxides without the need to regenerate Fe$^{3+}$ from Fe$^{2+}$ ions using AEAs such as nitrate. In addition to this pathway, Straub et al. (2006) provided evidence for humic compounds acting as electron transfer intermediaries in ferric iron reduction.

2.7.3.d Nitrogen cycle

Nitrogen makes up around 0.04 \% of all matter in the universe and globally occurs in trace amounts relative to the total mass of the earth (Audesirk and Audesirk, 1999). It constitutes around 3.3 \% of the human body (Starr and Taggart, 1987), up to 7 \% dry weight in plants and 8 to 14 \% dry weight of animal bodies (Mattson, 1980). Despite comprising 79 \% of our global atmosphere as strongly triple-bonded diatomic nitrogen molecules, most of the total global nitrogen stock (97.82 \%) is bound up in the mineralogy of rocks (Bartholomew and Clark, 1965, Daub and Seese, 1996). The global geochemical distribution of nitrogen is shown in Table 2-16.

<table>
<thead>
<tr>
<th>Category</th>
<th>Total mass x 10$^8$ tonne (trillion tonnes)</th>
<th>Percentage of total-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fundamental rocks</td>
<td>193 000 000</td>
<td>97.82</td>
</tr>
<tr>
<td>Atmosphere</td>
<td>3 864 800</td>
<td>1.96</td>
</tr>
<tr>
<td>Ancient sedimentary rocks</td>
<td>400 000</td>
<td>0.2</td>
</tr>
<tr>
<td>Terrestrial humus</td>
<td>820</td>
<td>Negligible</td>
</tr>
<tr>
<td>Sea bottom organic compounds</td>
<td>540</td>
<td>Negligible</td>
</tr>
<tr>
<td>Living organisms</td>
<td>28</td>
<td>Negligible</td>
</tr>
</tbody>
</table>

(i) Organic nitrogen

Nitrogen is one of the most abundant elements in the tissue of all organisms and as such is a critical nutrient that is required in significant quantities for the growth of plants and soil organisms, yet nitrogen occurs only in trace amounts in soils. For this reason, nitrogen is often perceived and experienced as a limiting nutrient in natural ecosystems as well as perturbed systems such as agriculture. Compounding this issue is the fact that the large stock of
atmospheric gaseous nitrogen is unavailable to most organisms except for certain bacteria. These bacteria are capable of transforming N\(_2\) into more plant-available inorganic forms such as ammonia via the processes of *nitrogen fixation* (Hellriegel, 1886), cited in (Nutman, 1987, Madigan and Martinko, 2006).

Unlike carbon, nitrogen is not ubiquitous in organic materials but is an essential part of organic compounds in living organisms. It appears in proteins, amine groups, coenzymes, nucleic acids such as DNA and RNA and chlorophyll (Starr and Taggart, 1987, Madigan and Martinko, 2006). In soils, organic nitrogen resides in humic substances as well as being part of living organisms and decomposing organic material. Major groups of organic-N material are summarised in Table 2-17, showing cellular material to be the dominant portion in soils.

Table 2-16: Organic nitrogen in soils (after Schultien and Schnitzer, 1998)

<table>
<thead>
<tr>
<th>Organic material</th>
<th>Details</th>
<th>Percentage of Total Organic N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinaceous substances</td>
<td>Proteins, peptides, amino acids</td>
<td>40 %</td>
</tr>
<tr>
<td>Heterocyclic compounds</td>
<td>Purines, pyrimidines (e.g. in DNA and RNA)</td>
<td>35 %</td>
</tr>
<tr>
<td>Ammonia</td>
<td>A quarter of this is fixed NH(_4^+)</td>
<td>19 %</td>
</tr>
<tr>
<td>Amino sugars</td>
<td></td>
<td>5 – 6 %</td>
</tr>
</tbody>
</table>

(ii) **Inorganic nitrogen**

The primary source of nitrogen for all forms of life is inorganic nitrogen and this mostly enters the food chain via microbial assimilative metabolism (Nason and Takahashi, 1958). Animals obtain their nitrogen requirements from the food they eat, whereas plants must obtain their supply from inorganic sources in soil and water. Inorganic forms of nitrogen include *gaseous di-nitrogen, ammonia, and ammonium, nitrate and nitrite* salts including ionic forms in solution. Nitrogen compounds are also present in many industrial products such as fertilisers, chemical reagents such as ammonia and nitric acid and explosives. Inorganic nitrogen exists in many oxidation states in nature (see Table 2-18). This large range of nitrogen oxidation states shows its capabilities as an active environmental oxidant through being able to accept five electrons in its reduction to nitrogen gas, or accepting eight electrons through reduction to ammonia (Campbell and Lees, 1967, Delwiche, 1970, Groffman, 2000, Canfield et al., 2005b).

Table 2-17: Oxidation states of inorganic nitrogen including hydrated forms (after Nason and Takahashi, 1958)

<table>
<thead>
<tr>
<th>Oxidation States of Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>+5</td>
</tr>
<tr>
<td>N(_2)O(_5)</td>
</tr>
<tr>
<td>HNO(_3)</td>
</tr>
<tr>
<td>NO(_3)</td>
</tr>
</tbody>
</table>
In an agricultural context, nitrogen cycles within the soil and atmosphere via the processes of: nitrogen fixation, ammonification, immobilisation, mineralisation, volatilisation and denitrification (Figure 2-5, Figure 2-6). Both inorganic and organic nitrogen species are created and transformed by predominately biotic and some abiotic processes (Johnson et al., 2005). The energy to carry out the biotically-driven processes comes from cellular respiration involving: (i) fermentation, and (ii) bio-catalysed redox reactions using oxygen (in aerobic conditions) or alternative electron acceptors (AEAs) as the terminal electron acceptors in anoxic environments.

![Agricultural nitrogen cycle](image)

*Figure 2-5: Agricultural nitrogen cycle (adapted from Johnson et al., 2005)*

The large range of oxidation potentials between most oxidised and reduced N-compounds provides much opportunity for electron exchange. As a consequence nitrogen compound redox cycling forms the basis for numerous microbial metabolic processes (Canfield et al., 2005b). Specific nitrogen transformations are described more fully in the following sections.

It is noted in Figure 2-5 there are four main pathways of nitrogen species loss from an agricultural nitrogen cycle system: (i) volatilisation of ammonia, (ii) leaching of nitrate and dissolved organic matter, (iii) organic nitrogen in crop removal, (iv) nitric and nitrous oxide gaseous emissions. Although immobilisation or assimilation of nitrogen into microbial biomass could also be argued to be a loss, the view here is that this nitrogen stays within the soil and is subsequently recycled. Urea is commonly the main source of nitrogen in cane soils (Figure 2.6) and this hydrolyses to ammonia which is then nitrified via several steps to nitrate. In the study site soils, nitrogen cycle processes are mediated by acidic soil conditions and anaerobic subsoils.
Nitrogen Fixation

Nitrogen fixation is the process whereby atmospheric di-nitrogen is transformed into inorganic compounds such as ammonia. The reduction of nitrogen, whether accomplished chemically or biologically, requires a large amount of energy to break the triple bond of the nitrogen molecule. Fixation occurs through both non-biological and biological means and the chemical reaction for this is the same for both the chemical and biological processes (Hubbell and Kidder, 2003) (Equation 2-19).

Equation 2-19: Nitrogen fixation

\[ \text{N}_2 + 3\text{H}_2 + \text{Energy} \rightarrow 2\text{NH}_3 \]

Non-biological fixation

There are two primary non-biological N-fixation processes: (i) the industrial Haber-Bosch process that uses gaseous nitrogen and hydrogen to produce anhydrous ammonia at high temperature and pressures, and (ii) atmospheric lightning transformation of atmospheric N\textsubscript{2} to produce nitrous oxide which then may be subsequently transformed to nitric dioxide and nitric acid vapour. Haber-Bosch anhydrous ammonia manufacture uses atmospheric nitrogen and
combines it with hydrogen from natural gas under heat and pressure to form anhydrous ammonia.

In the second non-biotic fixation reaction, the energy and heat of electrical discharges during thunderstorms breaks the bonds between the atmospheric nitrogen molecules, causing them to react with oxygen to form nitric oxide (NO) which then reacts with oxygen to form nitrogen dioxide (N₂O). This, combined with atmospheric moisture, produces nitrous and nitric acids which can precipitate as acid rain. In moist soils, these acids ionise, forming nitrate and nitrite ions which are readily taken up by plants and microorganisms (Christopherson, 1997, Brady and Weil, 2001).

**Biological nitrogen fixation**

Once thought to be carried out by a limited number of organisms in specific habitats (Streicher and Valentine, 1973), biological nitrogen fixation (BNF) is now known to occur in many different environments and the potential for BNF exists in any environment capable of supporting growth of microorganisms (Hubbell and Kidder, 2003). Blue-green algae, free living fermentative bacteria, symbiotic bacteria associated with leguminous and non-leguminous plants, symbiotic bacteria associated with animals such as termites and some fungi are all capable of fixing atmospheric nitrogen (Norris, 1962).

The ability of biological systems to fix nitrogen is dependent on them possessing a particular enzyme, *nitrogenase*, that catalyses the conversion of atmospheric nitrogen to a reduced ammonia form (Hubbell and Kidder, 2003), but not without considerable expenditure of ATP energy units by the microorganism. Living organisms use energy derived from the oxidation of carbohydrates to reduce molecular nitrogen to ammonia. This requires a continuous source of a strong reducing agent such as *ferredoxin* (an iron-sulfur protein), as well as adequate microbial energy reserves of ATP (Streicher and Valentine, 1973). The ammonia product is quickly assimilated by plants and microorganisms and becomes incorporated into proteins and other organic compounds supporting cellular growth (Kimbball, 2006).

The reduction of gaseous nitrogen by either chemical or biological means requires substantial quantities of energy. The industrial chemical process uses non-replaceable fossil fuels, whereas BNF transformations use energy from carbohydrates formed through the sunlight-driven photosynthetic activity of green plants. In many global environments carbohydrate substrate is readily available and thus forms a relatively inexhaustible source. Under suitable conditions, selected legumes and bacteria are capable of nitrogen fixation rates of 75 to 300 kg N ha⁻¹ (Hubbell and Kidder, 2003).

Symbiotic N-fixing bacteria on leguminous plants have long been recognised as a significant source of soil nitrogen (Russell, 1961), but other plants are now known to host similar bacteria. Brazilian sugarcane grown for over 100 years without fertiliser has long been suspected of harbouring nitrogen fixing bacteria in the rhizosphere (Neyra and Dobereiner, 1977), cited in
(Dong et al., 1994) and in Hawaii $^{15}$N studies showed that 70% of recovered sugarcane crop-N came from non-fertiliser sources (Takahashi, 1970), cited in (Dong et al., 1994).

Although little historical attention appears to have been given to sugarcane N-fixation research in Australia compared to Brazil, Dong et al. (1994) published a review of research revealing the presence of a unique N-fixing bacterial strains isolated from cane in Australia (Li and Macrae, 1991), as well as from Brazil and Mexico. The bacterium *Azotobacter diazotrophicus* is unusual in being more stress tolerant than most other N-fixing diazotrophs in being able to tolerate low pH as well as high sucrose and glucose concentrations. Most noteworthy was the microorganism’s ability to synthesise nitrogenase enzyme and express its activity in the presence of 80 mM of nitrate (Li and Macrae, 1991). It was also tolerant of oxygen and the presence of ammonium ions (NH$_4^+$) (Fu et al., 1988).

(iii) *N*-species transformation

*Ammonification and mineralisation*

The creation of ammonia (NH$_3$) and ammonia ions (NH$_4^+$), from inorganic and organic material respectively, occurs during oxic decomposition of nitrogen containing organic matter. This is carried out by a diverse range of microorganisms including bacteria, fungi and macrofauna such as earthworms. Organic matter generally contains significant quantities of nitrogen and microbes derive useful energy during this dissimilatory metabolic process. During this they also produce ammonium for N-assimilation to assist biosynthesis and growth (Bartholomew and Clark, 1965). Excess ammonium is secreted into the surrounding environment and becomes available for uptake by plants and microorganisms, or as substrate for another bacterially assisted process, that of nitrification.

Though commonly reported as a single step process, ammonification is a complex multi-enzyme processes (Ladd and Jackson, 1982), cited in (Paolo and Eldor, 2009). This transformation of organic nitrogen to ammonium is initially respiratory, however a portion of the ammonia may be assimilated for nutrition. Ammonium is an adequate source of nitrogen for many plant species, particularly those living in acidic environments.

As a positively charged cation, the ammonium ion may be held relatively strongly by forces resulting from ion-exchange reactions at the surfaces of clay minerals and organic matter in soils. As a consequence, ammonium is not effectively leached by water infiltrating the soil (Hillel, 1998). This contrasts with nitrate which is very mobile in soil water, potentially leading to significant leaching from agricultural systems subject to heavy fertiliser applications and also resulting in broad-ranging environmental impacts (OECD, 2001, Thorburn et al., 2005).

*Nitrification*

Although nitrification is still commonly stated to be the oxidation of ammonia to nitrite and nitrate by *chemoautotrophs* (Troech and Thomson, 1993), this is not how it has always been defined. Alexander (Alexander, 1965) comprehensively reviewed nitrification in 1965, finding
that *heterotrophs* could metabolically produce nitrite and nitrate and from substrates other than ammonium. An alternative definition of nitrification was advanced as early as 1960 which does not seem to have been adopted by subsequent researchers, namely: the biological conversion of nitrogen in organic or inorganic compounds from a reduced to a more oxidised state (Alexander *et al.*, 1960). The first mentioned more specific definition will be used in this review.

Nitrification occurs in two stages and is carried out by two specific groups of organisms of the *Nitrobacteriaceae* family of the order Pseudomonadales. The first step oxidation of ammonia to nitrite, termed *nitrosification*, is initiated by bacteria of the *Nitrosomonas* genera. The second stage bio-catalytic transformation of nitrite to nitrate is assisted by *Nitrobacter* (Alexander, 1965). In addition to the above, ammonia oxidising autotrophic bacteria of the genera, *Nitrosococcus*, *Nitrosospira*, *Nitrosococcus* and *Nitrosocystis* were also recognised (Breed *et al.*, 1957), cited in (Alexander, 1965, Paolo and Eldor, 2009). Whilst nitrification is predominantly a bacterial catalysed aerobic process, Bartholomew and Clark stated that nitrification capability is shared by many microorganisms including some obligate anaerobes, *Actinomycetes* and fungi (Bartholomew and Clark, 1965). There are also some reports asserting nitrification activity is dynamic and can change quickly in response to changes in soil conditions including pH (Bramley and White, 1989). However, the general consensus is that significant microbial production of soil nitrate does not occur in acidic soils below pH 5.0 (Gerardi, 2002) due to the general intolerance of nitrifying bacteria to acidity.

(iv) *Nitrate removal pathways*

After formation via nitrogen fixation, ammonification and nitrification transformations, nitrate is often progressively removed from the environment by physical, biological and biogeochemical means. These include (i) leaching and the microbially assisted reactions shown in Figure 2-7, (ii) biomass assimilation, (iii) sulfur-driven nitrate reduction to nitrogen gas or ammonia, (iv) anaerobic nitrite reduction to ammonia and nitrogen gas (anammox), (v) iron-driven denitrification, (vi) fermentative *dissimilatory nitrate reduction to ammonia* (DNRA) and (vii) respiratory denitrification (van Beek *et al.*, 1988, Guerrero *et al.*, 1981, Samuelsson, 1985, Brunet and Garcia-Gil, 1996, Sorensen, 1982).

These bio-catalysed pathways are shown graphically in Figure 2-7 and reviewed in the following sub-sections. Note that the blue arrows in Figure 2-7 indicate autotrophic pathways and purple arrows represent heterotrophic transformations. Iron disulfide was added to the *sulfur driven nitrate reduction* pathway based on the 2009 findings of Jorgenson (Jorgenson *et al.*, 2009).
Nitrate leaching

Nitrate leaching from agricultural land to groundwater poses a threat to water quality and also involves significant loss for agriculture (Jiao et al., 2004). Nitrate is an anion which does not bind easily to predominantly negatively-charged clay particles. As a consequence nitrate ions have been reported to be highly mobile in soils and particularly in high rainfall locales (Di and Cameron, 2002). In agricultural soils, soil nitrate typically derives from soil amendments such as nitrogenous fertilisers. Factors impacting on the susceptibility of this nitrate to leach either downwards or sideways includes: the presence of vegetation such as crops, the applied fertiliser chemical form and application rate, soil type and structure, the presence of macropores such as root channels, climate or seasonal conditions, groundwater height, and the presence of soil organic matter (Weier, 1994).

In an experiment investigating the impact of agricultural practices on nutrient leaching, Jiao et al. (2004) found that the type of fertiliser applied had more effect on total leaching than any other agricultural practice. Inorganic fertilisers leached more nitrate-N in dissolved form than organic based fertilisers (Jiao et al., 2004). Gupta (1982) found that greater water inputs increased leaching rates and depth of penetration and also that splitting fertiliser applications into multiple smaller amounts diminished the amount of nitrate lost through leaching (Gupta et al., 1982).

Nitrate as an alternative electron acceptor in low oxygen environments is susceptible to loss through reduction on entering saturated anoxic subsoil zones. Examples of this are found in the many reports of nitrate reduction occurring in aquifers in the presence of dissolved organic

**Biomass assimilation**

Although soil flora and fauna make up a significant proportion of the topsoil in agricultural soils and will take up nutrients as they become available, it could be argued that any nutrients they assimilate are potentially largely recycled rather than lost from the system. Nitrogen is required by all microorganisms for biosynthesis of amino acids and nucleotides and it may be obtained from organic and inorganic sources by moving it into the cell then converting it to ammonia and amino acids (Madigan and Martinko, 2006). Inorganic forms of nitrogen such as nitrate serve as a source and many plants, fungi and bacteria have the necessary enzymes to reduce nitrate to nitrite (nitrate reductase) and nitrite to ammonia (nitrite reductase). Other specialised microorganisms are able to use other processes, such as reducing nitrogen gas to ammonia using the enzyme nitrogenase, although this is costly in terms of energy requirements. Forming symbiotic associations with plants is another microbial strategy where the plant provides carbon and energy in exchange for fixed nitrogen (Paustian, 2000e).

**Anammox**

Anaerobic ammonia oxidation involves the bio-catalysed oxidation of ammonia to nitrogen gas coupled to the reduction of nitrite (Paolo and Eldor, 2009). This process has been shown to occur in \( ^{15} \text{N} \)-labelling experiments in water treatment facilities, but to date not in soils although Paolo states it seems reasonable to hypothesise the existence of the anammox bacteria in soils under anaerobic conditions. These bacteria were shown to be highly enriched in an experimental treatment plant, evidently growing autotrophically by obtaining energy from the conversion of ammonia and nitrite (via nitrite, hydroxylamine and hydrazine) to nitrogen gas (Mulder et al., 1995, Engstrom et al., 2005). The well known aerobic nitrifier, Nitrosomonas, has been reported to couple anaerobic ammonia oxidation to the reduction of nitrogen dioxide, resulting in the formation of NO and nitrite (Schmidt and Bock, 1997; (Kelly and Wood, 2006).

**(v) Nitrate reduction processes**

Agriculture is the most extensive anthropogenic source of nitrate in natural surface water and groundwater systems and although nitrate is stable under some conditions it is also highly mobile in systems subject to intermittent or permanent saturation (Korom, 1992). Saturation will rapidly drive a soil system anoxic and potentially lead to increased rates of nitrate reduction (Webster and Hopkins, 1996). Respiratory NO\(_3^-\) reduction includes microbial processes whereby nitrate is reduced in association with various electron donors by an energy gaining metabolism in anoxic or sub-oxic environments. At least five different nitrate coupled reactions have been identified in the literature.
Two different pathways were distinguished by Canfield (Canfield et al., 2005b):

(i) nitrate reduction to gaseous products (\(N_2O\) or \(N_2\)) by denitrification, with SOM

(ii) reduction to ammonium (\(NH_4^+\)) by nitrate ammonification or dissimilatory nitrate reduction to ammonium (DNRA)

Two specific processes were detailed by Burgin (2007), namely:

(iii) nitrate reduction with iron(II)

(iv) nitrate reduction with reduced S-species (including \(H_2S\), \(S^0\) and FeS)


(v) nitrate reduction by pyrite (\(FeS_2\))

These nitrate reduction processes are reviewed in more detail below.

**Denitrification**

Denitrification is an anaerobic microbial dissimilatory process whereby nitrate is fully sequentially reduced through various N-oxide species to nitrogen gas. The reactions are rapidly activated when soils become deficient in oxygen below 2 mg L\(^{-1}\), where nitrate is available (Payne, 1973, Wassenaar et al., 2006).

The definitive pathway associates nitrate reduction with carbon compound oxidation but the carbon source may be accessed autotrophically, or heterotrophically via an organic carbon source (Alewell et al., 2008). That is, the common pathway for denitrification is through use of organic matter (McCarty and Bremner, 1992) or dissolved organic matter as the electron donor (Siemens et al., 2003). McCarty and Bremner (1992) found SOC is decomposed so rapidly in surface soils that little is generally available for denitrification at depth (Starr and Gillham, 1993). The term denitrification is often used as a synonym for nitrate reduction in the literature (Jorgenson et al., 2009), but here the intention is to use this term solely for the process of complete nitrate reduction to \(N_2\) coupled with organic matter oxidation.

Nitrate is sequentially reduced in denitrification via a series of preferred N-species electron acceptors (Canfield et al., 2005b) (Equation 2-20). The nitrate reductase enzyme synthesis that enables nitrate reduction is activated only on the disappearance of oxygen and is not stored (Payne, 1973).

*Equation 2-20: Nitrogen oxides denitrification sequence*

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\]
Organic matter electron donors in denitrification include soil organic matter, plant residues, root exudates, manures, microbial residues (including metabolites, polysaccharides, proteins, lipids and lignin) and organic contaminants (Rivett et al., 2008) (Equation 2-21).

Equation 2-21: Organic matter coupled denitrification

\[ 5\text{CH}_2\text{O} + 4\text{NO}_3^- \rightarrow 2\text{N}_2 + 4\text{HCO}_3^- + \text{CO}_2 + 3\text{H}_2\text{O} \]

Many microorganisms denitrify nitrate under anaerobic conditions, including heterotrophic bacteria from the genus *Achromobacter*, *Chromobacterium*, *Corynebacterium*, *Microoccus*, *Pseudomonas* and *Spirillum*, and autotrophic bacteria from the genus *Acidithiobacillus*, *Paracoccus* and *Rhodobacter* (Payne, 1973). There is also a group of microorganisms that respire nitrate, but reportedly only to nitrite (Payne, 1973). These include many pathogenic enteric bacteria, but also some that live in soils such as *Actinobacillus* and *Agrobacterium* (renamed *Rhizobium*). It is noted that *Micrococcus denitrificans* is capable of autotrophic growth with hydrogen, nitrate or oxygen, and carbon dioxide.

The preferred denitrification pH range is 5.5 to 8.0 as low pH arrests the chain of reduction at the NO₂ or N₂O stage (Brady and Weil, 2001). Optimal temperature is 25 to 35 °C, but may occur in the range 2 to 50 °C. High salinity inhibits denitrification (>20 g L⁻¹ NaCl). Other inhibitory substances include heavy metals, pesticide derivatives and high concentration of organic compounds. In some instances, pesticides stimulated denitrification (Rivett et al., 2008). Furthermore, excess nitrate concentrations (>1mg N L⁻¹) affect denitrification by inhibiting formation of nitrogen gas and possibly terminating the sequential reactions at the N₂O stage. Nitrate increasing from 0 to 4 mg L⁻¹ decreased denitrification efficiency from N₂ to N₂O termination (Rivett et al., 2008). The presence of sulfate and thiosulfate has been shown to also inhibit denitrification in soils with the rate negatively correlated to sulfate or thiosulfate concentration (Rivett et al., 2008). The reduced sulfur form, sulfide, has been shown to promote dissimilatory reduction of nitrate to ammonium rather than denitrification (Hiscock et al., 1991), cited in Rivett, 2008).

Oxygen is the preferred electron acceptor over nitrate or other AEAs due to its higher potential free energy return to microorganisms. Therefore, denitrification proceeds only when the dissolved oxygen concentration falls below a 1 to 2 mg L⁻¹ threshold, however this may be mediated by bacterial habitat micro-site effects. Denitrifiers obtain energy for metabolism and growth from the oxidation of organic carbon, sulfide minerals or reduced iron and manganese. They also require other nutrients and trace elements. Relative concentrations of nitrate and SOC appear to control whether nitrate is depleted by denitrification or by dissimilatory nitrate reduction to ammonia (Rivett et al., 2008).
Dissimilatory nitrate reduction to ammonia

Specific nitrate reduction transformations to ammonia may be carried out by microorganisms (e.g. *Shewanella oneidensis MR-1*) under anaerobic conditions. In this respiratory nitrate ammonification, also termed *dissimilatory nitrate reduction to ammonium*, energy is gained by sequential reduction of nitrate to nitrite to ammonium. Bacteria exhibit considerable diversity, even within species, in being able to respire by denitrification or nitrate ammonification (Cruz-Garcia *et al.*, 2007). Nitrate ammonification is used by organisms to detoxify nitrite (NO$_2^-$) or at times as an electron sink during fermentation (Canfield *et al.*, 2005b), but some microorganisms are also true respirers (Welsh *et al.*, 2001).

Nitrate reduction coupled to iron oxidation

Postma (Postma, 1990) reviewed nitrate reduction by iron silicates and found little previous work in this area but did find some studies dealing with the reaction between dissolved Fe$^{2+}$ and nitrate (Equation 2-22). The rate of this reaction is reported to be temperature sensitive (Postma, 1990) and there is evidence that nitrite (NO$_2^-$) is more reactive with iron than is nitrate (Sorensen and Thorling, 1991) (Equation 2-23). Sorensen and Thorling (1991) also cited and reported evidence for ferrous iron binding to the ferri-oxhydroxide complex that stimulated the chemo-denitrification under slightly alkaline conditions.

*Equation 2-22: Nitrate reduction coupled to iron(II) oxidation (N$_2$)*

$$10\text{Fe}^{2+} + 2\text{NO}_3^- + 14\text{H}_2\text{O} \rightarrow 10\text{FeOOH} + \text{N}_2 + 18\text{H}^+$$

*Equation 2-23: Nitrite reduction coupled to iron(II) oxidation (N$_2$O)*

$$5\text{Fe}^{2+} + 2\text{NO}_2^- + 5\text{H}_2\text{O} \rightarrow 4\text{FeOOH} + \text{N}_2\text{O} + 6\text{H}^+$$

Straub (Straub *et al.*, 1996) reported that nitrate, nitrite and nitrous oxide readily oxidize iron(II) in vitro due to its solubility, but questioned whether this would occur to any significant extent under natural conditions. Testing with FeSO$_4$ as the sole electron donor, NO$_3^-$ was reduced with concomitant increase in ferric iron, with *Acidithiobacillus denitrificans* and *Pseudomonas stutzeri* identified as having this capability, but not *Thiomicrospira denitrificans* or *Paracoccus denitrificans*. Straub (1996) also cited evidence for abiotic nitrate reduction with ferrous iron oxidation and suggested that both biotic and abiotic reactions may take place depending on prevailing conditions, one of which could be the presence of metal catalysts such as copper, tin and silver ions (Ottley *et al.*, 1997). Ottley (1997) also cited other studies (Postma, 1990, Sorensen and Thorling, 1991) reporting that secondary iron(III) precipitates such as iron oxyhydroxides catalyse nitrate reduction.

2.7.4 Nitrate Coupled Pyrite Oxidation

The chemical complexity of an agricultural soil medium includes a multitude of organic and inorganic substances, and a plethora of potential biogeochemical reaction pathways. With the
recent work of Durr there is also confirmation of a substantial number of now largely identified soil microorganisms throughout the ASS profile to two metres (Durr, 2009). This creates a challenging environment in which to demonstrate an absolute connection between specific reactants and products in the field. Although many advances in soil biogeochemistry have occurred in the last 36 years, the words of Wilkins (1974) still bring a useful touch of realism and caution to a field where strongly stated assertions with regard to reaction pathways often prevail and are repeated in the literature:

_The mechanism of a chemical or geochemical reaction, that is the detailed manner in which it proceeds and particularly the number and nature of steps involved, is a product of our imagination. A correct mechanism cannot ever be proven, but a substantial amount of information may be gathered to test any hypothesised molecular processes (Williamson and Rimstidt, 1994) p.5443, citing (Wilkins, 1974)._  

The focus in this study is on acid-insoluble iron disulfide and its potential for increased rates of oxidation in the presence of soil nitrate in sugarcane agriculture. Whilst the intention is to investigate the potential for a direct enzymatic mechanism of nitrate coupled iron disulfide oxidation, the study aim does not exclude indirect processes involving, say, nitrate assisted chemical attack in the dissolution of soil pyrite. If there were some direct mechanisms, then these would be more efficient with close contact microbial contact with the mineral surface (Rawlings, 2004).

The terms _direct_ and _indirect_ are historical terms used at least as early as 1978 (Brierley, 1978). After the work of the Sand (Sand _et al._, 1995) and Schippers teams (Schippers _et al._, 1996) these terms became somewhat less relevant within the context of developments in other fields. Rawlings (2004) argues that the direct verses indirect microbial attack debate has been confused by a related question: 'do microorganisms affect the direct solubilisation of metals from minerals in a way that is greater than their ability to produce ferric iron and acid in the indirect mode?'

There is strong evidence that close microbial attachment does improve the metal dissolution or bioleaching rates (Tributsch and Rojas-Chapana, 2000, Rojas-Chapana and Tributsch, 2004, Rawlings, 2004, Pisapia _et al._, 2008, Zeigler _et al._, 2009). The affinity that bacteria such as _Acidithiobacillus ferrooxidans_ (Rodriguez-Leiva and Tributsch, 1988, Blake II _et al._, 2001, Rojas-Chapana _et al._, 1995) and _Leptospirillum ferrooxidans_ (Tributsch, 2001) have for mineral surfaces such as pyrite may appear to indicate they are attacking the mineral surface, but more recent evidence showed that mineral solubilisation is mostly physicochemical (i.e. _indirect_) (Crundwell, 1988, Edwards _et al._, 2001, Crundwell, 2001), cited in (Rawlings, 2004). This _indirect_ pathway is based upon the chemical oxidation of metal sulfides (including pyrite) by ferric ions.
Crundwell (2003) sought to end the longstanding debate by comprehensively reviewing the experimental literature and concluded by proposing an integrated solution as follows. Crundwell argued that close attachment of bacteria with the pyrite mineral surface may increase the oxidation rate through either (i) the formation of a porous layer of sulfur (product layer diffusion), or (ii) increasing the pH at the mineral surface which increases the rate of dissolution, but in both cases the leaching mechanism was that of chemical dissolution by ferric ions (Crundwell, 2003). Further developments, however, indicated that the direct vs. indirect process differentiation was becoming blurred.

Rose-Chapana and Tributsch (2004) described pyrite oxidation activity by *Leptospirillum ferrooxidans* which oxidises only iron, but creates a specific biofilm environment to electrochemically corrode the pyrite surface, thereby producing electrons for Fe$^{3+}$ reduction (Rojas-Chapana and Tributsch, 2004). Zhu *et al.* (2008) reported increased rates of pyrite oxidation in the presence of cysteine which attaches to the mineral surface and changes its chemical properties. There is also evidence that cysteine alone can corrode the pyrite surface (Zhu *et al.*, 2008). It is noted that *Thiobacillus denitrificans*, bacteria that biocatalyse nitrate reduction, contain cysteine and S-sulfo-cysteine synthases (Hensel and Truper, 1976). A comprehensive review of pyrite surface reactivity by Murphy and Strongin (2009) revealed several other pyrite dissolution mechanisms likely to mediate not only rates of pyrite oxidation, but also its susceptibility to dissolution by substances other than molecular oxygen and ferric ions. Water may form a coordinate covalent bond with the pyrite surface Fe atoms via donation of electron density to the empty iron d$_{z^2}$ orbitals.

Water combined with oxygen substantially increases the pyrite oxidation rate in a context where each substance alone oxidises pyrite slowly or not at all. The second mechanism relates to natural surface defect sites in pyrite which contain monosulfides molecules. These may serve as cathodic sites allowing more efficacious electrochemical dissolution of the pyrite (Murphy and Strongin, 2009). It should be noted that many microbially mediated reactions are involved in further oxidation of the intermediate sulfide oxidation products to sulfate (Rohwerder and Sand, 2007). Chemical oxidation of pyrite by purely chemical means is possible with molecular oxygen (Colmer and Hinkle, 1947) and ferric ions (Lowson, 1982). Nitrate has also been widely implicated in iron monosulfide (FeS) oxidation (Schippers and Jorgensen, 2002a, Haaijer *et al.*, 2006, Haaijer *et al.*, 2007, Tugtug and Pavlostathis, 2007) but direct iron disulfide oxidation coupled to nitrate reduction appears less well supported although widely postulated (Pauwels, 1994, Pauwels *et al.*, 1998, Pauwels *et al.*, 2000, Schwientek *et al.*, 2008, Jorgenson *et al.*, 2009, Zhang *et al.*, 2009). With some exceptions however (Jorgenson *et al.*, 2009), most of these reports are really describing nitrate removal associated with sulfate appearance in pyritic sediment/aquifer profiles. Thus there is scant evidence directly associating nitrate
reduction with pyrite oxidation in these studies, nor is there much hard evidence for any specific reaction pathways.

In reviewing nitrate attenuation in groundwater systems, Rivett et al. (2008) cites studies supporting simple and complex organic matter; reduced iron (Fe$^{2+}$) and reduced sulfur compounds (including pyrite FeS$_2$), as potential electron donors associated with nitrate reduction (Rivett et al., 2008). They commented that the susceptibility of pyrite to nitrate induced oxidation depends on its microscopic structure (Kolle et al., 1985), cited in (Rivett et al., 2008) with finer particles being more reactive.

Although Mn(II) / Mn(IV) cycling has been described as being an important geochemical couple in natural systems, it is reported that nitrate and nitrite reduction does not redox-couple with Mn(II) under any conditions (Schippers et al., 2005). Manganese(IV) does oxidise ammonia and organic-N substances to produce Mn(II) which is readily re-oxidised by oxygen (Luther et al., 1997).

Nitrate reduction coupled to organic matter oxidation reactions (e.g. denitrification) are recognised as being catalysed by many different microorganisms, including subclasses of the Proteobacteria as well as various Archaea hyperthermophilic and halophytic branches. This reduction using an electron transport chain is carried out across the cell cyto- and peri-plasmic membranes (Madigan and Martinko, 2006) using nitrate dissimilatory reductase (Nar) to produce nitrite. Nar is expressed in low oxygen conditions and (unlike the nitrate assimilatory reductase, Nas) is not inhibited by ammonia (Zumft, 1997). With continuation of the reduction, nitrite reductase reduces nitrite to nitric oxide and nitric oxide reductase and nitrous oxide reductase complete the reduction to nitrogen gas (Madigan and Martinko, 2006). Due to its potential higher energy return, organic matter is likely to be used if it is available (Achtnich et al., 1995).

The role of nitrate in iron oxidation has been reasonably well researched in the literature. Postma (1990) reported widespread nitrate reduction in the presence of Fe(II) bearing silicates (i.e. Fe bearing amphibole/pyroxene fractions) in the pH range 2 to 7 (Postma, 1990). Soluble ferrous iron in soils may be chemically oxidised by oxygen but also by nitrate and possibly manganese(IV) oxide under anaerobic conditions. Ferric ion production via oxidation of ferrous ions has been reported to occur with nitrate as the electron acceptor. Therefore, in sub-oxic / anoxic soil systems, nitrate can replace oxygen in regenerating ferric ions that are the primary oxidant of pyrite (Straub et al., 1996). Whilst nitrate’s role in acid-insoluble iron disulfide oxidation is still unclear, in the absence of oxygen it is highly likely for nitrate to be the preferred electron acceptor for the regeneration of ferric iron. Even in the presence of ferric ions, a strong oxidant of pyrite, there is some evidence that nitrate is the preferred electron acceptor although this would be mediated by reactant concentration (Ehrlich and Newman, 2008).
Pathways of nitrate oxidation of reduced sulfur compounds other than acid-insoluble iron disulfide are reasonably clear and unambiguous. Nitrate has also been nominated as an alternative electron acceptor in the oxidation of intermediate sulfur compounds (i.e. elemental sulfur > thiosulfate > tetrathionate > sulfite) in sulfide to sulfate oxidation (Canfield et al., 2005d). In the presence of FeS and H₂S combined, large amounts of ammonium ions were abiotically (i.e. chemically) formed from nitrate reduction. FeS alone caused only minor conversion and none with H₂S alone. This indicates that nitrate ions cannot co-exist in anaerobic pyrite-forming sulfate-reducing conditions (Blochl et al., 1992). Under these conditions, obligate sulfate-reducing bacteria such as Desulfovibrio sp. can use inorganic alternative electron acceptors, including nitrate and nitrite (Dalsgaard and Bak, 1994).

Nitrate reduction in pyrite bearing aquifers has been widely investigated and described. These reports (Pauwels, 1994) are often accompanied by the postulated nitrate coupled pyrite oxidation reaction (Equation 2-24), viz.:

**Equation 2-24: Nitrate coupled anoxic pyrite oxidation**

\[5\text{FeS}_2 + 14\text{NO}_3^- + 4\text{H}^+ \rightarrow 7\text{N}_2 + 10\text{SO}_4^{2-} + 5\text{Fe}^{3+} + 2\text{H}_2\text{O}\]

In the Pauwels (1994) study it was acknowledged that the observed nitrate decline in the profile could also have been partly due to organic matter mineralization with nitrate as the electron acceptor. The pyrite / nitrate reaction (Equation 2-24) shows the sulfide moiety chemically oxidized by nitrate to sulfate, and the iron(II) solubilised to ferrous ions. This is similar to the molecular oxygen pyrite oxidation reaction (Equation 2-13) with nitrate replacing molecular oxygen. However, some evidence is weighted against this reaction scenario. Jorgenson et al. (2009) cite Stumm and Morgan (1996) in suggesting that a direct nitrate reduction coupled pyrite oxidation chemical pathway is kinetically highly unlikely. Supporting this view, Schippers et al. (2001) could not detect any pyrite oxidation by nitrate in marine sediment samples at pH 8 (Schippers and Jorgensen, 2001). Re-iterating the findings of the Schippers group (2001), Haaijer et al. reported that pyrite did not function as an electron donor for microbial nitrate reduction in continuous reactor experiments (Haaijer et al., 2007).

An interesting point was noted in the Schippers et al. (2001) paper describing pyrite oxidation by manganese(IV) dioxide for the first time experimentally. This was not reported as a direct reaction, but an indirect one where ferric ions were the oxidant for pyrite dissolution and the Mn(IV) regenerated ferric ions using the oxidation Fe²⁺ product (Schippers and Jorgensen, 2001). This raises queries around what it is about ferric ions that enables them to directly oxidise the sulfur moiety in pyrite that is different to manganese(IV) and nitrate ions, which apparently cannot carry out this reaction.

The Jorgenson et al. (2009) paper is one of the few focussing on anoxic microbial pyrite oxidation coupled to nitrate reduction. Their paper acknowledges the widely reported O₂ and Fe(III) pyrite oxidation pathways clarified above, but also reiterates the assumption of a nitrate
reduction pathway. This pathway is shown by the nitrate reduction half reaction in Equation 2-25. Note that this half reaction consumes protons.

\[ 2\text{NO}_3^- + 12\text{H}^+ + 10\text{e}^- \rightarrow \text{N}_2 + 6\text{H}_2\text{O} \]

With respect to pyrite oxidation, Jorgenson et al. (2009) write that it is generally assumed (added emphasis) that pyrite and other reduced sulfur compounds are important electron donors for in situ nitrate reduction processes in anoxic groundwater environments, citing ((Postma et al., 1991, Korom, 1992, Kolle et al., 1983, Kolle et al., 1985, Bottcher et al., 1985, Robertson et al., 1996b, Tesoriero et al., 2000, Garcia-Gil and Golterman, 1993, Korom et al., 2005)). Jorgenson et al. (2009) suggested a potential reaction pathway for this reaction (Equation 2-24).

The Jorgenson et al. (2009) paper also acknowledges the role of ferrous iron and organic matter as electron donors in the anaerobic reduction of nitrate (Equation 2-26 and Equation 2-27). Nitrate also anoxically couples with, manganese(IV) (Equation 2-28) and with iron(III) (Equation 2-32).

\[ 5\text{Fe}^{2+} + 2\text{NO}_3^- + 12\text{H}_2\text{O} \rightarrow 5\text{Fe(OH)}_3 + 0.5\text{N}_2 + 9\text{H}^+ \]

\[ 5\text{CH}_2\text{O} + 4\text{NO}_3^- + 4\text{H}^+ \rightarrow 5\text{CO}_2 + 2\text{N}_2 + 7\text{H}_2\text{O} \]

\[ \text{FeS}_2 + 7.5\text{MnO}_2 + 11\text{H}^+ \rightarrow \text{Fe(OH)}_3 + 2\text{SO}_4^{2-} + 7.5\text{Mn}^{2+} + 4\text{H}_2\text{O} \]

Jorgenson et al. (2009) cites Stumm and Morgan (1991) in rejecting on kinetic grounds a chemical pathway for direct anaerobic nitrate-coupled pyrite oxidation (Stumm and Morgan, 1991). They suggest that microbial catalysis may enable electron transfer but acknowledge that no studies have yet demonstrated this. They then describe experimental results of anoxic biocatalysed nitrate reduction using pyrite as the primary electron donor. This takes place in sandy sediment and they ascribe 50 % of the nitrate reduction to oxidation of the pyrite. In situ measurements at the Jorgenson et al. (2009) study site indicated that 65 to 80 % of nitrate reduction in the anoxic aquifer zone is due to pyrite oxidation. It is clear from these results that: (i) nitrate is being reduced in association with iron disulfide dissolution; (ii) nitrate reduction accompanying pyrite oxidation raised the reactor solution pH; and (iii) nitrate-reduction reactions other than the reported nitrate-coupled pyrite reduction are occurring. Jorgenson et al. (2009) conclude that microbes can control groundwater nitrate concentration by denitrification using pyrite primarily as the electron donor at the oxic-anoxic interface, thereby determining the position of the redox boundary between nitrate-containing and nitrate-free subsoil zones.
The Jorgenson et al. (2009) anoxic incubation experiment, conducted using natural pyritic sandy sediments with potassium nitrate solution as the source of nitrate ions, did not identify the microorganisms purportedly involved. However their argument was supported by the assumption that the primary active reaction was nitrate-reduction coupled with pyrite oxidation (Equation 2-24). This reaction produces ionic ferric sulfate, nitrogen and water (i.e. alkalinity through incorporation of protons) thereby producing ferric iron (in low pH conditions) potentially capable of chemically oxidising pyrite directly. Chemical oxidation of pyrite by ferric ions is shown by Equation 2-29, where 1 mole of pyrite reacts with 14 moles of ferric ions, producing 16 moles of protons (Fowler et al., 1999).

\[ \text{Equation 2-29: Ferric ion oxidation of pyrite} \]
\[ \text{FeS}_2 + 8\text{H}_2\text{O} + 14\text{Fe}^{3+} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+ \]

It can be calculated using Equation 2-24 that 5 moles of pyrite reacts with 14 moles of nitrate to produce 5 moles of ferric ions, consuming 4 moles of protons in the process. Therefore, if nitrate-coupled pyrite oxidation was to occur and ferric ion oxidation of pyrite was occurring concurrently, then ferric ions could quickly become limiting depending on the kinetics of each reaction. This raises the question of another potential source of ferric ions and whether Acidithiobacillus ferrooxidans could assist regeneration of ferric from ferrous ions under these anoxic conditions.

Despite some reports of them being aerobes (van Bremen, 1972, Gormely and Duncan, 1974), Pronk et al. (1992) demonstrated *A. ferrooxidans* growth in anoxic environments, thus showing them to be facultative anaerobes (Pronk et al., 1992). However, *A. ferrooxidans* appeared incapable of oxidising ferrous ion to ferric using other electron acceptors. Under anaerobic conditions they were reported to reduce Fe\(^{3+}\) ions to Fe\(^{2+}\) using elemental sulfur as electron donor (Pronk et al., 1992). In another report, unspecified chemolithotrophic bacteria were shown to anaerobically oxidise Fe\(^{2+}\) to Fe\(^{3+}\) (Benz et al., 1998). Another potential pathway was reported by Pauwels et al. (Pauwels et al., 1998) who interpreted results of an aquifer tracer experiment of nitrate coupled pyrite oxidation as being partly due to nitrate oxidation of ferrous ions to ferric ions by Equation 2-30.

\[ \text{Equation 2-30: Oxidation of ferrous ions by nitrate} \text{(Pauwels et al., 1998)} \]
\[ \text{NO}_3^- + 5\text{Fe}^{2+} + 6\text{H}^+ \rightarrow 0.5\text{N}_2 + 5\text{Fe}^{3+} + 3\text{H}_2\text{O} \]

Therefore there are two potential indirect mechanisms for anoxic pyrite oxidation through Fe\(^{3+}\) creation in anoxic acidic soil systems. The first is created by the low pH \(<4\) conditions themselves through solubilisation of ferric ions from iron oxides and oxihydroxides as well as amorphous iron compounds in the soil.
Although the solubility of Fe$^{3+}$ ions increases markedly below pH 4, ferric ion solubility reportedly has a log-linear relationship with pH between pH 3.1 to 7.3 (Byrne and Luo, 2000). This suggests that even at circum-neutral pH some Fe$^{3+}$ could be available as reactant.

The second mechanism uses nitrate reduction coupled to ferric ions oxidation (Benz et al., 1998, Pauwels et al., 1998).

Support for direct nitrate-coupled pyrite oxidation pathways comes from thermodynamics and calculation of Gibbs free energy for potential sulfide oxidation reactions (Table 2-19). These values are 'standard' Gibbs free energy at 25°C and 1M concentration. As temperature and reactant concentration are two of the factors in the Nernst equation (Equation 2-11) used to calculate this, the Gibbs free energy values for field conditions will be different, but the standard value relativity is nevertheless informative. Given molar concentrations, nitrate oxidation of pyrite has a free energy advantage of around 70 % or just over 1000 kJ/mol over pyrite oxidation by molecular oxygen (Table 2-19). While this does not take into account ferric ion oxidation of pyrite, it does support the nitrate-pyrite coupling thesis somewhat. As these Gibbs free energy values show potential energy to be gained by full reduction of a substance, this complete reduction is often not available to single organism species using one enzyme (Stumm and Morgan, 1996).

**Table 2-18: Gibbs free energy values for potential FeS and FeS$_2$ oxidation reactions (after Stumm and Morgan 1996; Lowson 1982)**

<table>
<thead>
<tr>
<th>Potential reactions for oxidation of FeS and FeS$_2$</th>
<th>Gibbs free energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 FeS + 9 NO$_3^-$ + 8 H$_2$O $\rightarrow$ 5 Fe(OH)$_3$ + 5 SO$_4^{2-}$ + 4.5 N$_2$ + 2 H$^+$</td>
<td>$\Delta G_f^0 = -3817$ kJ/mol acidifying</td>
</tr>
<tr>
<td>2 FeS$_2$ + 6 NO$_3^-$ + 4 H$_2$O $\rightarrow$ 2 Fe(OH)$_3$ + 4 SO$_4^{2-}$ + 3 N$_2$ + 2 H$^+$</td>
<td>$\Delta G_f^0 = -2439$ kJ/mol acidifying</td>
</tr>
<tr>
<td>FeS$_2$ + 4.25 O$_2$ + 2.5 H$_2$O $\rightarrow$ Fe(OH)$_3$ + 2 SO$_4^{2-}$ + 2 H$^+$</td>
<td>$\Delta G_f^0 = -1435$ kJ/mol acidifying</td>
</tr>
<tr>
<td>FeS$_2$ + 7.5 MnO$_2$ + 11 H$^+$ $\rightarrow$ Fe(OH)$_3$ + 2 SO$_4^{2-}$ + 7.5 MnO$_2$ + 4 H$_2$O</td>
<td>$\Delta G_f^0 = -1199$ kJ/mol</td>
</tr>
<tr>
<td>FeS$_2$ + 14 Fe(OH)$_3$ + 15 HCO$_3^-$ + 11 H$_2$ $\rightarrow$ 15 FeCO$_2$ + 2 SO$_4^{2-}$ + 34 H$_2$O</td>
<td>$\Delta G_f^0 = -806$ kJ/mol</td>
</tr>
<tr>
<td>FeS + 4.5 MnO$_2$ + 7 H$^+$ $\rightarrow$ Fe(OH)$_3$ + SO$_4^{2-}$ + 4.5 Mn$^{2+}$ + 2 H$_2$O</td>
<td>$\Delta G_f^0 = -751$ kJ/mol acidifying</td>
</tr>
<tr>
<td>FeS + 2.25 O$_2$ + 2.5 H$_2$O $\rightarrow$ Fe(OH)$_3$ + SO$_4^{2-}$ + 2 H$^+$</td>
<td>$\Delta G_f^0 = -750$ kJ/mol acidifying</td>
</tr>
<tr>
<td>FeS + 8 Fe(OH)$_3$ + 9 HCO$_3^-$ + 7 H$_2$ $\rightarrow$ 9 FeCO$_2$ + SO$_4^{2-}$ + 20 H$_2$O</td>
<td>$\Delta G_f^0 = -515$ kJ/mol</td>
</tr>
<tr>
<td>FeS + 26 Fe(OH)$_3$ $\rightarrow$ 9 Fe$_2$O$_3$ + SO$_4^{2-}$ + 38 H$_2$O + 2 H$^+$</td>
<td>$\Delta G_f^0 = +596$ kJ/mol acidifying</td>
</tr>
<tr>
<td>FeS$_2$ + 44 Fe(OH)$_3$ $\rightarrow$ 15 Fe$_2$O$_3$ + 2 SO$_4^{2-}$ + 64 H$_2$O + 4 H$^+$</td>
<td>$\Delta G_f^0 = +942$ kJ/mol acidifying</td>
</tr>
</tbody>
</table>

Many other reactions and interactions are also possible in a soil system. Nitrate can potentially be involved in many iron cycle reactions including those relating to ferric iron. Ferrous ions can react with nitrate to produce iron(III) oxyhydroxides such as lepidocrocite and goethite (Rivett et al., 2008) (Equation 2-31).

**Equation 2-31: Ferrous ion reaction with nitrate**

$$10\text{Fe}^{2+} + 2\text{NO}_3^- + 14\text{H}_2\text{O} \leftrightarrow 10\text{FeOOH} + \text{N}_2 + 18\text{H}^+$$
Lepidocrocite is reported to stimulate further ferrous iron oxidation by nitrate (Sorensen and Thorling, 1991). Nitrate is also reportedly able to react with ferric ions to produce magnetite (Fe₃O₄) plus substantial amounts of protons (Equation 2-32). Magnetite is an Fe(II)-Fe(III) composite of wüstite (FeO) and hematite (Fe₂O₃) (Rivett et al., 2008).

\[
15\text{Fe}^{3+} + 2\text{NO}_3^- + 13\text{H}_2\text{O} \rightarrow 5\text{Fe}_3\text{O}_4 + \text{N}_2 + 28\text{H}^+
\]

2.8 Concluding Remarks

After decades-long contention it is now reasonably well accepted that aerobic pyrite oxidation occurs through chemical oxidation of the sulfide moiety producing a series of intermediate sulfur-species to sulfate. Apart from molecular oxygen, another primary oxidation pathway uses Fe³⁺ ions to oxidise the sulfide moiety of the iron disulfide. Microorganisms help to sustain this pyrite oxidation by regenerating Fe³⁺ ions through Fe²⁺ oxidation. Although there is considerable support for discounting direct microbial action in pyrite dissolution, the view here is that some previous quite specific experimental results are difficult to explain by any other mechanism than this.

Accepting that ferric ions are one of the main oxidants of pyrite, oxidation may only continue if the supply of Fe³⁺ is maintained. There is substantial support for mechanisms whereby ferric ions are regenerated chemically (i.e. oxidised) from ferrous ions by oxygen and manganese(IV), and biochemically by nitrate. The ferrous ions in this instance are generally assumed to be the residual component resulting from initial oxidation of the pyrite sulfide moiety. The primary pyrite oxidation pathway thus takes place using soluble Fe³⁺ ions. Although the critical point for ferric ion solubility has been widely reported as being around pH \( \leq 3.6 \), there is some indication that Fe³⁺ becomes increasing available from pH 7.

Ferric iron also forms part of the numerous iron oxides normally present in oxidising ASS systems and this Fe(III) reportedly occurs in crystalline, amorphous and complexed forms in these soils. The assumption here is that, as the soil becomes more acid, ferric ions could be expected to increasingly solubilise from iron oxide sources within the sediment. As iron oxides are reported to be the product of pyrite oxidation under certain conditions, it can be postulated that situations may arise where pyrite oxidation could become (at least partially) self-sustaining in a localised virtually-closed acidic geochemical ‘system’. This suggests that ferric ions could start becoming available much earlier in the pyrite oxidation cycle than previously understood.
Much of the pyrite investigation to date has only considered aerobic oxidation, with far fewer experiments being carried out under anaerobic conditions. Many of these were carried out with non-soil pyritic samples or samples from specific environments such as deep marine sediments and often under varying pH conditions ranging from circum-neutral to slightly alkaline.

It is evident that nitrate is a mobile biogeochemically active substance in both aerobic and anaerobic soils and saturated sediment systems. Although mediated by other soil substance concentration, due to its high Gibbs free energy value, nitrate is often in a position of being the electrochemically preferred electron acceptor in sub-oxic and anoxic environments. This reportedly holds even in the presence of ferric iron but possibly less so in the presence of high concentrations of Fe$^{3+}$ ions. Nitrate reportedly also has the capability to chemically and biochemically redox couple with a broad range of naturally occurring materials in soil and sediment systems, including: reduced iron, reduced monosulfides species, reduced manganese and organic matter. Despite substantial experimental support in the literature for direct and indirect nitrate coupled pyrite oxidation mechanisms, other rigorous experiment work appear to contradict these affirmative findings. Whilst there is uncertainty around whether nitrate oxidises pyrite directly, there is considerable evidence showing that it readily oxidises the intermediate sulfur species resulting from pyrite oxidation. These sulfur species are elemental sulfur, thiosulfate, tetrathionate, and sulfite.

Thus, from the literature it is clear there are a number of unresolved issues with respect to nitrate coupled pyrite oxidation. These include:

- Whether nitrate reduction is directly coupled to pyrite oxidation and, if so, by what reaction pathway(s)
- The type of system within which the transformation occur. It is suggested here that a simple laboratory experiment with relatively few chemical reactants cannot begin to replicate the heterogeneity of natural soil systems. A chemical experiment may assist demonstration of a specific reaction occurring but the view here is that much caution needs to be applied when translating these results to natural systems
- The acidity in the investigated system. Because soil pH has such a dominant influence on microbial species present; on biogeochemical functioning; on solubility of reactants and products; and on what reactions potentially may or may not occur, then soil solution pH must be an important experimental consideration
- The presence or absence of organic and inorganic catalysts or complexes. Reports indicate that both microorganisms and common soil substances may enable, accelerate, or act as electron transfer intermediaries in many typical ASS reactions
• The potential for cyclic oxidation-reduction processes to occur in natural systems, particularly those with a redoxcline zone. Here, biogeochemical cycling at the oxic-anoxic interface may occur in a potentially self-sustaining manner.

• The potential for multiple biogeochemical reactions and transformations to be occurring, not just sequentially, but simultaneously in micro-environments that may provide relatively dissimilar environmental conditions.

This review of literature provides the background information required to examine nitrogen transformation and changes in agricultural soils. Given the interdisciplinary nature of this study the review has been wide ranging but this was considered necessary due to the complexities inherent in a study involving agricultural operations in oxidising acid sulfate soils.

In keeping with the stated study aim, the intention of the following chapters is to examine the potential for nitrate to increase the rate of pyrite oxidation in the study soils. It will do this aided by field site description, study soils characterisation and evaluation of selected soil properties potentially related to and impacting on nitrate reduction and pyrite oxidation. Subsequent chapters examine soil nitrogen changes within a sugarcane block over a crop cycle, and investigate the potential for nitrate reduction coupled to pyrite oxidation in the context of laboratory incubation experiments using study site soil samples.
3 Field Site Description

3.1 Overview

This chapter describes the study region and details the location as well as examining regional climate, geology, geomorphology, soils and land-use history of the region. The primary purpose of the current study was to evaluate specific biogeochemical interactions between soil pyrite and fertiliser derived nitrate. To minimise the effects of extraneous variables it was decided to conduct the field study at one field site location. This field site was chosen primarily for the consistency of its soil horizons and the uniform nature of the acid sulfate soil profile as well as its history of long term nitrogen fertiliser use in cane production.
3.2 Site Location

The study site is located on a cane farm at McLeods Creek, approximately 18 km by road north-east of Murwillumbah in the lower Tweed River valley, NSW, Australia (Latitude: 28° 19' S, Longitude: 153° 26' E, Elevation 0.5m AHD). This is a sub-tropical zone with normal rainfall in excess of 1500 mm per year.

3.3 Geology and Geomorphology

The Tweed field-study region lies on the eastern extremity of the Clarence-Moreton Basin, a very large structural and sedimentary basin upon which many other structures have been imposed. This basin is believed to have developed as a result of late Jurassic – Triassic (210-145 Myr) trough subsidence along a north - south axis running through the present-day towns of Grafton, Casino and Kyogle and on into southern Queensland (Packham, 1969). The basin is also believed to be part of a more extensive Tasman Depression that covered much of present-day central Queensland (Graham, 2001). Fluctuating environmental conditions and intermittent slow subsidence with complementary uplift of the adjoining highlands provided the setting for the subsequent deposition of sand and mud sediments into the shallow embayment during this Mesozoic era (Packham, 1969).

Infilling of the Clarence-Morton Basin laid down the bedrock underlying the contemporary regional landscape. This bedrock comprises thick-beded conglomerates and sandstones along with thinner beds of siltstone and claystone. This region then underwent around 120 million years of erosion before the next major building processes with the Miocene volcanics beginning 24 million years ago (Wells and O’Brien, 1994, Graham, 2001). With the Australian continental plate moving north from Gondwana, the whole region was moving over a volcanic hot-spot that created volcanic activity in a long arc down the east-coast from mid-north Queensland to Victoria in the south. Whilst there were many outflows in the Tweed region, the dominating influence on the current field site was that of the Tweed Shield volcano ca. 20 Myr BP (Wells and O’Brien, 1994).

The Mt Warning Central Volcanic Complex comprised a massive cone shield of fine-grained surface basalts overlying subsurface gabbros, related to basalts, formed by slower cooling. These are now exposed due to massive erosion since their formation. Graham (2001) postulates that the apparent high rainfall in the region promoted the rapid cutting back of the Oxley and Tweed rivers into the central zone around the crater.

This allowed the river system to capture more and more of the water running off the sloping shield, leading to even faster erosion of the river valley into the bedrock, particularly the adjacent continental shelf exposed during the final glacial periods during the Pleistocene.
Since the last glacial maximum around 19,000 years ago the ocean surface has risen approximately 120 m to present day levels (Osmond et al., 1965). There was a relatively rapid sea-level rise of approximately 10 mm per year during the global warming phase that ended this glaciations but this rise slowed around 7,000 years ago and the rise continued to the present-day level. Indications are that sea-level rise stabilised at present levels around 2,000 years ago (Schubel et al., 1971, Church et al., 2001). However, a comprehensive review by Morelock (Morelock et al., 2005), suggested that a possible sequence of high sea-level scenarios occurred whereby sea-levels had risen from 2 to 6 m above present levels beginning around 130,000 years ago.

Post-glacial sea-level rise initiated ‘drowning’ of the previously cut-down river valleys. The geomorphology of some river valleys predicated their infilling with varying combinations of fluvial and marine sediments. The Tweed River valley in northern NSW Australia is a typical estuarine floodplain where infill consolidation of the river valley is believed to have resulted from both tidal and fluvial processes gradually depositing the fine-grained material that also contains significant levels of pyrite (Scheibner, 1998).

Several factors mediated the geomorphological development of the estuary floodplain (Figure 3.2). The extent of erosion occurring in the Tweed catchment during the Holocene and Pleistocene led to infilling of the estuary in a reasonably low-energy tidally-dominated environment constrained and protected by sand barriers (Roy, 1984). It also supported coastal progradation. Graham (2001) conjects that this low energy environment was supported by the then contemporary headlands formed by Terranora ridge to the north-west of Stotts Island in the Tweed River (see Figure 3.2) and the Duranbar – Eviron ridge to the south-east of the river (Graham, 2001). Spurs to the centre of the valley from these ridges would have further supported the creation of many quieter backwater tidal environments. The Pleistocene dune extending north-east from the end of the Duranbar – Eviron ridge provided even more protection for the estuary and appears to have served as the foundation for bar formation and further prograding processes which created Fingal Head peninsula and supported the infilling of the Tweed Broadwater created by the north arm cutting and Fingal Head peninsula.

Although the early geomorphology indicates river valley infilling or drowning in a relatively open estuary, the maturation of the Tweed valley geomorphology would appear to be shaped by forces and processes associated with a barrier estuary (Schubel et al., 1971, Roy, 1984). These could provide numerous bars, spits and protected sand flats in Tweed Broadwater mouth of the estuary leading to the river and tidal flows being braided and de-energised. Van Oploo (2000) carried out extensive transects across the McLeods Creek locale, evaluating from the findings that:
Excluding the levee soils of the Stotts Channel transect where an AASS is absent, both AASS and PASS layers occur throughout the site and occur at a predictable elevation, 0.35 m to -0.7 m AHD, reflecting either uniform deposition in a relatively flat, low energy environment, such as a shallow estuarine lake and/or a common control on the oxidation of the PASS (van Oploo, 2000).

![Map of the Tweed River valley estuarine floodplain](image)

*Figure 3-2: Study region - Tweed River valley estuarine floodplain*

The physical environment supported the laying down of fine-grained tidal and fluvially-transported clays. The composition of this fine-grained material would have been mediated by ocean storms and river flooding events laying down larger grained material in thin bands evident in some regional soil profiles today. Although the fine clay material at the field site is generally remarkably uniform to depths greater than 20m in places (van Oploo, 2000), many areas at the margins of the old estuary including local back-swamp sites have bands of other material such as sand and shells interspersed with this marine clay (van Oploo, 2000, Reilly, 2003b). Present-day soil profiles containing a greater proportion of fine sand and other materials, seen in the upper McLeods Creek locale (van Oploo, 2000) and on the other northwest side of the current river course at North Tumbulgum (Donner, 2001), could have resulted from intermittent higher energy tidal and fluvial forces during storm events.
Fluvial forces dominating in the latter stages of estuarine floodplain evolution led to river flooding events shaping the infilled estuary through creation of levee banks and deposition of alluvium over the pyritic marine clays. These alluvial deposits, readily observed over most of the valley today, grade gently down away from the river (van Oploo, 2000).

Low deposition rates of flood overbank material in back-swamp zones created low lying vegetated swampy areas away from the relatively higher natural levees associated with the main channels. Conditions were thus suitable for the laying down of limnic or sedimentary peat deposits (peaty loams) (Smith, 2001) which today characterise many low lying areas in the Tweed catchment.

Fluvial sediments are probably of diverse origin as the river has incised Palaeozoic metasediments and igneous rocks ringing the edge of the Clarence-Moreton Basin (Packham, 1969), but in the upper reaches the river is eroding Jurassic sandstone/mudstone sediments interspersed with coal measures which are overlain by predominantly basic Tertiary volcanics of the Lamington and Mt Warning groups (Wells and O'Brien, 1994) which appear to have contributed much to the extensive agricultural areas along the Tweed River today.

3.4 Land-use History

Although Australia is thought to have been settled by people at least 45 000 years ago (Fryer-Smith, 2002) it is unclear when humans may have settled in the Tweed River region. There is some evidence of indigenous occupation of the northern NSW / southern Qld region from at least 6,000 years ago (Graham, 2001). Graham speculates that this lack of human habitation evidence prior to 6 000 years ago may only indicate that these early settlers lived close to the coast which was 30 to 40 kilometres further east for most of the last 25 000 years due to the sea-level being up to 120 m lower than at present. Ocean levels are thought to have reached their present height between 6,000 and 3,000 years ago at the end of the last glacial maximum (Yokoyama et al., 2000).

After the explorer Matthew Flinders’ visits to Moreton Bay (Qld) in 1799 (Graham, 2001), the Northern Rivers region was visited by Europeans in the early 1820s and was settled several decades later (NSW-Sugar, 1998). Early farming was mixed, with a focus on grazing, dairying and banana production, but from the mid 1860s sugarcane was increasingly grown on a commercial scale in the Tweed valley. Initially cane was grown on hill slopes to minimise the damaging effects of intermittent frosts, but the fertile soil along with the opportunities presented by the rapidly expanding cane milling capacity in the region in the 1870s soon led to increased use of river flat land for sugarcane growing (NSW-Sugar, 1998). As the Tweed River valley is relatively small and physiographically confined, this demand for land promoted by greater cane mill capacity has resulted in a gradual shift from remaining mixed farming and grazing, to sugar production (Graham, 2001).
Chapter 3: Field Site Description

Sugarcane cropping is now well established, highly mechanised and very efficient in the region, providing substantial social and economic benefits to the area. The regional sugarcane management body, NSW Sugar Milling Cooperative, has proactively worked with all stakeholders to manage some of the significant environmental issues arising from intensive agriculture on acid sulfate soils (NSW-Sugar, 2000b, NSW-Sugar, 2000a).

3.4.1 Sugarcane Cultivation

At the study site, sugarcane is cultivated on one or two year cycles under a plant and ratoon system (i.e. plant regrowth from the harvested cane-stools). Plant cane-blocks are prepared by a plough-out of the old cane stools followed by cultivation and reforming of the mounded rows ready for planting. In addition to the cane ‘setts’ planted in the row, a Plant-mix combined fertiliser is often added to aid effective striking and growth of the new cane. Around six weeks after planting, a heavy side-dress application of up to 200 kg N ha$^{-1}$ of urea nitrogenous fertiliser is added along the cane rows (Weier, 1994, Quirk, 2006).

Sugarcane plants grown intensively on these floodplain soils have a complex three-way root system including rope roots that may extend beyond 2 m in depth. Despite these deep roots, 85% of cane roots reportedly occur in a ball shaped mass in the upper 0.6 m of soil (Smith et al., 2005). Cane roots, along with most other plants, are very sensitive to soil oxygen restriction (Banath and Monteith, 1966), highlighting the importance of maintaining soil structure in these intensively cultivated and machinery tracked soils. When the deep sugarcane rope-roots die, they decompose and mineralise (van Oploo, 2000) to potentially form channels that increase soil aeration and water infiltration. It must also be assumed that this also provides for increased exchange of chemically active substances between soil horizons, particularly under the influence of dynamic watertable movement.

Whilst there are still differing views as to historical groundwater relations, the current groundwater has been shown by White (1993) and Wilson (1995; 1999) to be highly variable, declining to 1.5 m depth under sugarcane during dry periods, but then responding rapidly to rainfall to rise to the near surface after intense storms. Groundwater under sugarcane cropping is affected by cane crop evapotranspiration, leading to substantial groundwater decline in dry conditions (White et al., 1993, Wilson, 1995);

(i) Soil amendment

Sugarcane soils are subject to various inputs, including fertilisers, lime, herbicides and pesticides. While some herbicides and pesticides are targeted specifically, many others are reported to more generally affect soil biological functioning (Weckert, 2004), including that of bacteria and fungi. Lime is used to reduce and correct acidity in soils and can assist in developing soil structure through increases in organic matter and soil biota (Brady and Weil, 2001).
When hydrolysed, the urea fertiliser mainly used on sugarcane (Weier, 1994) produces ammonia, ammonium ions and bicarbonate ions, stoichiometrically shown in Equation 3-1:

\[
\text{CO(NH}_2\text{)}_2 + 2\text{H}_2\text{O} \leftrightarrow \text{NH}_3 + \text{NH}_4^+ + \text{HCO}_3^- \]

Soil amendment with fertilisers not only adds plant nutrients, but also substantial quantities of active and reactive chemical agents creating breakdown products that are known to significantly affect soil biogeochemical functioning. Bicarbonate has an alkalinising effect on soil (McNab and Jerie, 1989), but this is potentially neutralised by the acidifying effects of nitrate creation. The presence of bicarbonate has been linked to deficiencies and toxicity in some plants due to limiting plant calcium and increasing plant phosphorus – the linked to trace-metal deficiencies like iron (Miller, 1960, Brown, 1960). High ammonium bicarbonate concentrations have also been linked to poor seedling growth in many plant species (Brown, 1960) and this salt is often potentially available very close to sugarcane plant roots within 6-8 weeks after planting.

3.5 Hydrology and Drainage

The Tweed River has a relatively small drainage basin area of 1 032 km$^2$, with a floodplain area of 117 km$^2$ (Lin et al., 1998); (White, 2001). Although the main river channel itself is relatively stable, the Tweed River floodplain hydrology has been highly modified over the past 150 years. Along with flood mitigation works such as river-levee augmentation, over 250 one-way floodgates have been installed in the Tweed catchment. Today there is an extensive network of drainage channels across the region including cane-block field drains and mole drains (Tulau, 1999).

Within the McLeods Creek catchment, there are over 100 km of drains that ultimately flow into the Tweed River most via McLeods Creek which has been widened and straightened to create a primary drainage channel (Wilson, 1995). Some field drains flow directly into the creek. A network of larger lateral drains collects most of the flow from field drains and directs this into the main channel. Drainage outlet controls are in place over much of this catchment to maintain drain and groundwater levels (Wilson, 1995, Kinsela and Melville, 2004a). A large freshwater drain to the south and east of the current study field site intercepts water-flow from the adjacent hills and channels this water to the lower reaches of the creek before it enters the Tweed River (Wilson, 1995).
Chapter 3: Field Site Description

The lower Tweed River estuarine floodplain generally and the McLeods Creek field site specifically, sits effectively at 0 m Australian Height Datum (AHD), which is the datum for mean sea-level. This indicates the groundwater has nowhere to flow to and regionally would be expected to stay reasonably level. However the work of White et al. (1993) and Wilson (1995; Wilson et al., 1999) both show the effect of artificial drainage level control in mediating natural groundwater heights in adjacent cropping areas. Nearby higher water level drains may create a potential gradient extending tens of metres horizontally down to a lower ground water table.

Cane blocks on sugarcane farms are now predominantly graded using laser-level guided machinery, allowing floodwaters to quickly run off into the intensive drainage system (Quirk, 2001) assisted by one-way floodgates. Rapid removal of floodwaters from cropping areas is assisted by the use of pumps at these floodgates on the headland drains (Wilson, 1995). The study site soils have been extensively studied but field sampling reveals that two soil horizons, with four distinct characteristics, appear in the profile. These are described in the next section.

3.6 Soils Description

Sugarcane topsoils in the Tweed study region are predominantly Quaternary gravels, sands, silt and clay deposited on a broad flat floodplain through hill slope colluvial and riverine fluvial processes (Wells and O’Brien, 1994). Soil substrates in some areas of the valley have an acid sulfate character and appear to have been laid down under estuarine conditions in the last 10 000 years (Dent, 1986a, Scheibner, 1998).

Sampled soils generally have fine textures, with loams, silty loams and clays predominating. Topsoils tended to be dark coloured with generally a sharp boundary to the underlying acid sulfate clay subsoil. An acid to strong acid soil trend is clearly evident in the top 0.8 m of this duplex profile, but changes to more neutral conditions below 1.3 m depth. The soils are classified as Sulfidic or Sulfuric Hydrosols under the Australian Soil Classification System (Isbell, 2002). Back-swamp soils known locally as ‘peats’ approach an Organosol classification but due to the acid reaction trend and the presence of sulfidic clay in the subsoils, these are classified here as Sulfidic Hydrosols (Isbell, 2002).

Cane soils in the McLeods Creek catchment appear to have a reasonably consistent profile in having an alluvial topsoil horizon, a sulfuric actual acid sulfate soil (AASS) B1 subsoil horizon and a potential acid sulfate soil (PASS) B2 subsoil horizon below this across much of the local floodplain. Van Oploo (2000) reported results of several long transects in north-south and east-west directions across the catchment, showing that only on the levees close to the main Tweed river channel is the AASS horizon absent. Both AASS and PASS horizons occur at relatively low elevations of -0.35 m to -0.7 m AHD respectively, reflecting either uniform deposition in a
relatively flat low energy environment such as a shallow estuarine lake and/or a common control on the oxidation of the PASS (van Oploo, 2000).

Comprehensive investigation of soil profile cores supports the view that originally only two horizons were present: (i) a deep lower layer of unoxidised pyritic gel-clay and (ii) river alluvium (Wilson, 1995, van Oploo, 2000). However, Kinsela and Melville challenge the two horizon postulate, suggesting instead that the current upper AASS horizon may have been laid down in a low-energy lacustrine environment influenced predominantly by fluvial forces and terrestrial materials. Under this scenario, this horizon would then include less pyritic material and proportionally greater terrestrial sediments (Kinsela and Melville, 2004a).

Contemporary soil profiles at the field study site comprise four horizons (Figure 3-3 and Table 3-1). The A layer of loamy-clay alluvial topsoil is of medium texture and appears to be relatively permeable to water. Underlying this is oxidised pyritic clay in two quite different physicochemical states: (i) fully oxidised acid sulfate soil zone (B$_1$ horizon) of medium permeability due to the disturbance by soil chemical transformation and root incursion, and (ii) a transition zone of partially oxidised sulfidic B$_2$ soil that is reported (e.g. White et al., 2005) to be relatively impermeable to water flow. Underlying these horizons is the unoxidised potential acid sulfate soil (B$_3$ horizon) that at the study field site contains up to 5 % pyrite. Soil hydraulic conductivity (designated by $K$ in centimetres per second) is one widely accepted indicator of water flow through porous material. Published figures indicate that hydraulic conductivity of the fine-grained pyritic gel-clay here could be as low as $10^{-8}$ cm/s, or nearly one cm/day (Fetter, 2001).

![Figure 3-3: Representative soil horizons at the field-study site](image)

Table 3-1: Soil Profile overview - field-study site

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Depth (m)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0 – 0.3 varies</td>
<td>River alluvium topsoil</td>
</tr>
<tr>
<td>B$_1$</td>
<td>0.3 – 0.7</td>
<td>Fully oxidised (sulfuric) acid sulfate soil</td>
</tr>
<tr>
<td>B$_2$</td>
<td>0.7 – 1.4</td>
<td>Transition zone of partly oxidised (sulfuric) acid sulfate soil</td>
</tr>
<tr>
<td>B$_3$</td>
<td>1.4 -</td>
<td>Unoxidised (sulfidic) acid sulfate soil</td>
</tr>
</tbody>
</table>
Selected soil properties are presented in chapter 4 and comprehensive soil profile descriptions are detailed in Appendix 12.

3.7 Concluding Remarks

The study site sits is situated on an estuarine floodplain at a height close to sea level. Its climate is subtropical and it normally receives rainfall of around 1 500 mm per year allowing it to support continuous sugarcane cropping. This field site was chosen for the uniformity of its ASS profile and the characteristic nature of its partly oxidised soil profile. Soil characterisation, description carried out during this study confirmed this to be duplex acid sulfate soil with a shallow alluvial clay-loam topsoil overlying deep partially-oxidised pyritic gel-clay of estuarine origin. The field site soil is not complicated by sand, shell, or peat bands throughout its profile. Consequently this soil could be considered to be an ‘archetype’ for acid sulfate soils with all the classic ASS characteristics manifesting in the top 1.5 m of the profile. As such, it presents as an ideal field site for this study.
4 Selected Soil Properties

4.1 Soil Acidity and Oxidation-Reduction Potential

Despite previous extensive soil geochemical characterisation work in this locale by van Oploo, (van Oploo, 2000) many of the measured soil parameters are highly dynamic and specific to time of sampling and particular sampling location. Hence, there was a need to measure and monitor selected soil properties at the study site.

The central thesis of the work in this and the two following chapters is that soil physical, biogeochemical and hydrological properties largely determine the movement and transformation of nitrogen fertilisers and their products in these soils. This chapter focuses on some primary soil properties that can assist in evaluating these processes: (i) acidity (pH) and oxidation-reduction potential (ORP), and (ii) soil organic carbon (SOC). These soil property investigations are presented in the following two sections, each containing its own aims, method, results and discussion.

4.2 Soil Acidity

4.2.1 Aims and Objectives

Soil acidity (pH) and ORP (Eh mV) trends down the profile impact on and reflect many soil transformations, particularly some associated with nitrate reduction. The aim was to evaluate these characteristics at the study site and understand how they affect soil processes associated with nitrate coupled pyrite oxidation. The objective was to measure pH and redox potential in the nitrogen trial (N-trial) cane-block profile at the beginning, middle and end of the cane cycle, corresponding to September 2006, January 2007 and September 2007 respectively.

4.2.2 Methods

The soil profile on Plot 15 (Mid-N treatment: 10.7 kg N/ha) was sampled and tested initially at 0.1 m intervals to 1.5 m depth and, on the two later occasions, at selected intervals to 1.8 m depth. Replicate soil samples were taken from three locations 10 m apart along a single row in the centre of grower R. Quirk’s cane-block 704. Soil samples were extracted with a bucket auger to 0.8 m, then a tapered gouge auger down to 1.8 m depth. Soil pH and ORP measurements were carried out immediately at the time of soil sampling in a 1:1 ratio soil:water paste (Thomas, 1996). A minimum of three replicate measurements were carried out on each sampling occasion. Standard errors (s.e.) were calculated from the formula: s.e. = standard deviation / square root of number of measurements. Significant differences were tested at the 95% confidence interval level using the statistical t-test.
A Horiba D-54 multifunction meter (Horiba, 2006b) with separate pH, ORP and temperature electrodes measured soil acidity, oxidation-reduction potential and soil temperature. The pH electrode was an Ionode IJ44 double junction glass spear-point electrode (Ionode, 2007) and was calibrated before each daily use using buffered pH standards of pH 4.0 and 7.0. The redox probe was an Ionode IJ64 Ag/AgCl double junction platinum tip electrode (Ionode, 2007). This electrode was regularly checked in ORP calibration solution at 400 mV. Soil ORP readings were adjusted to Eh values by adding 210 mV as per manufacturer’s instructions.

4.2.3 Results and Discussion

4.2.3.a Soil acidity

Results of soil profile replicated pH and Eh measurement on three occasions over a 12 month cane crop cycle are shown (Figure 4-1 and 4-2). Error bars show ± standard error (s.e.) (n=3). Tabulated pH and Eh results are given in Appendix 10.

![Figure 4-1: pH trends in the N-trial cane-block soil profile (bars = ± s.e.)](image-url)
Results for soil acidity (Figure 4-1) show pH varies substantially down the profile to 1.7 m depth, with high acidity in the upper horizons grading to a more neutral pH state at depth. Differences between sampling periods were evident and three aspects are noted.

Firstly, maximum acidity in the profile appears in the 0.8 to 1.0 m zone. From upper profile values of pH 4.0 to 4.6 at 0.1 m depth the pH declines to lows of pH 3.2 to 3.7 at 0.8 to 1.0 m depth. It then increases uniformly to higher pH values between 6.0 and 6.8 at 1.7 m depth in the PASS zone. Thus the topsoil is generally acid, the mid-zone at 0.9 m is very acid and the lower horizon at 1.7 m more neutral to slightly acid.

Secondly, between the three soil-testing events, soil pH in the upper, middle and lower parts of the tested soil profile changed in different ways (Table 4-1). The negative sign and figures in red indicate pH decline while figures in black denote a pH increase between the two sampling dates.

<table>
<thead>
<tr>
<th>Soil sampling data</th>
<th>pH ± s.e. (0.1 m depth)</th>
<th>pH ± s.e. (0.9 m depth)</th>
<th>pH ± s.e. (1.7 m depth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sep. 2006 to Jan. 2007</td>
<td>- 0.44 ± 0.14</td>
<td>+ 0.26 ± 0.32</td>
<td>+ 0.77 ± 0.07</td>
</tr>
<tr>
<td>Jan. 2007 to Sep. 2007</td>
<td>+ 0.15 ± 0.12</td>
<td>- 0.55 ± 0.24</td>
<td>- 0.42 ± 0.15</td>
</tr>
</tbody>
</table>

During the first period (Sep. 2006 to Jan. 2007) the 0.1 m topsoil pH decreased in contrast to a pH increase in the 0.9 and 1.7 m soil zones. This was reversed in the second period between Jan. 2007 and Sep. 2007, when the 0.1 m depth topsoil became slightly less acidic and the 0.9 and 1.7 m depth horizons became relatively more acid. Over the 12 months cane crop cycle the 0.1 m topsoil pH decreased by 0.29 pH units in contrast to soil at 0.9 m depth which increased by 0.29 pH units. At 1.7 m depth the PASS soil pH increased by 0.35 pH units.

Thirdly, over the full year cane crop cycle the soil profile pH minimum shifts to a lower point in the soil profile, moving from 0.8 m (pH 3.21) to 1.0 m (pH 3.16).

4.2.3.b Soil redox potential (Eh)

Redox potential also varies markedly down the soil profile (Figure 4-2). Although there were differences between the three datasets, generally these differences were not significant because substantial standard errors resulted in overlap between results for the three periods.

Results showed the cane soil to have aerobic properties in the upper soil profile to 0.8 m but more anaerobic around 1.5 m depth. Measured Eh values ranged between 600 to 660 mV in the upper profile from 0.1 to 0.8 m depth before declining to 150 - 237 mV in the 1.5 to 1.7 m depth zone. A non-significant slightly raised Eh potential is indicated around 0.4 m depth relative to that at 0.1 m. The pronounced decline in redox potential begins between 0.6 and 0.8 m depth in the soil.
Between sampling events substantial Eh differences were evident at 1.5 and 1.7 m depth. The 1.5 m depth soil experienced a significant Eh decline of 88 mV [$t(4) = 4.67, p < .001$] from September 2006 to January 2007. Soil Eh at this depth then rose significantly by 83 mV [$t(4) = 4.109, p < .01$] in the next period January to September 2007. During this same period, the pH rose significantly by 0.40 pH points [$t(4) = 1.733, p < .1$]. It is noted that acidity pH is generally negatively correlated with redox potential Eh in these data.

Results indicate a typical scenario of pyrite induced acidity in the upper part of the study soil profile with a pH minimum around 0.9 m depth in the AASS transition zone. This is associated with relatively high oxidation-reduction Eh potentials in the upper part of the measured profile, indicative of oxic conditions. Below approximately one metre depth, soil pH rapidly moves higher towards pH neutrality whilst the ORP Eh readings indicate increasing anoxia at depth.

Ongoing acid creation is a factor in soils containing significant quantities of pyrite. Other researchers (White et al., 1993, White et al., 1997b, Lin et al., 1998, Johnston et al., 2004, Kinsela and Melville, 2004a) have investigated pathways of acidity loss from sugarcane ASS, highlighting evapotranspiration as the primary driver of acid generation in ASS through watertable lowering, which allows air entry into the upper part of the sulfidic horizon. Soil
reaction will generally reflect the actual acidity rather than the potential acidity in the surrounding soil and, in the case of ASS, this varies by soil buffering capacity.

Under low inflow conditions, ASS oxidation products in the 0.7 to 1.1 m zone may move upward via groundwater capillarity and evapotranspiration forces. It may then be stored in the non-sulfidic AASS horizon in iron and aluminium sulfate compound forms such as jarosite. The creation and dissolution of these oxidation products and their temporary storage in the jarosite layer can impact significantly on acid outflow. The stored acidity may be moved naturally into drains, but possibly in limited amounts due to the slow kinetics of jarosite transformations (Lin et al., 1998).

During prolonged rain events heavy enough to induce substantial groundwater rise, the actual acidity may be carried away via the mole/field drain system or by overland flow along the graded cane-block surface to headland drains. Although acidity varied over the cane cropping cycle and the cane-block was subject to some high rainfall events, the study soil pH did not change significantly over the logged period. This indicates that a moderate acid balance exists within this soil system. It is notable that no major pH or Eh trends or shifts occurred in the soil profile despite the below-average recorded rainfall during the field N-trial period. There is an increased risk of greater pyrite oxidation during periods of relatively low rainfall when the watertable may decline into the PASS gel-clay zone.

However, one minor shift was the observed Sept. 2007 minimum of pH 3.16 at 1.0 m in the profile. This pH minimum is 0.2 m lower in the profile than for the same month the previous year. This could be interpreted as a deepening of the pyrite oxidation front over the course of the 2006-07 cane crop growing cycle on this block or could simply reflect soil heterogeneity differences in different sampling locations.

Platinum electrodes used for ORP measurements may not always accurately reflect the redox potential of specific transformations in soils but they can usefully indicate the general oxic status of the soil if it is close to electrochemical equilibrium. These electrodes can also indicate shifts in soil biochemical transformations and functioning as shown by rapid changes in measured pH and Eh values below the redoxcline around 1.0 m depth. The most obvious outcome of this soil testing is to highlight this 0.8 to 1.0 m depth soil profile zone as being the most chemically active in this ASS profile. This soil testing also confirms the 0.8 to 1.4 m depth zone as being the AASS transition zone where pyrite is still oxidising across a 0.6 m deep soil horizon front.
4.3 Soil Organic Carbon

4.3.1 Aims and Objectives

As soil organic carbon (SOC) plays a significant part in soil physical and biochemical processes, the aim here is to evaluate organic matter in association with nitrate transformation and cycling in the study soils. The objective was to sample these soils down to 1.7 m and analyse them for soil organic carbon (SOC).

4.3.2 Methods

(i) Field sampling and sample preparation

In September 2007 replicate field site soil samples were collected at 0.1 m intervals from hand augured holes to 1.7 m in the study cane-block. Soil was stored moist in evacuated resealable plastic bags and kept initially in a cooled container in the field. It was then transferred to refrigerated cabinets at around 4°C until analysed in a laboratory within seven days. Soil samples were prepared for SOC determination according to guidelines in Nelson and Sommers (Nelson and Sommers, 1996) with the exception that the samples were dried relatively quickly in a fan-assisted oven-drier at 45°C for 48 hrs to minimise subsoil pyrite oxidation. On drying the soil was rough crushed to pass a 5 mm sieve, then mixed thoroughly and sub-sampled to provide a portion for laboratory analysis. These approximately 100 g sub-samples were ground in a mortar and pestle to pass a 2 mm sieve. A 25 g subsample of each was hand ground to pass a 0.42 mm sieve. This was stored in air-tight numbered glass containers. Soil organic carbon was analysed in triplicate for selected depths in the soil profile.

(ii) Laboratory analysis

The laboratory method is derived from a wet oxidation procedure originally developed by Walkley and Black (1934) and later adapted and modified by many researchers including Walkley (Walkley, 1947) and Heanes (Heanes, 1984). The underlying method is that of Walkley (1947) but it also uses the Heanes method of heating the sample to 135°C before setting aside for cooling. A mixture of potassium dichromate and concentrated sulfuric acid is heated to oxidise organic matter to CO₂ (Equation 4.1) (Rayment and Higginson, 1992).

**Equation 4-1: Potassium dichromate reaction for SOC determination**

\[ 2 \text{Cr}_2\text{O}_7^{2-} + 3\text{C} + 16\text{H}^+ \rightarrow 4 \text{Cr}^{3+} + 8\text{H}_2\text{O} + 3\text{CO}_2 \]

This is a colourmetric procedure using back-titration with ferrous sulfate solution in conjunction with Ferroin indicator to determine residual dichromate. Organic carbon was determined by calculation. The full procedure and tabulated results are given in Appendix 1.
4.3.3 Results and Discussion

Mean SOC results here show relatively high soil carbon levels in the upper horizons, declining to lower values in the subsoil but with a mid-zone showing markedly depleted SOC values (Figure 4-3). Error bars show standard error of the mean (n=3). From a high of over 6% SOC in the topsoil alluvium, SOC decreases rapidly from 6% (at 0.3 m) to 1% at 0.5 m depth. From this point it increases to a subsoil maximum of 3.3% at 1.2 m and then maintains approximately 2.9% SOC to 1.8 m depth.

Soil organic carbon in the ASS profile reflects previous research findings by van Oploo (2000) in showing relatively high soil carbon levels in the upper horizons, declining to relatively lower levels in the acid sulfate soil subsoil. Of note here are: (i) the general low SOC values in the acidic AASS zone from 0.5 to 1.0 m soil depth, and (ii) the unexpectedly higher SOC concentrations in the subsoil below 1.0 m depth.

The study site is in a back-swamp locale in the Tweed estuarine floodplain and as such is likely to have peaty topsoils typical of these areas. This is confirmed in van Oploo’s study (2000) in this same McLeods Creek catchment. Thus, the high SOC content of the topsoil is typical of these former back-swamp areas. These ASS subsoil sediments would have been formed in low energy organic-rich backwater areas of the estuary where organic matter in excess of pyrite formation requirements could easily become embedded in the sedimentary profile (van Oploo 2000; Graham 2001).

The apparent depleted SOC zone between 0.4 and 1.2 m in the profile is less amenable to explanation. While the slope of the SOC line above and below this zone invites reconstruction of a postulated ‘original’ SOC profile, shown completed by the dashed blue line in Figure 4-3 [Postulated SOC (1)], other evidence discounts this explanation. The primary reason rests on the fact that this is a duplex soil profile, with transported river alluvium sitting on top of fine-grained estuarine clay sediment.
Given the two different origins and modes of formation, the view here is that these two soil horizons are likely to have quite different SOC concentrations. Flood overbank material is known to have relatively high SOC values (i.e. 2.8 to 3.8 % SOC at less than 0.3 km from the main channel (Reilly, 2001). In the several thousand years since the sea-level last stabilised, it is reasonable to assume that this topsoil could have become even more enriched by decaying back-swamp vegetation. The clay subsoil was reportedly laid down in low energy brackish backwater conditions. Therefore it is suggested that the topsoil and ASS originally had different SOC contents with the alluvial topsoil being > 6 % and the acid sulfate subsoil in the range 2.5 to 3.5 %. This is indicated by the second darker broken line in Figure 4-3.

Whilst this explanation is reasonable, it is also inferred and does not effectively answer queries about the relatively lower SOC levels between 0.4 and 1.0 m depth in the study soil profile. Three explanations for this are proffered here. The first is that the measured soil carbon could simply reflect the condition as originally laid down under estuarine conditions. However, there is evidence that sea-level rise slowed around 7500 years (-2 m) BP, so it is reasonable to assume that this would encourage the laying down of greater quantities of sedimentary material including organic matter, thereby enhancing SOM rather than diminishing it.

The second relates to the sulfuric acid generated through pyrite oxidation and the impact of this in oxidising the soil organic matter. Sulfuric acid is an oxidising acid and readily acts on organic matter to reduce it (Arruda, 2007). Thus, over time and with continuous acid production, the organic matter would be diminished in the pyrite oxidising zone. An interesting aspect of the data results occurs around the 0.8 m profile depth where SOC values begin to increase again from the local lowest value of 0.6 – 0.8 %. Results of soil mineralogy investigation in a later section (5.2) shows this to be the depth at which pyrite begins to appear in the soil profile and can therefore assumed to be close to the redoxcline, the main oxidation front. Soil acidity measurements support this by showing lowest pH in the cane-block soil between 0.8 and 1.0 m.

The third explanation pertains to the role of organic matter as an electron donor in soil oxidation-reduction reactions. Organic matter is oxidised mainly to carbon dioxide in these processes. Whilst organic matter oxidation is a natural process, it is suggested that this SOC depletion could also be exacerbated by other electron acceptors such as nitrate. Nitrogenous fertilisers have been applied to these soils for up to 70 years (Reilly 2001) which is arguably a long enough period to create an effect such as this.

### 4.4 Concluding Comments

Soil evaluation in this chapter provides more detailed information about soil pH, Eh, and soil carbon that can inform interpretation of field and laboratory work. Acidity mediates many biocatalysed soil reactions and information about soil redox potential informs interpretation of
the oxic/anoxic status of the soil profile. As soil carbon is a highly preferred electron donor in soil systems, information gained here will assist evaluation of study soil redox biogeochemical processes.
Chapter 5: Mineralogy and Clay Characterisation

5 Soil Mineralogy and Clay Characterisation

5.1 Introduction

The nature and properties of soil largely supports and mediates the biogeochemical processes that occur and the rate at which these proceed. Soil matrix mineralogy provides many of the substrates for geochemical cycle transformations. Soil constituents such as clays and amorphous material may store and enable exchange of soil cations and anions. These processes are also supported or constrained by the soil physical structure including the size and assembly of its pore spaces and soil particles. Given the nominal 0.2 to 2 µm diameter and 1 to 10 µm length size range of microorganisms (Stanier et al. 1977), microbial activity must necessarily be confined to those parts of the soil profile where the habitat is suitable. This is not only to physically accommodate the cells, but also to allow diffusion of air, water and substrates for microbial assimilation and dissimilation. Therefore it was deemed important that these cane-block ASS properties be examined here.

This chapter presents results of these investigations in three sections: (i) cane soil mineralogy, (ii) clay type, and (iii) PASS gel-clay microstructure. It presents aims and objectives, methods and a combined results and discussion for each section, then provides a brief summary of chapter outcomes.

5.2 Cane-block Soil Mineralogy

As soil minerals provide reactants for soil chemical reactions as well as nutrient and dissimilatory substrates for microbial growth and respiration, the aim here was to investigate the mineral status of the study site cane soil and assess its properties as an aid to evaluating soil N-species processes. The specific objective was to quantify cane soil-minerals, including pyrite, at selected depths using X-Ray Diffraction (XRD) techniques. The primary focus of this investigation was the redoxcline at 0.8 to 1.0 m depth and the chosen sampling depths reflect this focus.

5.2.1 Methods

Field site cane-block soil samples previously collected were used for mineralogy analysis. As the AASS transition and PASS zones were of most interest here, soil samples from depths of 0.6m, 0.9m, 1.1m and 1.7m in the profile were prepared for XRD analysis. Soil samples of around 50 g were oven dried at 45 °C for 24 hours to minimise pyrite degradation which is possible at higher temperatures. Initial crushing and grinding in a hand mortar and pestle allowed the sample to first pass a 420 µm sieve. Two gram subsamples were then milled with acetone in an agate mortar to 2 µm size and allowed to dry.
Chapter 5: Mineralogy and Clay Characterisation

The prepared powder was loaded into a cavity-type side loading sample holder in such a way as to produce a well packed specimen whilst minimising preferred orientation. Mineral analysis was carried out with a Siemens D501 Diffractometer. These results were interpreted using the Bruker AXS software package Diffracplus Eva 10 (2003) for identification and Siroquant V3 software for quantification. This diffractometer was equipped with a graphite monochromator and scintillation detector using CuKα radiation. To assist quantification of amorphous material, 20 % wt /wt corundum (1 μm Al₂O₃, Baikowski) was added to each sample and thoroughly mixed. Samples were suspended on a side-packed sample holder and scanned at 2 to 70° 2-theta using a step width of 0.02° and a scan speed of 1° per minute.

5.2.2 Results and Discussion

Cane-block subsoil mineralogy comprised five main groupings of minerals: clays, quartz, plagioclase, amorphous material and pyrite (Table 5-1 and Figure 5-1). XRD results show soil mineral proportions generally in the order:

\[
\text{Quartz} = \text{Clays} > \text{Amorphous} > \text{Plagioclase} > \text{Pyrite}
\]

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Pyrite</th>
<th>Quartz</th>
<th>Plagioclase</th>
<th>Amorphous</th>
<th>Clay</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>0</td>
<td>33.6</td>
<td>13.2</td>
<td>12.5</td>
<td>40.7</td>
</tr>
<tr>
<td>0.9</td>
<td>1.3</td>
<td>36.7</td>
<td>16.3</td>
<td>11.4</td>
<td>34.2</td>
</tr>
<tr>
<td>1.0</td>
<td>1.5</td>
<td>39.2</td>
<td>12.9</td>
<td>16.1</td>
<td>30.3</td>
</tr>
<tr>
<td>1.7</td>
<td>4.9</td>
<td>22.9</td>
<td>5.5</td>
<td>31.1</td>
<td>35.6</td>
</tr>
</tbody>
</table>

Pyrite concentration increased in a linear fashion from zero at 0.6 m to 4.9 % at 1.7 m depth. The amount of quartz in the cane-block subsoil increased from 34 % (0.6 m) in the upper ASS profile to 39 % at 0.9 m before declining to approximately 23 % at 1.7 m depth. Plagioclase concentration increased briefly from 13 % (0.6 m) to 16 % (0.9 m) before declining to 5.5 % at depth. Apart from a small decline between 0.6 m and 0.9 m depth, amorphous material in the oven-dried soil sample increased with depth from 12 % to comprise just over 30 % at 1.7 m. Clay content mirrored the amorphous material by declining initially from 41 % (0.6 m) to 30 % (0.9 m) and then increasing to 36 % at 1.7m.

Figure 5-1: Cane-block soil XRD analysis
Chapter 5: Mineralogy and Clay Characterisation

The field site was specifically chosen for the uniformity of its soil horizons and properties and this is reflected in these results. Although there is reported variation in soil properties across the Tweed River region, the initial observation was that this study soil represents a simple ‘classic’ acid sulfate soil profile. This has alluvial loam topsoil overlaying fully oxidised then partially-oxidised sulfuric medium-clay horizons. Underlying this is a deep sulfidic horizon containing unoxidised pyritic gel-clay.

Results for soil pyrite clearly show the AASS-PASS transition zone beginning between 0.6 and 0.8 m depth. Assuming uniform pyrite concentration increase with depth, interpolation of the above sulfide-S results estimates a pyrite concentration of 3.9 % at 1.5 m depth. This equates to around 20.6 mg sulfide-S g⁻¹ OD soil. As shown in Section 8.5, the mean sulfide-S content in PASS gel-clay from this same 1.5 m depth in the profile was found to be 20.44 ± 0.23 (s.e.) mg sulfide-S g⁻¹ OD soil (n=10). These two sets of results therefore validate each other.

Estuarine sediments are likely to contain quartz (sand) material. Although the sand in this sample cannot be detected by touch, it makes up one of the larger mineral components in this clay subsoil at 23 to 39 % of the OD material. Given its apparent small size it could readily be transported by both tidal and fluvial processes in the developing estuary. Gel-clay micro-morphology examination (Section 5.4.3) confirmed the particle size of this soil as being in the colloid, clay and silt size range. The reduced quartz content of 23 % at 1.7 m cannot be explained directly here. However, Galan et al. (1999) found increased proportions of quartz in natural mineral samples treated with acid sulfate water from sulfidic mine spoil runoff. Untreated similar samples showed lower quartz content (Galan et al., 1999). Another possible explanation for the different quartz content in the profile is variations in sediment composition during infilling of the estuary.

Plagioclase is also present in 5 to 16 % concentrations. This is a sodium calcium aluminium silicate weathering material likely to have been fluvially transported from the upper catchment during its major erosive phases. Amorphous material is also present in significant quantities with the least amount (11 – 12 %) in the upper 0.6 to 0.9 m zone, rising to 31 % at 1.7 m depth. The Bruker Diffracplus Eva software used here evaluates amorphous content as any non-crystalline organic and inorganic material in the soil. This includes various oxides, hydroxides and oxyhydroxides of metals including iron, diatomaceous material, volcanic glass (Lal, 2006), organic matter including humic matter, inert carbon or char material and mineralised organic matter (Saint-Germaines et al., 2002). Within the sulfuric horizon, to around 1.0 m depth amorphous material is approximately 50 % lower relative to that in the PASS zone, again indicating potential impacts of AASS chemical properties on diminishment of this non-crystalline material.

Given the proximity of the older Mt Warning volcanic complex as well as the developmental
history of Tweed estuary, it is very likely that amorphous materials such as volcanic glass debris and silicon-based diatomaceous matter could be present in quantity in these floodplain sedimentary soils. The Mt Warning complex volcanic activity preceded the Tweed River valley infill by close to 20 million years. Colloidal coal material could also be present as the Tweed River and its tributaries are also cutting into coal measures in the catchment headwaters (Wells and O'Brien, 1994, Graham, 2001).

Clays form the other large component of these soils, ranging from 30 to 40% on an OD basis between 0.6 and 1.7 m depth. Clays comprise many different types and these are investigated further in the following section.

5.3 Cane-block Clay Characterisation

5.3.1 Aims and Objectives

Although extensive soil profile analysis was done previously in this study locale by at least two other researchers, Wilson (Wilson, 1995) and van Oploo (van Oploo, 2000), it was considered appropriate and necessary here to carry out clay type analysis to examine specific subsoil mineralogy at this study field site. The aim here was to determine constituent clay type within the sugarcane block AASS-PASS transition profile (i.e. 0.6 to 1.7 m) in order to better evaluate soil biogeochemical properties and processes and aid interpretation of clay micromorphology.

5.3.2 Methods

Finely ground subsoil samples prepared for the XRD soil mineralogy analysis described in the previous section were used here for clay-type analysis. However it was first necessary to separate out the clay from the total soil sample. Clay separation was performed by aqueous settling according to the Millipore Filter Transfer Method (Moore and Reynolds, 1997).

Finely ground soil was added to a measuring cylinder filled with distilled water and a small amount of dispersant and agitated for 15-20 seconds with a sonic probe. The suspension was then allowed to settle for a predetermined period before a <2 μm subsample of the upper measuring cylinder suspension was withdrawn with a syringe and filtered under vacuum through a 0.45 μm membrane. When all water had been removed, the membrane was removed from the filtering apparatus and the clay film transferred to a glass plate and allowed to dry.

The resulting <2 μm clay fractions were analysed on a Siemens D501 Diffractometer in four treatments: (i) Mg-saturation (scan range 2-42° 2θ, step width 0.02°, scan speed 1° per minute); (ii) saturation with ethylene glycol (2-32° 2θ, 0.02°, 1° per minute); (iii) heating to 350 °C (2-28° 2θ, 0.02°, 1° per minute); and (iv) heating to 550 °C (2-28° 2θ, 0.02°, 1° per minute).
Results were interpreted using the Bruker AXS software package Diffracplus Eva 10 (2003) for identification and Siroquant V3 for quantification using the bulk scan. Results are presented in the following sub-section with full XRD output available in Appendix 8.

5.3.3 Results and Discussion

X-ray diffraction analysis of clay fractions provided site-specific mineralogy information for the study soils (Figure 5-2 and Table 5-2). Three main clay types were shown to be present and clay proportions in the soil profile generally varied in the order: smectite > kaolinite > illite.

Several aspects of these results are noted. The first relates to significant changes in clay concentration in the soil profile. This is indicated in this data at 1.0 m depth but the true turning point could be masked by the fact that no other XRD clay analysis was carried out between 1.0 and 1.7m. Clay concentration transitions all appear around 1.0 m depth, a feature also observed in the mineralogy results. The other aspects of note here are the high smectite content of the study soils along with the relatively low illite proportions, particularly at depth.

![Figure 5-2: Cane-block soil clay type](image)

Table 5-2: XRD Cane-block soil clay analysis at selected depths

<table>
<thead>
<tr>
<th>Depth</th>
<th>Kaolinite</th>
<th>Smectite</th>
<th>Illite</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>8.0</td>
<td>27.9</td>
<td>4.8</td>
</tr>
<tr>
<td>0.9</td>
<td>12.6</td>
<td>17.7</td>
<td>3.9</td>
</tr>
<tr>
<td>1.1</td>
<td>16.7</td>
<td>12.9</td>
<td>0.7</td>
</tr>
<tr>
<td>1.7</td>
<td>13.9</td>
<td>21.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Of the total 30 to 42% clay in the study soils, smectite (a 2:1 lattice expanding clay) makes up the greater proportion in varying from 28% (0.6 m) to 13% (1.0 m) before increasing somewhat at depth to 21.7% (1.7 m). Kaolinite content generally increases with depth from 8 to 14%, but has a local maximum of nearly 17% at 1.0 m depth. Illite declines from nearly 5% at 0.6 m to 0% at depth but with a very sharp decline between 0.9 and 1.1 m depth.

Jackson et al. described a weathering sequence of clay-sized mineral particles in soils (Jackson et al., 1948). They postulate that the dominance of particular minerals within a sequence give indications as to the stage of weathering of the composite material.
The mineral weathering stages they presented follow the order:

quartz → illite → mica-intermediates → montmorillonite → kaolinite

Beyond the kaolinite weathering stage, Jackson et al. described mineral product stages of iron oxide development such as gibbsite and hematite including goethite and limonite. Thus, the results here could be interpreted as an intermediate weathering progression from illite to montmorillonite (i.e. smectite) to kaolinite, with the illite in decline and the kaolinite content rising with increased weathering in the upper soil profile. This does not fully explain the clay content variation with depth, but geochemical and other factors may help to explain this.

The variable clay-type content down the current tested profile could result from differences in the original deposited sediment as this was built up over geological time. It is easy to conject that changes in source material over time could lead to differences in clay content in this soil profile. However, these observed differences may also result, at least partly, from geochemical changes in the clay itself under the influence of, say, soil acids. A study by Galen et al. (1999) testing the effects of acid mine drainage water on clay sediments found that the clay composition changed markedly after treatment. Two size fractions were measured in the Galen et al. (1999) study: (i) \(<63 \mu m\), and (ii) \(<2 \mu m\). After the acid sulfate solution treatment, illite content increased from 67 \% to 76 \% in the \(<63 \mu m\) fraction, and from 22 \% to 74 \% in the \(<2 \mu m\) group. Kaolinite content increased from 21 \% to 24 \% and from 8 \% to 26 \% in the larger and smaller size fractions respectively. Smectite content fell from 12 \% to zero and from 70 \% to zero, respectively (Galan et al., 1999). The indication from the Galen work was that ASS acid sulfate products can significantly impact on clay concentrations in ASS through increasing the illite and kaolinite content and diminishing the smectite clay content.

Whilst the Galen et al. (1999) work does not fully explain the results here, it does go some way to explain the observed data at the redoxcline around 1.0 m depth in the study soil where sulfuric acid is being created at the oxidation front. Illite and kaolinite levels are raised and smectite content diminishes, thus supporting the view that clay content and transformations are perhaps not fixed in these soils. Galen et al. (1999) also observed release of clay octahedral-occupied metal ions: Fe, Al and Mg, as well as tetrahedral Si and Al upon the breakdown of smectite in the treated samples, thereby increasing the concentration of these ions in the aqueous solution. This could explain a source and raised levels of metal ions at similar depths in these subsoils reported by van Oploo (2000).

One of the most striking aspects of these current findings is the apparent geochemical control exercised by the AASS-PASS interface zone at 1.0 m soil depth. All three identified clay types here showed substantial shifts in concentration between 0.9 and 1.1 m profile depth, reiterating the apparent critical biogeochemical nature of the redoxcline – the oxic-anoxic boundary in these soils.
5.4 Gel-clay Micromorphology

In the early 1990s acid sulfate soils in Australia began to be more widely recognised as a significant environmental issue (Melville et al., 1991). Intensive research work was initiated by four main research institutions: the CSIRO, University of NSW, Australian National University and Southern Cross University. It aimed to characterise these reactive soils and to understand the environmental impacts of natural and perturbed biogeochemical processes within them. This work was significantly aided by the pre-existing and emerging body of ASS research from Europe, particularly in The Netherlands, led by researchers such as Pons (Pons, 1972), van Bremen (van Breemen, 1972), Nordstrom (Nordstrom, 1982b) and including others elsewhere such as Lowson (Lowson, 1982).

From the review of literature presented earlier in chapter 2, it is evident that the Australian research effort has been very productive, but in 2003 it was also obvious that some ASS properties were still not well understood. Despite the significant investigative work of van Oploo (2000) on soils in the same locale as the study field site, knowledge gaps still existed with respect to the structure of the clay gel and to specific mineralogy of gel-clays at the study site. In particular, the microstructure of the gel-clay had not yet been described. Thus the intention of the work here was to investigate this property of the ASS gel-clay and this was done using scanning electron microscopy (SEM).

5.4.1 Aims and Objective

The principal aim of this section was to characterise PASS gel-clay micromorphology to better understand its potential role in mediating biogeochemical reactions including pyrite oxidation. Objectives included:

- To develop methodologies suitable for representative imaging of the moist pyritic gel-clay micro-structure
- To examine and characterise the size, shape and micro-structural formation of the PASS clay micelles

One of the challenges with this new work was the identification of the 'true' microstructure (as opposed to artefact structures) of the gel-clay in a context where the true clay structure formation was not known.

5.4.2 Equipment and Methods

The apparatus and equipment used in this part of the current study was available in the ANU Scanning Electron Microscopy facility which included facilities for cryogenic work.
Apart from the main SEM instrument, associated apparatus included: sample holders, electrically conducting cryo-adhesive and cryogen vessels suitable for liquid nitrogen handling. The specification of the SEM equipment was as follows:

**Cambridge S360 SEM,** 1987, general purpose SE and BSE imaging, cryoSEM and X-ray analysis.
- Lanthium Hexaboride cathode,
- Tracor Northern EDXA detector (Be window, 147eV) with Moran EDXA processing system
- 4-segment backscatter detector
- Oxford CT1500B Cryotrans cold stage / sputter coating unit
- Point-to-point measurement capability intrinsic to microscope
- Turbomolecular and sputter ion pumps
- 70 mm ("220") roll film
- videographic printer, and
- ImageSlave 1024 x 768 slow-scan image acquisition.

**Methods**

The procedure involved adhesively mounting the moist gel-clay soil sample on a stub, then submitting this to rapid freezing, shearing, sublimation etching and gold sputter-coating. The final stage was examination of the exposed surface using SEM imaging.

A relatively undisturbed and unoxidised moist gel-clay sub-sample (<0.5 g) from 1.8m depth was attached to an SEM stub holder using a 50:50 mixture of Tissue-Tek adhesive and colloidal graphite. Supercooled nitrogen liquid-ice slush (at around -210 °C) was created by boiling off some of the liquid under vacuum for several minutes in a polystyrene cup. In this procedure, heat of vaporisation is extracted from the liquid thereby cooling it from the standard liquid nitrogen boiling point of approximately -196 °C to around -210 °C (Echlin, 1992, Heady, 2003). This half-frozen nitrogen mixture was then used to rapidly cool the small clay sample at a rate fast enough to minimise the formation of large ice crystals, thus minimising distortion of and damage to its natural-state micelle formation.

The sample was then quickly transferred to the SEM cold stage which was then rapidly brought down to operating vacuum (5 x 10⁻⁷ Torr) and temperature (approximately -175 °C or 98 K). Following shearing (by breaking off part of the protruding frozen soil section to obtain a fresh unperturbed observation face) the sample was moved into the SEM main chamber for sublimation etching down to a depth of 10-15μm. This latter procedure was achieved by raising the main chamber temperature to around -85 °C (188 K) for 4 to 7 minutes to remove interstitial water directly as water vapour. The sample was viewed and monitored at a low beam voltage of 2 kV during this procedure.
When sufficient etching had occurred, the sample was transferred to the cold stage for sputter coating with gold after re-cooling to around -150 °C. It was then moved back to the main chamber for imaging and analysis. Beam voltage varied between 2 and 15 kV for this.

5.4.3 Results and Discussion

Continued iterative refinement of the sublimation etching technique involving the creation of many snap-frozen PASS samples resulted in images of progressively greater structural clarity. In addition, repeated sample preparation allowed the development of experience in recognising artefacts within the sample. It appeared that most structural artefacts arose from poor initial freezing and sublimation techniques. This progressive development of the procedure is shown in a sequence of SEM images, shown in Figure 5-3 through to Figure 5-7.

Figure 5-3 shows a high SEM magnification image of the frozen gel-clay with microcrystalline ice formation. The top bar is 2 μm in length. No large crystals are evident, nor is abnormal crystal growth evident in the image.

When this snap-frozen sample is warmed slightly under vacuum, water is removed as water vapour by sublimation. Figure 5-4 shows the SEM sample with surface ice removed but with solid water still below the surface. To see detail of the clay particles, this subsurface water has to be removed by sublimation deep-etching to a depth of around 16 to 20 μm.

Figure 5-5 displays evidence that surface and subsurface water has been removed to sufficient depth to reveal the relatively undisturbed open structure of the gel-clay.
Chapter 5: Mineralogy and Clay Characterisation

A high-magnification image of the fully etched gel-clay shows the fine pore-space open structure of this material (Figure 5-6). From the 2 μm measurement bar at the top of the image it is clear these spaces are generally of the order of <3 μm and that the matrix material varies from sub-micron up to 5 μm in size. Also obvious in the image are the different arrangement of the clay platelets. Some of these are in laminar association and others approaching an edge to face card-house arrangement. Rather than being formed from uniform material, Figure 5-6 and other backscatter electron mode analysis (not shown here) shows evidence of different mineralogy within the gel-clay matrix.

The final image here (Figure 5-7) shows a brackish water diatom observed on one of the sample surfaces after fracture. This provides further evidence of the estuarine origin of this clay material. Of note here are the clay micelles lying on the diatom shell confirming not only the size of this material but also the laminar characteristics of these thin clay platelets.

Development of any new procedure inevitably involves much investigation and, in this instance, the evaluation of many SEM images. As highlighted above, changing the procedure gives different outcomes that are often challenging to interpret in a context where the ‘true’ microstructure of the gel-clay is not known.

However, some specific albeit indirect information about these clays may be used to inform interpretation in this instance. The in situ PASS gel-clays typically have porosities of around 50-60 % with gravimetric water contents up to 120 % OD soil weight (van Oploo, 2000, Reilly, 2008). This high water content property therefore gives clues as to how the gel-clay microstructure could appear in imaging. Clay ‘card-house’ structure, alluded to by van Olphen (Van Olphen, 1951), described by Ingles (Ingles 1968, cited in (Bennet and Hulbert, 1986) and poorly imaged in sedimentary clays by Dent (1986) thus provides an appropriate starting point for this gel-clay micelle structure evaluation.

Van Olphen (1951) attributed the rigid structures created in suspensions of montmorillonite clay micelles as resulting from electrical attraction between oppositely charged flat and lateral
surfaces of the micelle plates. Charges on clay particles may be pH dependent, but in acid conditions the lattice layer surface is normally negatively charged as the exchangeable cations are the counter ions of the electric double layer. Conversely, the edges of the platelets where the aluminium hydrosilicate layers are disrupted are positively charged (Van Olphen, 1951), possibly due to the displacement of inter-lattice monovalent cations by the trivalent aluminium cation (Hillel, 1998). It is this edge-to-face electrical charge attraction that supports the open ‘card-house’ structure seen in Figure 5-6, but other platelet associations are also evident. Kaolinite clay has a different lattice structure that does not generate disparate edge and face charges. This means that kaolinite micelles tend to laminate into parallel layers. Some of these laminar structures are evident in Figure 5-6 as would be expected from the mix of clays found in the study cane soil. The view here is that these tightly-packed kaolinite laminar components could potentially impact on soil water flow, dispersion and diffusion properties, but no testing for this was carried out in this study.

Confirmation of the estuarine origin of these subsoils comes from discovery of brackish-freshwater zone specific diatoms in the PASS gel-clay samples. These were identified as *Diploneis* sp. These diatoms have been observed in marine-brackish and freshwater epipelon communities in supra-tidal and intertidal areas incorporating sub-tidal shallow marine basins, back levee marshes and intertidal sandy shoals (Vos and de Wolf, 1988, Vos and Wolf, 1993). The McLeods Creek study site fits the brackish back-levee marsh habitat description. It has a protected position next to the adjacent headland projecting out almost to the present-day river channel from the coastal highlands to the east.

*Chapter Summary*

Analysed pyrite concentrations in the soil profile confirmed earlier research indications of a pyrite free upper-AASS zone with a transition AASS zone where pyrite levels increased from 0 to 4.9 % between 0.7 m and 1.7 m. These findings highlight the three-level geochemical structure of this estuarine clay sedimentary material. It is clearly divided into (i) a pyrite-free oxidised AASS zone from 0.3 to 0.7 m depth, (ii) a partly oxidised AASS transition-zone of increasing pyrite concentration from 0.7 to 1.4 m depth, and (iii) a zone of unoxidised potential ASS subsoil extending below 1.4 m.

The cane-block subsoil also contained a substantial proportion of amorphous material. Whilst many different materials comprise the amorphous component in soils, the non- or poorly-crystalline iron oxide group could potentially impact on pyrite oxidation in these soils. Under acid conditions, these iron oxides may provide a ready supply of soluble ferric irons. Finding smectite clays was also of interest here for two reasons. The first relates to fixed and variable charges these clays may acquire which supports edge-to-face micelle structure formation. The second issue has to do with the role of soil mineral salt solutions in
maintaining the card-house microstructure found here in the study subsoil gel-clay. Any soil conditions likely to diminish the surface to edge charge difference of these smectite clays is also likely to induce collapse of this structure. Changing the ionic strength of the soil solution is one way this structure collapse may occur (White et al., 2005).

Despite many scans of multiple gel-clay samples, only one instance of framoidal pyrite form was found in the PASS gel-clay samples. This suggests that the predominant pyrite form is micron-sized euhedral particles evenly distributed throughout the gel-clay material. This information is useful in other parts of this study, particularly the experimental work section involving analysis of the sulfide content of the PASS pyritic gel-clay.

5.5 Concluding Comments

The combined results provide selective information on the physical, mineral and chemical properties of the study soil that can be applied in the later work evaluating the redox chemistry of nitrate coupled pyrite oxidation in the study soil. Of particular note here is information about pyrite concentrations in the cane-block soil including its postulated single particle form; the gel-clay microstructure detail including confirmation of gel-clay porosity; the high content of smectite clay; and the high amorphous material content.
Chapter 6: Thermal and Hydrological Properties

6 Thermal and Hydrological Properties

6.1 Air and Soil Temperature

Soil temperature data has not been reported for these regional soils. As soil temperature and its daily and seasonal variation directly affects soil biochemical reaction kinetics it is important that soil temperature data be collected at the field site. Soil temperature was a focus for Australian researchers in the 1950s and 1960s in an attempt to understand the interactions of temperature on many soil and crop growth processes (Bristow, 1988, Watson, 1980, West, 1952, Rose, 1968). Soil profile temperature is reported to be one of the key factors in root growth (McMichael and Quisenberry, 1993), but this may be species and variety dependent. In reviewing soil environment impacts on root growth, McMichael and Quisenberry (1993) found that the optimum temperature for root growth ranged from around 15 to 30 °C for common crop plants including legumes. Biological reactions and transformations in soil are also closely correlated with temperature. This also affects urea hydrolysis which is reported to have an activity peak between 35 and 45 °C after increasing linearly from 5 to 35 °C (Hamid et al., 2004).

6.1.1 Aims and Objective

The primary aim here was to investigate soil temperature as one of the selected factors in cane-block soil ecology and biochemical reaction kinetics. As winter air temperatures at the field site can at times fall below zero, the specific objective was to evaluate soil temperature in the cane-block profile over the period of the second nitrogen field trial through use of embedded self-contained temperature loggers to evaluate the impact of these low (and high summer) air temperatures within the cane-block soil profile. This data would be compared with air temperature recorded at the field site over same period. Whilst it could be assumed from air temperature records that these soil profile to depth remains mesophilic, the lack of available data confirming this prompted the collection of this information in this study.

6.1.2 Methods

The field sampling design incorporated use of self contained waterproof, miniature temperature loggers (iButton temperature loggers [(Maxim, 2008)]) installed at depths of 0.3 m, 0.8 m and 1.3 m into the undisturbed cane-block profile. These loggers are approximately 16 mm diameter and 5 mm thick, and have a service life of 10 years. The soil temperature loggers were fitted to the end of purpose-made 19 mm outside diameter polyvinyl chloride (PVC) tubes for installation into 18 mm diameter augered holes in the profile. These installation tubes were insulated and sealed at both ends, the base seal being a 50 mm long O-ring sealed nylon plug sitting just clear of the metal logger case. Installation involved inserting the installation tube to
the full depth of the hole to ensure the recorder sensor was in close contact with the soil. Tubes were installed into a compacted mounded section of the cane row to minimise water ingress during rain events.

When set up to record temperature at 60 minute intervals, the loggers can record data for around 147 days with an accuracy of ± 0.5 °C. Data downloading involved withdrawing the rod carefully from its hole, cleaning the iButton logger with a cloth, attaching a 1-Wire clip (Maxim, 2008) to the logger base and downloading the data via a USB adapter to a notebook computer. The temperature logger assembly was then reinserted into the soil as before and soil tamped around the tube to seal it.

An additional iButton temperature logger recording air temperature was fitted to the underside of the field site rain-gauge logger installed as part of this study. This tipping-bucket rainfall recorder was fitted onto a 3 metre high by 10 cm diameter PVC pipe within the cane-block and close to the soil-temperature loggers. Although this air temperature logger was not installed within a standard meteorological cabinet it was installed so as to be continually shaded but still exposed to free air flow and well away from any heat absorbing/emitting material. Data downloading was accomplished as previously described for the soil temperature loggers, the exception being that the 1-Wire clip could be attached in situ with no requirement to remove the logger for data downloading. Of the four loggers installed, the 30 cm recorder failed during the first logging period and was replaced with an upgraded version measuring to ± 0.125 °C accuracy on a subsequent site visit.

Loggers were installed at the end of January 2007 and removed prior to cane harvesting on 17th September 2007. The 30cm soil temperature logger failed in the first four weeks of the logging period. Due to the infrequent field trip schedule as well as the difficulty in obtaining a replacement, this logger was not replaced until June 2007. Air and soil temperature was logged at 2-hourly intervals over the field site investigation period. Each of the iButton loggers was checked for accuracy and precision before use and re-checked at the end of the field study. This latter procedure involved all four loggers being set up together in a controlled temperature environment and allowed to equilibrate. Static temperatures were compared with a pre-calibrated laboratory thermometer. All loggers showed ≤ 0.5 °C error at this calibration check.

Manual downloading of temperature data from each recorder occurred approximately every three months in a tab-delimited file format and this file was archived to retain an original copy. Data format conversion was carried within a spreadsheet application (MS Excel, Microsoft Corporation) and graphing and charting was generated in MS Excel (Microsoft Corporation) and SigmaPlot (Systat Software, Inc.) computer applications.

Temperature data comprised approximately 2750 readings for each logger. Non-linear modelling of these temperature data can reveal potential seasonal variation lag-effects evident between dependent and linked parts of the biosphere such as between atmosphere and soil.
Fitted lines were applied to recorded air and 30 cm soil temperature data. The fitted lines were 5th order or higher polynomials.

### 6.1.3 Results and Discussion

Data from all four temperature recorders is plotted in Figure 6-1, with summary results presented in Table 6-1.

<table>
<thead>
<tr>
<th></th>
<th>Mean (°C)</th>
<th>Maximum Temperature (°C)</th>
<th>Minimum Temperature (°C)</th>
<th>Range (Max-Min) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Temperature</td>
<td>17.1</td>
<td>33.0</td>
<td>-3.0</td>
<td>36.0</td>
</tr>
<tr>
<td>Daily Mean Temperature</td>
<td>17.1</td>
<td>27.9</td>
<td>6.4</td>
<td>21.5</td>
</tr>
<tr>
<td>30 cm Soil Temperature</td>
<td>n.d.</td>
<td>n.d.</td>
<td>11.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>80 cm Soil Temperature</td>
<td>20.0</td>
<td>25.0</td>
<td>15.0</td>
<td>10.0</td>
</tr>
<tr>
<td>130 cm Soil Temperature</td>
<td>20.9</td>
<td>24.0</td>
<td>17.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

As would be expected, air temperature shows the highest daily variability (up to 27.5 °C) (Reilly, 2007) and the largest range (36 °C in this eight month period) of all the logged datasets. The lowest recorded air temperature at the field site (at 3m height above ground) was -3 °C at 0600 hrs on 18th July 2007 and the highest air temperature of 33 °C in this record occurred on 31st January 2007 at 1200 hrs.

The incomplete 30 cm soil depth dataset displayed the next highest daily variability. Soil profile temperature data from 80 and 130 cm depth loggers showed minimal or no diurnal variation. Seasonal lag effects relative to air temperature were evident in all logged soil data and these were positively correlated with soil depth. Deeper soil horizons respond more slowly to seasonal maximum and minimum mean air temperature (Table 6-2).

<table>
<thead>
<tr>
<th></th>
<th>Air Temperature</th>
<th>Modeled Mean Air Temperature</th>
<th>Modeled 30cm Soil Temperature</th>
<th>80cm Soil Temperature</th>
<th>130cm Soil Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum Temp.</td>
<td>33.0</td>
<td>17.1</td>
<td>n.d.</td>
<td>20.0</td>
<td>20.9</td>
</tr>
<tr>
<td>Date (Max. Temp.)</td>
<td>31 Jan. 07</td>
<td>27 Jan. 07 (BOM)</td>
<td>n.d.</td>
<td>11 Feb. 07</td>
<td>9 Mar. 07</td>
</tr>
<tr>
<td>Lag period (Max. Temp.)</td>
<td>n/a</td>
<td>0 days</td>
<td>n.d.</td>
<td>12 days</td>
<td>38 days</td>
</tr>
<tr>
<td>Min. Temp.</td>
<td>-3.0</td>
<td>11.2</td>
<td>13.0</td>
<td>15.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Date (Min. Temp.)</td>
<td>18 Jul. 07</td>
<td>14 Jul. 07</td>
<td>22 Jul. 07</td>
<td>5 Aug. 07</td>
<td>27 Aug. 07</td>
</tr>
<tr>
<td>Lag period (Min. Temp.)</td>
<td>n/a</td>
<td>0 days</td>
<td>8 days</td>
<td>22 days</td>
<td>44 days</td>
</tr>
</tbody>
</table>
Chapter 6: Thermal and Hydrological Properties

Figure 6-1: Air and soil profile temperature
Fifth order polynomial fitted lines for mean daily air and 30 cm soil temperature data allowed analysis and comparison of temperature maxima and minima at different soil depths. These fitted lines are referred to the *modelled air temperature* and *modelled 30 cm soil temperature* respectively. Modelled air temperature thus better indicates the low frequency seasonal shifts in air temperature that induces similar but delayed soil temperature variation at depth in the soil profile. The range of this seasonal temperature variation diminishes with soil depth and seasonal soil temperature maxima and minima lagged behind those for modelled air temperature at all soil depths logged.

During **summer**, modelled air temperatures peaked around 25 °C in late January 2007 which induced soil temperature maxima of 25.0 °C at 80 cm depth on 11 February 2007 (12 days after the modelled air temperature peak) and 24.0 °C at 130 cm depth on 9 March 2007 (38 days after the modelled air temperature maximum). In **winter**, a modelled air temperature minimum of 11.2 °C occurred on 14 July 2007. This was associated with soil temperature minima of 13.1 °C at 30 cm depth on 22 July 2007 (a lag of 8 days); 15.0 °C at 80 cm depth on 15 August 2007 (a 22 day lag); and 17.0 °C at 130 cm depth on 27 August 2007 (44 days after the modelled air temperature minimum).

Short-term volatility in mean daily air-temperature (the solid black line in Figure 6-1) is very evident in this data-record. An irregular two to ten day pattern in this mean daily air temperature record is evident and is attributed here to cyclic weather pattern influences on air temperature. More regular diurnal temperature variation in the 30 cm soil depth temperature record (Figure 6-2) is associated with solar radiation heating during the day and cooling during the night.

Soil at 30 cm depth tracked daily air temperature variations but with markedly reduced amplitude and a discernible delay of around 8 to 10 hours. Between 12\textsuperscript{th} and 13\textsuperscript{th} July 2007 an air temperature high of 20.5 °C at 1400 hrs invoked a 30 cm depth soil temperature peak of 13.64 °C at 2200 hrs, a lag of 8 hours. An air temperature overnight low of 0.5 °C at 0200 hrs was associated with a 30 cm depth soil temperature minimum of 12.76 °C at 1200 hrs, a delay of 10 hours. Therefore, a daily air temperature variation of 20.0 °C was associated with 30 cm soil temperature fluctuation of 0.88 °C. Over the whole logged period 30 cm soil temperature diurnal fluctuations rarely exceeded 1.0 °C.
Figure 6-2: Air and 30cm soil temperature showing soil temperature diurnal lag

The data record for the deeper soil temperatures loggers at 80 cm and 130 cm show imperceptible response to daily air temperature fluctuations, but these two data loggers have a recording accuracy of 0.5 °C, in contrast to the 30 cm logger’s 0.125 °C accuracy. Therefore the deeper temperature loggers may not be sensitive enough to record these small diurnal variations.

The collected data confirms the mesophilic properties of the study soils. Air temperatures did fall below freezing several times in mid to late winter (July and August), inducing 30 cm soil depth temperature decline below 15°C for these two months. Despite the low air temperatures, soil at 30 cm depth did not fall below 11°C (Table 6-1). It could be expected that mesophilic microorganism activity in the topsoil zone may slow during these two winter months, but only in the surface layers exposed to freezing temperatures is this activity likely to temporarily cease. It is possible that near surface temperatures may in fact not have fallen to freezing due to the mediating effect of the sugarcane plant canopy and the ongoing heat flux from within the soil. Soil surface temperature was not logged in this study.

Two different types of soil fluctuations are evident: (i) the diurnal, driven by the daily temperature rise and fall, and (ii) the seasonal, affected more by mean temperature variation from summer to winter. Both effects lessen with soil depth with the diurnal cycling affected much more by this than the seasonal. Even with limited data for 30 cm depth it is clear that the upper soil layers are more responsive to air temperature variation than the deeper layers.
Whilst air temperature movements clearly drive most soil temperature variations, it may also be inferred from the data that heat flux from the deep soil zones mediates the amplitude and timing of these daily and seasonal fluctuations. Results established that the study soil to 1.3 m depth operates in the mesophilic temperature range for the eight month logged period from mid-summer to the following spring. However, the data also indicated study soil temperatures to be in the lower half of the mesophilic range, from around 4 to 28 °C. Apart from this latter qualification there appears to be no obvious extraordinary temperature conditions present that could adversely or otherwise impact on soil biogeochemical processes in the study soil.

### 6.2 Soil Hydrology

#### 6.2.1 Overview

This section describes the investigation into soil water relations in the field site soil profile. This evaluation included: logging rainfall inputs to the cane-block; evaluation of groundwater movement; monitoring of soil moisture; and evaluation of adjacent drain impacts on cane-block soil water.

Soil moisture is a key mediating factor in multiple biogeochemical processes in soils including those affecting the growth of plants and microorganism functioning (Ponnampерuma, 1972). Plant roots require adequate moisture but also an oxic environment for survival and optimal growth. Saturation in the root-zone rhizosphere invariably creates conditions limiting ingress of oxygen (Ponnampperuma, 1984). Conversely, too little moisture in the root-zone can give rise to plant stress and poor growth, with extreme dryness often resulting in death of the plant.

Moisture is also essential to soil biological activity and is thus a primary factor in the support of major global biogeochemical nutrient cycling. As much of this cycling involves biocatalysed oxidation-reduction reactions, water plays a crucial part in soil substance transformation and is also closely correlated with reaction kinetics (Ponnampemura, 1984, Yadov et al., 1987). In order to evaluate hydrological impacts on study soil biogeochemical processes, three specific parameters were monitored over most of the cane harvest cycle at the field site.

The first of these, rainfall, was chosen to quantify direct inputs into the cane-block hydrological system. Being on the edge of the tropical cyclone belt in northern Australia, the Tweed region is prone to the influence of broad tropical lows which bring pervasive rain to large areas of the Queensland and northern NSW coasts (BOM, 2007). However, rain cells vary greatly in size and intensity (Stow and Dirks, 1998, Chubey et al., 1999) and whilst regional precipitation may often appear to derive from large pervasive low air-pressure systems, some research suggests that the rainfall is mostly associated with localised rain cells less than five kilometres in diameter (Goldhirsh and Musiani, 1986). Weather data from the nearest
government weather station 18 km distant from the field site may therefore not accurately reflect precipitation on the study field site, so a standard rain gauge was installed at the nitrogen field trial location.

The second parameter, soil moisture, allows evaluation of soil profile wetting and drying dynamics including quantification of rainfall wetting fronts and plant moisture loss (i.e. plant transpiration effects). It also allows estimation of water-filled pore space and observation of capillary-rise effects. The chosen moisture logger measured volumetric soil moisture in 20 cm increments to 80 cm depth in the profile. One location in the centre of the study cane-block was monitored. Moisture data was logged continuously at two hourly intervals from late January to mid-September 2007.

The third soil aspect monitored was groundwater height. The ground watertable marks the zone of total saturation in the soil and to a large extent determines soil oxic / anoxic properties. Groundwater height is mediated by water inputs, storage, losses and outflows. Precipitation at the field site is considered to be the dominant soil input although horizontal throughflow could not initially be totally excluded. Groundwater and water-filled pore space (WFPS) in the upper profile are considered to be the main storage mediums in these soils. Evaporation and transpiration losses are postulated to contribute most to soil moisture losses with surface runoff and mole drainage adding most to any outflows. The reported low permeability of the PASS gel-clay and the low lying nature of this landscape with its near sea-level groundwater surface indicate that deep percolation losses are minimal in the study site soil.

This soil hydrology section includes aims and objectives, followed by methods and results sections for each monitored parameter. It concludes with a single integrated discussion of combined results

6.2.2 Aims and Objectives

The overall aim was to investigate hydrological properties of the field site cane-block to: (i) identify and quantify fertiliser-derived nitrate leaching in the cane-block profile, and (ii) evaluate soil biogeochemical conditions. Specific objectives included:

a) Logging of precipitation falling onto the field site cane-block
b) Direct monitoring of ground watertable height
c) Direct evaluation of rainwater infiltration dynamics down to around 1.8 m. in the cane-block soil profile
d) Quantification of the above infiltration rates if this was found to occur
e) Estimation of soil evapotranspiration rates under sugarcane
f) Evaluation of any capillary rise effects in this cane-block soil
6.2.3 Methods and Equipment

Very specific data logging equipment was chosen to collect the above data. This consisted of: (i) a recording rain gauge, (ii) five capacitance water level loggers and (iii) a capacitance soil moisture probe with an integrated data logger measuring volumetric soil moisture. Before installation at the field site, all equipment was calibrated as per manufacturer’s instructions. Water and moisture data-logging equipment was set up on the cane-block as shown in Figure 7-5 in chapter 7. After installation, these sensors were levelled to enable comparison and reporting of all results relative to Australian Height Datum (AHD). The following sub-sections give a brief overview of this equipment.

6.2.3.a Rainfall

The Davis Tipping Bucket Rain Gauge (Dataflow, 2006b) used to measure daily and accumulated rainfall was designed to meet the guidelines of the World Meteorological Organisation. This rain gauge was installed at the top of a 4 m x 0.1 m diameter PVC pipe buried 1 m into the field site cane-block allowing the rainfall logger to sit approximately 3 m above ground. This was well clear of a fully grown 1 year old plant-cane leaf canopy.

Rainfall was recorded in 0.2 mm increments in real time with one second precision for eight months during the major part of the sugarcane crop cycle. Logger internal memory was sufficient to record continuously for around nine months, but in practice data was downloaded every two to three months. External regional data for comparison with the field site readings was obtained from the closest government weather station, Murwillumbah Bray Park (BOM Stn. No. 058158), 17 km away. Long term climate data from 1887 to 1972 was also obtained to allow realistic comparison of current rainfall with long term averages for the region. This data was recorded at the now closed weather station at Condong Cane Mill (BOM Stn. No. 058013), 11 km distant from the field site.

Downloaded logger data was copied and the original file archived. Working data files were imported into a computer spreadsheet (Excel 2007, Microsoft Corporation) in tab-delimited form for processing, plotting and analysis. Where relevant, data was imported into Sigma-Plot software (Systat, 2009) for specific chart plotting.

6.2.3.b Soil moisture content

The installed Data Flow Systems Green Light-Red Light (GL-RL) Soil Moisture Recording System (Dataflow, 2006c) consisted of a 16 mm diameter by 900 mm long glass-fibre rod constructed with four paired ring sensors at 20 cm intervals from the base.
This rod was designed to fit inside a bottom-sealed 19 mm outside diameter and 16.5 mm inside diameter PVC tube inserted into the soil. An 18 mm diameter soil auger was used to bore an 85 cm. deep hole at the desired location at the centre of the cane-block. The 19 mm diameter outer tube was then driven into this hole to resistance and the installation completed by inserting the close-fitting sensor pole and sealing the two parts together to prevent moisture ingress. The installation procedure was designed to eliminate voids around the tube that could induce errors into the sensor readings.

Calibration was initially carried out using the factory supplied calibration constants for field site soil type at each sensor depth of 20, 40, 60 and 80 cm. Three months after installation replicated soil samples were collected at the four sensor depths approximately three metres from the moisture logger. Gravimetric and volumetric soil moisture was then calculated using the methods of Topp (Topp and Ferre, 2002). Results of this allowed adjustment of the moisture logger calibration constants. In practice, minimal re-adjustment was required. Recalibration at this time allowed retrospective adjustment of all previously collected data. Logged soil moisture data was downloaded every two to three months along with rainfall and groundwater height data.

6.2.3.c Groundwater

Capacitance groundwater level probes used here consisted of a 2.5 m long assembly comprising a 0.1 m brass-rod weight attached to a 2.3 m length of Teflon covered wire that was connected to a small sealed tube logger head (Dataflow, 2006a). Probe calibration involved marking the capacitance-probe wire at 0.25 m and 2 m and noting the logger readings after full immersion in water to these two points. These readings were then used to calculate an offset and slope factor for input to the logger. This was repeated for the other four groundwater loggers.

All groundwater height loggers were installed into 2.4 m long open-base piezometer tubes. Preparation of these standard 0.1 m diameter UPVC tubes involved cutting multiple angled slits into the bottom third of the tube to allow groundwater ingress. The tubes were installed to the side of cane-row mounds into tight-fitting 2.3 m deep augured holes. Clay packed around the tube exit minimised surface-water ingress down the side of the tubes. A proprietary reducer fitting holding a 50 mm diameter 0.5 m long riser tube supported the tubular logger head above the ground. The capacitance wire could thus measure water level to 1.8 m depth into the profile as well as recording flooding events up to 0.3 m height above ground.
Two level surveys using an Ashtech ProMark 2 differential GPS surveying system allowed the piezometer tubes to be levelled to better than ± 8 mm accuracy (Magellan, 2009). Calculated reduced levels were later tied into multiple local AHD reference marks previously surveyed by a regional professional survey company. This enabled reporting of watertable reduced-level heights in metres AHD. Downloading and processing of the logger data was carried out in a similar fashion to the other hydraulic data collected here with the difference being that raw groundwater heights were reduced to the AHD level datum.

6.2.4 Results and Discussion

6.2.4.a Rainfall

Long term monthly average rainfall records and 2006-07 rainfall data from the nearest Bureau of Meteorology registered site at Murwillumbah Bray Park (BOM Stn. No. 058158) allowed useful comparison with field site monthly rainfall data. These are shown together in Figure 6-3.

Rainfall during the field-study period differs markedly from historical averages for this locale, not only in total precipitation, but also in its monthly distribution. Historical averages show peak rainfall periods December to March, declining quickly in April and then declining more slowly to a low rainfall period in September. By contrast, the contemporary pattern (as shown by the field site data) is more variable, showing very low rainfall in unconnected months and with no definite peak rainfall period evident.

Rainfall logged at the field site showed reasonable accord with the official Bureau of Meteorology (BOM) rain-gauge at Murwillumbah, approximately 18 km away but in every month but one, (February 2007), rainfall at the field site exceeded the BOM readings.
Over the eight month recording period field site precipitation exceeded the Murwillumbah recorded rainfall by 65.5 mm or nearly 12%. Using BOM Murwillumbah data for missing months from October 2006 to January 2007, the annual rainfall at McLeods Creek was 975 mm, a substantial decline relative to the region long term average of over 1 720 mm per annum (BOM, 2001).

Rainfall recording was carried out continuously for 229 days at the field site with total rainfall of 597.2 mm recorded on 125 separate rainfall days. Daily rainfall at the field site is shown in Figure 6-4. The record has periods of up to 26 days when rainfall did not fall on the field site cane-block.

Ranked distribution plots give a much clearer indication of frequency of daily rainfall events ranked by size. The plot of field site rainfall during the field trial shows the highly skewed nature of the distribution where the majority of daily rainfall was less than one millimetre per day (Figure 6-5). Daily rainfall greater than 15 mm occurred on only 13 separate days out of the 229 days logged (Table 6-3).

<table>
<thead>
<tr>
<th>Rainfall intensity (mm per day)</th>
<th>Percentage of total received rainfall</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (&lt;\ 1\ mm)</td>
<td>60.0%</td>
<td>75 of 125 rainfall days</td>
</tr>
<tr>
<td>1 mm to (&lt;\ 8\ mm)</td>
<td>23.2%</td>
<td>29 of 125 days</td>
</tr>
<tr>
<td>8 mm to (&lt;\ 15\ mm)</td>
<td>6.4%</td>
<td>6 of 125 days</td>
</tr>
<tr>
<td>(\geq\ 15\ mm)</td>
<td>10.4%</td>
<td>13 separate rainfall-days</td>
</tr>
</tbody>
</table>

As daily rainfall statistics in Table 6-3 do not always give a realistic indication of precipitation likely to affect groundwater, the data was analysed to determine total rainfall per event.
A total of 16 rain events in this record, varying from one to five days duration, occurred where 15 mm or greater total precipitation fell on the cane-block. Significant events (here taken to be greater than eight mm) occurred in the record on 19 occasions. Analysis is presented in a later section 6.2.4.c showing relationships between these events and groundwater rise.

From an ecological functioning and agricultural cropping perspective, rainfall is only effective where evaporative and transpiration loss is less than the precipitation gain. BOM pan evaporation data (BOM, 2007) plotted with actual rainfall data at the study site shows substantial deficits over the Field Trial 2 study period (Figure 6-6). Over this logged period, the total moisture deficit was nearly 113 mm.

Pan evaporation is only one measure used to determine the evaporation component of evapotranspiration. In the context of a sugarcane crop with its closed canopy, pan evaporation is possibly not a particularly accurate indicator of soil evaporation. It does however indicate periods when soil moisture deficits are likely to occur. Thus from this data, it is expected that significant groundwater decline would have occurred from February to May 2007 and again in July 2007. These deficits are generally reflected in groundwater logged data (Figure 6-11), shown in section 6.2.4.c.

Current-study rainfall data showed considerable variation from historical averages for this region. Four features are immediately evident from this data. Firstly, rainfall for the field trial period was highly variable, departing significantly from the long term monthly means. Secondly, the total rainfall received at the field site during the logged period (597 mm) was around half that of the historical average (1156 mm) recorded at nearby Condong. Thirdly, the decision to install a study site rain gauge appears justified given the observed differences between the Bureau of Meteorology (BOM) Murwillumbah Bray Park data and the field site readings. In several instances, the BOM Bray Park rainfall varied up to 100% relative to that received at the field site. Lastly, although long term trends may not safely be inferred from a single year’s data, there are indications that the low rainfall period here has shifted from its historical low in September to around April-May. This, however, may simply reflect the inherent variability in the local climate during this recorded period.

It is evident from the ranked distribution plot that the most frequent precipitation input at the study site was less than 1 mm per day. Despite this there were 13 instances during the 8 month logged period where rainfall of 15 mm or greater per day fell on the cane-block, an average of 1.6 events per month over the logged period.
In practice these rainfall events were irregular. BOM data from the Murwillumbah recording station (BOM, 2007) for months from late September 2006 to January 2007 is shown in Table 6-4 Study site logged rainfall data is shown in Table 6-5.

**Table 6-4: Rainfall events greater than 15 mm per day (by month) (BOM data)**

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>nil</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 6-5: Study site rainfall events greater than 15 mm per day (by month)**

<table>
<thead>
<tr>
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</tbody>
</table>

This full year data then indicates that 23 rainfall events ≥ 15 mm per day occurred on the study cane-block over the full sugarcane crop cycle. Five of these events occurred in the first three months of sugarcane crop cycle when applied nitrogen fertiliser is likely to be most vulnerable to mobilisation. This information becomes more important when examined in the context of the combined impacts of evapotranspiration and precipitation on soil moisture and the watertable height.

### 6.2.4.b Soil moisture

As expected, data from rainfall and soil moisture logging showed close linkages between rainfall and soil moisture in the cane-block. Other factors also influence moisture dynamics. Generally the amount of moisture in the study soil increased with depth from below field capacity in the topsoil to saturation at the ground watertable in the AASS or PASS subsoil. Generally the upper soil layers responded faster to rainfall inputs and to a greater extent than layers deeper in the profile. Volumetric soil moisture plotted with cumulative rainfall (Figure 6-7) clearly indicates the impact of rainfall events in increasing volumetric soil moisture. However, this soil response to rainfall varies with depth.

The upper soil profile to 60 cm depth showed faster and generally greater response to rainfall but this zone also lost moisture more rapidly through evapotranspiration relative to soil at 80 cm depth. Only under the influence of very high rainfall events did the 20, 40, and 60 cm soil depths saturate, shown in Figure 6-7 by horizontal sections at the base of a near-vertical wetting front line. After a 68 mm rainfall event in June 2007, soil at 60 cm became saturated for nine days before evapotranspiration de-saturated the soil at that depth. During this same event, soil at 80 cm became saturated to 64% volumetric moisture and remained at this level until the end of the field trial in September 2007.

In September 2007 just over 200 mm of precipitation fell on the study cane-block over seven days and the 20, 40 and 60 cm loggers recorded soil saturation for relatively brief periods. The period for which soils stayed saturated increased with depth.
Topsoil was more vulnerable to evapotranspiration (ET) loss and rainfall variation than deeper soil layers (Figure 6-7). Evapotranspiration moisture loss, shown by upward trending lines in this figure, is not greatly evident in the soil at or below 80 cm depth. The exception to this occurred in the early part of the record when groundwater was very low. It is noted that sugarcane roots mainly occupy the upper soil profile to 60 cm depth where most transpiration moisture is likely to be depleted. This is supported by the general lack of ET loss in the 80 cm deep soil zone in Figure 6-7.

The assumption here that soil moisture loss may be attributed to evapotranspiration is supported by detailed analysis of the data showing soil moisture diurnal variations. These are evident in the early part of the record from February to March, even down to 80 cm depth. Figure 6-8 shows daily soil moisture rise and fall is in the order of 0.5 % volumetric soil moisture with a daily moisture minima at 1500 hrs and a maxima at around 0700 hrs. This indicates soil moisture depletion occurs between 0700 and 1500 hrs during the day with regeneration occurring overnight between 1500 and 0700 hrs. It can also be seen in Figure 6-8 that the 60 cm soil zone is losing moisture faster than at 80 cm soil depth.
Soil moisture response to rainfall varied with depth. Upper layers responded first with increasing time delay evident deeper in the profile (Figure 6-9). The amount and intensity of rainfall also affected the speed of the wetting front down the profile.

After an 18 day dry period, Figure 6-9 shows soil moisture response to a 68 mm rainfall event in late June 2007. It is noted that soil wetting initially moves relatively slowly down the dry soil profile. Rain onset was at 0000 hrs. Soil at 20, 40, 60 and 80 cm depth showed raised soil moisture response at 0100 hrs (1 hr delay); 0400 hrs (4 hours delay); 0900 hrs (9 hours
elapsed time), and 0930 hrs (9.5 hrs elapsed time) respectively. In contrast to the upper two
sensor depths, the soil at 60 and 80 cm depths barely responded to initial rain onset. It was not
until after approximately 25 mm had fallen over 22 hours and the rain intensity had increased
markedly that the 60 and 80 cm soil depths displayed significant increases in moisture to soil
saturation. The data indicates this occurred within 3 to 5 hours from onset of increased rainfall
intensity beginning around 2100 hrs on 25th June 2007.

Results of this soil moisture evaluation show that moisture in the sugarcane plant root zone
to 60 cm depth is highly variable and rarely saturates for periods long enough to cause anoxia.
Conversely, study data indicates soil at and below 80 cm depth is more likely to be saturated
and is consequently more liable to being anaerobic. Indications are that capillary rise from the
watertable is the main agent maintaining soil moisture below 80 cm depth. It is only when the
groundwater has declined to very low levels greater than approximately 1.3 m below ground
level does the soil at and below 80 cm depth de-saturate and potentially become aerobic. These
oxic/anoxic state and shifts have implications for soil chemical and biochemical functioning.

6.2.4.3 Groundwater

Five groundwater height loggers were installed at the Field Trial 2 cane-block site (Figure 7-
5). The first three of these (WT Loggers 1, 2 and 4) were installed along the north-south
centreline of the cane-block at distances of 10 m, 60 m and 110 m from the headland drain to
the north of the cane-block. Loggers 5 and 6 completed an L-shaped return to a field drain to
the west side of the block. Loggers 4, 5 and 6 were therefore at a similar distance (110 m) from
the northern headland drain.

Logged watertable height plots in Figure 6-10 display individual data for each of the five
loggers. These indicate groundwater movement up to 1.4 m. From early March until late June
2007 it is evident that the data plot for WT Logger 1 shows highly variable reading anomalies.
This was found to be due to moisture having entered the logger electronics after the April 2007
field trip. This problem was repaired in late June. WT Logger 1 data from this four month
period was not used in the study analysis. All other groundwater logger datasets show high
correlation with each other \(r^2 = 0.99\).

Watertable vertical movement was of two different types: (i) small amplitude but high
frequency movement, and (ii) larger but irregular lower frequency movement. The former was
attributed to diurnal watertable fluctuation and the latter to rainfall (for the rising arm) and
evapotranspiration between significant rainfall events (for the declining component).
Figure 6-10: Groundwater logger data plots

When co-plotted with rainfall (Figure 6-11), groundwater rise was observed to respond to significant rainfall events greater than approximately 15 mm per day. During dry periods the data shows considerable watertable drawdown by evapotranspiration. Analysis of significant rainfall events associated with groundwater rise (Table 6-6) shows groundwater height increase is associated with the relatively higher rainfall events.

Table 6-6: Substantial rainfall events (> 8 mm/day) over the Field N-trial 2 period

<table>
<thead>
<tr>
<th>Date of major rainfall event</th>
<th>Total rainfall (mm)</th>
<th>Groundwater Depth (mm) (below ground level)</th>
<th>Groundwater rise?</th>
<th>Groundwater rise (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 – 25 Aug 07</td>
<td>212.8</td>
<td>720</td>
<td>Yes</td>
<td>770</td>
</tr>
<tr>
<td>25 – 26 Jun 07</td>
<td>69.2</td>
<td>930</td>
<td>Yes</td>
<td>670</td>
</tr>
<tr>
<td>4 – 7 Sep 07</td>
<td>52.4</td>
<td>400</td>
<td>Yes</td>
<td>320</td>
</tr>
<tr>
<td>5 – 8 Mar 07</td>
<td>52.2</td>
<td>1240</td>
<td>Yes</td>
<td>420</td>
</tr>
<tr>
<td>11 – 14 Feb 07</td>
<td>34.6</td>
<td>1040</td>
<td>Yes</td>
<td>120</td>
</tr>
<tr>
<td>4 – 7 Jun 07</td>
<td>31.0</td>
<td>1140</td>
<td>Yes</td>
<td>330</td>
</tr>
<tr>
<td>6 – 9 Apr 07</td>
<td>26.8</td>
<td>1330</td>
<td>Yes</td>
<td>220</td>
</tr>
<tr>
<td>13 – 14 May 07</td>
<td>16.8</td>
<td>1340</td>
<td>Yes</td>
<td>240</td>
</tr>
<tr>
<td>7 – 8 May 07</td>
<td>13.0</td>
<td>1360</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>28 Feb – 2 Mar 07</td>
<td>10.8</td>
<td>1190</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>22 – 24 Jul 07</td>
<td>10.0</td>
<td>600</td>
<td>Yes</td>
<td>20</td>
</tr>
<tr>
<td>12 – 13 Mar 07</td>
<td>8.6</td>
<td>870</td>
<td>No</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 6-11: Cane-block mean-groundwater response to rainfall events
Watertable rise height shows strong correlation with daily rainfall amount \((r = 0.83)\). Daily precipitation of less than 15 mm per day mostly resulted in no rise in the watertable regardless of groundwater depth (Table 6-6). An exception was the 20mm groundwater rise associated with 10 mm rainfall on 22 to 24 July 2007 when the watertable was very high in the profile.

Groundwater response to rainfall varied by rain intensity as well as watertable height (Figure 6-12). Watertable levels plotted with rainfall shows the rate of groundwater rise changing in parallel with rainfall intensity. In addition, the watertable response time lessened as the watertable rose higher in the profile. There was a strong negative correlation \((r = -0.76)\) between watertable height and watertable response time to rainfall changes in this data. Little rain had fallen in the three weeks prior to this 68 mm June rainfall event.

![Figure 6-12: Changing soil profile response to wetting](image)

From rain onset the initial interval for groundwater at around 1.1 m depth to respond to rainfall infiltration was approximately 10 hours (Figure 6-12). This duration is possibly affected by the intermittent nature of the first 15 mm of rainfall over 9 hours as well as the watertable depth. Onset of a second more intensive precipitation period took around 5.5 hrs to impact on the watertable at 1.0 m depth. The next change in intensity resulted in a 3.8 hrs watertable response delay. Rainfall cessation resulted in a 2.8 hr groundwater response lag.
(i) Evapotranspiration Effects

Although of relatively small magnitude (<0.15 m), diurnal groundwater movement was clearly evident in all watertable recordings but these were more pronounced toward the centre of the cane-block (Figure 6-13). In this figure, WT Logger 6 is adjacent to a dry field drain at the edge of the cane-block. Regular daily watertable oscillation became more obvious during dryer periods with observed ranges from 0.01 to 0.15 m. Daily groundwater minima typically occurred around 0600 hrs with the daily maxima evident at approximately 2000 hrs.

![Cane Block Groundwater - diurnal fluctuations (May-June07)](image)

Groundwater Low: ~ 0600hrs, High: ~ 2000hrs

Figure 6-13: Diurnal groundwater movements

Comparison of individual piezometer data plots showed differences in these diurnal cycles, indicating the influence of other forces (data not shown here). Tidal fluctuations in the adjacent Tweed River and McLeods Creek (adjusted for distance inland) plotted with piezometer watertable data gave clear indications of tidal influence on groundwater levels closest to the river and northern headland drain. Preliminary observation indicates these groundwater perturbations are of the same order of magnitude but of different frequency to the diurnal evapotranspiration effects on the watertable.

Detailed examination of groundwater and soil moisture data shows evidence of close coupling between these two parameters. Diurnal groundwater declines closely matched soil moisture rises higher in the soil profile (Figure 6-14). Groundwater diurnal fluctuation minima occur at 0600 hrs associated with soil moisture maxima between 0630 to 0730 hrs. Conversely,
groundwater maxima at 2000 hrs occur in conjunction with soil moisture minima at 1530 hrs. These data therefore indicate groundwater drawdown occurring overnight between 2000 and 0700 hrs whereas soil moisture decline higher in the soil profile takes place during the day between 0700 and 1530 hrs. Soil moisture data from summer months (not shown) indicates moisture decline over a longer daylight period between 0630 and 2000 hrs.

![Graph showing soil moisture and watertable depth over time](image)

*Figure 6-14: Groundwater and soil moisture coupling*

(ii) *Cane-block drainage controls on watertable height.*

Other watertable data shows evidence of rapid draining of groundwater once a specific height is reached in the cane-block profile (Figure 6-15). This height corresponded to -0.64 m AHD or approximately 0.5 m below the ground surface. Investigation of invert levels of the side and end drains indicated they were the controls for this draining. Groundwater height plots during the August 2007 high rainfall events clearly show rapid draining of groundwater from the upper soil profile above the height of the outlet for the mole and field drains (Figure 6-15). Preliminary calculations show substantial volumes of soil water were potentially exported from the cane-block.

The ground watertable rose past the drain control height at 0200 hrs on 20-08-07 and fell past this point at 1400 hrs on 27-08-07. Between these times 166 mm of rain fell on the study cane-block. Evapotranspiration was determined from recorded data as being 3 mm per day equivalent rainfall or less than 26 mm in 8.5 days. Over the cane block area, this equates to a total of 3 878 400 litres of water (less 595 780 litres estimated evapotranspiration) potentially drained from the upper layers of the cane-block. Over 8.5 days the net outflow approaches 386 200 litres day$^{-1}$ or around 165 000 litres ha$^{-1}$ day$^{-1}$.
Figure 6-15: Field drain control of watertable drainage

Watertable heights from the three piezometers along the centreline of the cane-block show substantial headland drain impacts on cane-block groundwater surface close to this drain (Figure 6-16). Indications are that this influence extends at least 60 m into the cane-block. On 24 April 2007 when the groundwater surface 60 m from the main northern drain was at one of its lowest recorded heights of -1.66 m AHD, the groundwater surface at the 10 m piezometer tube was reading -1.23 m AHD, that is: 0.43 m higher than the 60 m piezometer reading.

Figure 6-16: Headland drain influence on cane-block watertable height
Current study fieldwork objectives included evaluation of soil nitrogen species in the soil profile as well as evaluation of cane-block leaching potential. Field study results in this section demonstrated the relatively high hydraulic conductivity of the oxidised AASS zone. Field data also showed the relative ease with which precipitated water greater than 15 mm per day can infiltrate the moist soil profile to a relatively low groundwater surface in the AASS/PASS transition zone. Rainfall infiltration to around 1 m depth typically occurs in less than 10 hours in relatively dry soil and around five and a half hours in soils at field capacity.

Very intense rainfall onto already moist soil potentially results in a major portion of the precipitation running off the laser-graded cane-block surface. There was no indication of this in the recorded data despite several high intensity rain events. The data does indicate that the cane-block and farm drainage systems prevented groundwater rising to the soil surface thus preventing any significant cane-block surface runoff. The critical level for this drainage control was the field drain invert at -0.64 m AHD (approximately 0.5 m below ground level). The assumption here is that this rapid draining was largely facilitated by the mole drain system. Preliminary calculations estimate the lateral flow from the cane-block to be nearly 4.5 litres per second over the whole cane-block over an 8.5 day rainfall event totalling 210 mm.

These combined results indicate two primary factors impacting on watertable dynamics in these soils. The first is sugarcane evapotranspiration which acts to draw down the watertable. The second is rainfall infiltration that raises groundwater levels. In terms of study data known and estimated cane block inflows and outflows, there are indications that an imbalance exists. Field site recorded rainfall and BOM pan evaporation data showed a deficit of 113 mm rainfall equivalent. Pan evaporation data does not include a cane plant transpiration component which would make this deficit larger. In addition to this, recorded data shows a watertable rise over this period making the water deficit appear even larger. All this information supports conjecture about another major source of water input to the cane-block to balance this estimated deficit.

A potential explanation can be seen in study data showing a hydraulic gradient (indicative of inflow) between a higher headland drain and the adjacent cane-block watertable. At this Field Trial 2 study site there are water-filled headland drainage channels at each end of the 354 m long cane-block. When the watertable is low under a growing sugarcane crop, watertable data indicates that cane-block groundwater is being recharged from the headland drains. This could account for at least some of the estimated soil water deficit.

Additional support for this postulate comes from Field Trial 1 cane yield data showing cane row harvest yields increasing consistently right across a 60 m wide cane-block towards the adjacent main drain, completely masking any nitrogen fertiliser treatment effect. One obvious explanation is that water seeping into the cane block creates a sloping equipotential groundwater surface across the cane-block. Field Trial 1 cane harvest results showed N-treatment Plot yields
increasing across the cane-block in a curvilinear fashion closely matching this postulated sloping groundwater surface.

6.3 Concluding Comments

Integrated results of investigation into soil-thermal and hydrological properties here provided information enabling more informed evaluation of soil ecology and biogeochemical functioning at the study field site. In this it met one of the study objectives to characterise the study soils within a context of the main study aim to evaluate the impacts of fertiliser nitrate on rates of pyrite oxidation in a typical agricultural acid sulfate soil.

It is clear from the results that soil at and below 80 cm depth is semi-permanently saturated and therefore largely anaerobic for much of the year under reasonably average rainfall regimes for this area. This is supported by soil redox potential data presented earlier in section 4.2.3.b. This directly determines whether oxic or anoxic (bio)chemical processes prevail in this AASS transition zone.

Collected soil hydrology data also provided information crucial to the evaluation of fertiliser-derived nitrate leaching rates. Observed fast infiltration rates demonstrate the soil’s capability to rapidly transport nitrate ions down the profile to the saturated zone. It is suggested that diffusion mechanisms could then dominate to move soil nitrate deeper into the profile. It is suggested this postulated downward diffusion component could be limited by the upward flow of water from the watertable to the surface through mechanisms of evapotranspiration and capillary rise. Cane soil profile nitrogen species are investigated in the following chapter.
7 Cane-block Soil Nitrogen

7.1 Introduction

One of the study objectives was to investigate fertiliser-derived soil nitrogen changes and movement in sugarcane acid sulfate soils within a broader study aim of evaluating the potential for nitrate to oxidise pyrite in the cane-block acid sulfate soil profiles. In addition to the soil characterisation described in chapters 4, 5 and 6, a fieldwork program was implemented to concurrently collect the necessary data to broadly evaluate the fate of soil nitrogen in these cane soils.

As little was known about applied soil nitrogen in these profiles this investigation was carried out using two separate nitrogen treatment field trials. referred to here as Field Trial 1 and Field Trial 2. The purpose of the first nitrogen treatment trial was to gather initial information on soil nitrogen species over a sugarcane crop cycle. It was also used to evaluate sampling, extraction and analysis methodologies as well as soil sample transport and storage methods. Information and experience gained in this trial allowed a more refined and directed approach in the second field trial.

The following sections provide an overview of aims and objectives, experimental design, field and analysis methods, results and discussion for each nitrogen field trial.

7.2 Nitrogen Field Trial 1

With the cooperation of McLeods Creek sugarcane grower Robert Quirk a nitrogen fertiliser treatment trial was designed to allow evaluation of primary nitrogen species in the soil profile under a growing sugarcane crop. The interplay of other issues such as declining crop yields, nitrogen pollution of surface and ground water and concern about gaseous emission losses of nitrogen species, lent weight to the imperative to also gather information on the crop soil response to significantly different amounts of nitrogen soil amendment.

7.2.1 Aims and Objectives

The overall aim of this initial field nitrogen trial was to gather background information relating to fertiliser-derived nitrogen concentrations in these soils including nitrogen speciation and movement in the soil profile.

Specific objectives were:

- Sampling and analysis of soil-amended ammonium and nitrate in the cane-block soil (to at least 1.5 m depth) at least three times over a sugarcane crop cycle
- Evaluation of methods for sampling, storage and analysis of cane-block soil samples
These aims were supported with a trial design incorporating three different N-treatments.

7.2.2 Experimental Design

Field Trial 1 was designed to allow replicated sampling of a three-treatment soil nitrogen trial on a sugarcane block over a crop cycle. The cane-block (Quirk, Blk 603) had been fallow for one cane season and was due to be planted with new cane. This block measured approximately 60 m by 280 m. To minimise cane-block edge effects and treatment overlap effects, two features were incorporated into the trial design:

a) A three row buffer strip was left along each side of the cane-block. Although this plot was amended with fertiliser at the highest treatment rate, it was not used for soil sampling due to its proximity to the adjacent field drain. This buffer strip was subsequently not identified as a plot and is referred to here as the ‘Edge buffer’.

b) To minimise potential treatment contamination from the adjacent treatment plot, soil sampling was only taken from the centre row of the three cane-row wide plot. With a row width of 1.6 m between centres, soil sampling locations for different N-treatments were never closer than 4.8 m distance although a cane row with a differing N-treatment was approximately 3 m distance from any treatment plot centre-row sampling location.

Treatment plots were three cane rows wide running the length of the cane-block. Three significantly different CK44 (Incitec-Pivot, 2009) nitrogen fertiliser treatments were applied to each 3-row plot side-by-side in the order: Low-N, Mid-N and High-N treatments corresponding to 63, 125 and 250 kg CK44 fertiliser ha\(^{-1}\) respectively. This equates to 5.3 (Low-N), 10.7 (Median-N), and 21.3 (High-N) kg NH\(_4\)\(^{+}\) -N ha\(^{-1}\) respectively, with this sequence being replicated across the cane-block. Fertiliser specifications are detailed in Appendix 14.

There were no nil-treatment control plots in this first trial. The width of the cane-block allowed for two separate three-treatment modules to be implemented across the cane-block. Replication was achieved at sampling points 50 m each side of the main sampling line 150 m from the east end of the block. Randomisation was not used at the N-treatment layout level. In the field trial design, threefold replication in most sampling and analysis procedures helped to minimise gross errors, reduce sub-sampling errors and minimise the effects of natural heterogeneity in the cane-block soil. In reporting results, standard error (defined as standard deviation / square root of number of measurements) was used here as the indicator of sample spread. Significant differences, where reported, were tested using a standard one tailed t-test. Association between two sets of variables was determined using Pearson’s correlation coefficient.
The Field Trial 1 cane-block layout is shown in Figure 7.1. The fieldwork schedule is detailed in Table 7-1.

![Figure 7-1: Field Trial 1 cane-block layout and sampling locations](image)

### Table 7-1: Field Trial 1 fieldwork schedule

<table>
<thead>
<tr>
<th>Field trip date</th>
<th>Purpose</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Sep 2003</td>
<td>Grower offers Blk 603 for N-Trial Field trial design sent to grower</td>
<td>Plough-out block harvested in June 2003. Full trash blanketed and sprayed with 3 kg urea in solution / ha to decompose cane trash.</td>
</tr>
<tr>
<td>26 Sep 2003</td>
<td>Cane planting Amendment with CK44 fertiliser in 3 treatments: 2 cwt/acre, 1 cwt/acre, 0.5 cwt/acre</td>
<td>Low-N: Fertiliser rate: 63 kg / ha (5.3 kg N / ha Mid-N: Fertiliser rate: 126 kg / ha (10.7 kg N / ha High-N: Fertiliser rate: 251 kg / ha (21.3 kg N / ha)</td>
</tr>
<tr>
<td>5-7 Nov 2003</td>
<td>Soil-N sampling / extraction Soil description</td>
<td>Plots 10,11,12 (Note: Plot 13 was assigned to an adjacent cane block for comparison purposes)</td>
</tr>
<tr>
<td>17-19 Jan 2004</td>
<td>Soil-N sampling / extraction</td>
<td>Plots 10,11,12,14,15,16</td>
</tr>
<tr>
<td>15-16 Jun 2004</td>
<td>Soil-N sampling / extraction Soil description</td>
<td>Plots 14,15,16 (Note: Plots 10, 11, and 12 suffered storm damage to the cane making them impossible to traverse)</td>
</tr>
<tr>
<td>Sep 2004</td>
<td>Harvest data collection</td>
<td></td>
</tr>
</tbody>
</table>

### 7.2.3 Field and Laboratory Methods

Field methods used in this field trial fell into different categories including: (i) soil sampling and storage, (ii) soil extraction, and (iii) laboratory analysis of ammonium and nitrate. These are described in the following subsections.
7.2.3.a Soil sampling, storage and transport

Soil sampling and handling was generally carried out in accordance with guidelines in Rayment and Higginson (Rayment and Higginson, 1992). Soil samples were retrieved 0.1 to 0.15 m from the centreline of the cane row using a 0.1 m diameter bucket auger (Dormer, 2000). These holes were dug to around 0.7 m, then a gouge auger (Dormer, 2000) used for further samples to 1.8 m depth. Three cores were taken from each treatment plot and these soil samples were analysed separately (i.e. not amalgamated). Assuming that changes in chemical speciation are most likely to occur between soil horizons, samples were collected at the top and bottom of each of horizon in the cane-block soil profile to 1.5 m depth. Whilst the depth of each horizon varied slightly depending on the location within the cane block, typical sample depths were 5 cm, 30 cm, 40 cm, 75 cm, 105 cm, and 1.5 cm.

Around 250 grams of soil from the selected depth were collected and placed in small thick-walled polypropylene (PP) resealable plastic bags. Sample soil was hand compressed to exclude air before bag sealing and the filled sample bags were temporarily stored in a cooled insulated box during field collection. Field soil sampling was carried out in batches taking no more than 45 minutes to collect. These were then taken to a field laboratory (<1 km distant) for storage in a refrigerator at 3 °C. prior to extraction of critical ions. As nitrate and ammonium analysis results from frozen and thawed soil sample are susceptible to wide divergence from true soil concentrations (Esala, 1995), care was taken not to freeze any samples used for later analysis.

7.2.3.b Soil extraction

Extraction of ammonium and nitrate ions from soil samples was carried out using the method of Bremner (Bremner, 1967). This procedure uses 2M potassium chloride (2M KCl) to displace soil ions into the extract solution. A measured representative 3 g sample of moist soil was added to a 30 ml polyethylene (PE) capped centrifuge tube and topped up with a measured volume of 2M KCl in the ratio of 1 g of moist soil to 10 ml of KCl solution. Mass and volumes were recorded to allow reporting of N-species results on an oven dry soil (OD) basis. At the same time a nominal 30-50 g of each soil sample was also weighed out onto annotated trays for drying at 105 °C for soil moisture determination.

Extract tubes were tumbled on a rotary shaker for 1 hour at around 30 rpm to extract relevant ions. Tubes were then centrifuged at 2000 rpm for 6 minutes. The resulting clear supernatant was filtered through a 0.47μm polycellulose filter under vacuum. As polycellulose filters may potentially leach small amounts of nitrates (Halmhain and Danachair, 1974), filters were washed with 2M KCl before use. After decanting into annotated clean centrifuge tubes, the supernatant soil extracts were stored in a refrigerator at 3 °C until transported back to the Canberra ACT laboratory for analysis.
For this latter trip, extract tubes were batch-packed securely in thick PP bags and then placed into high-efficiency insulated and cooled boxes. These transport boxes also contained the chilled soil samples to provide significant thermal mass to minimise warming during the 14 hour trip. On arrival, all samples were transferred to a refrigerator set at 2-3 °C for storage until used for N-species analysis.

7.2.3.c Laboratory procedures: ammonium and nitrate-ion analysis

Samples were analysed for nitrate (NO$_3^-$) and ammonium (NH$_4^+$) ion concentration in 1:10 2M KCl extracts. Due to the availability of a Hach DR/2010 spectrophotometer (Hach, 2004a), Hach screw-topped vial kits and a Hach thermostatically-controlled heating block, colourmetric analytical procedures were adopted for both nitrate and ammonium determination in the cane-block soil samples. However, the procedures for each ion analysis were different.

(i) Ammonium-ion analysis

The adopted ammonium analysis method was a modified semi-macro procedure described by Mulvaney (Mulvaney, 1996) for a 25 ml soil extract sample. This procedure is based on the Berthelot reaction (Kempers, 1974) using sodium nitroprusside and hypochlorite reagents. The original Mulvaney method was here scaled down for semi-micro analysis, using 1 ml of extract sample plus appropriately-scaled reagent volumes to fit 10 ml screw-topped borosilicate glass tubes. These tubes fitted the available heating block and could also be directly used as cuvettes in the final colourmetric analysis stage (Hach, 2004a). The development of a semi-micro procedure also had the benefit of using significantly less sodium nitroprusside reagent containing cyanide.

Primary ammonium-N standards of varying concentrations to span the range of expected soil ammonium results were made up weekly to check and calibrate this procedure. These were refrigerated at 4 °C after use. Two ammonium-standards and a nil-ammonium control were run at the beginning and end of each 20 sample batch to ensure the integrity of results.

The ammonium-ion analysis procedure essentially consists of reaction and colourising reagents being added to the soil extract and heating this mixture at 37 °C for 30 minutes to allow colour development. Sample tubes are then inserted into the pre-calibrated spectrophotometer and evaluated for ammonium ion concentration in mg NH$_4^+$-N/L at a wavelength of 667 nanometres (nm). The full semi-micro analysis procedure is described in Appendix 2.

(ii) Nitrate analysis

The nitrate-ion analysis colourmetric procedure is based on a cadmium-reduction method using Hach Company pre-mixed dry reagents (NitraVer 5 Powder Pillows) to produce colour in the sample extract in a glass cuvette (Hach, 2004b). This method uses cadmium metal to reduce nitrates in the sample to nitrite. The nitrite ion then reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. This salt couples with gentisic acid to form an
amber coloured solution. Test results were colourmetrically determined at 500 nm on a Hach 2010 spectrophotometer. See Appendix 4 for a description of the full procedure.

Analysed nitrate concentrations were recorded in mg N-species-N per litre (mg N/L) of extract solution. Ammonium and nitrate N-species concentrations reported were all subsequently standardised and reduced to micrograms N per gram of oven dried soil (OD), written here as: $\mu g$ 'N species' - N g$^{-1}$ OD.

### 7.2.4 Field Trial 1 Results

Soil sampling in the field trial cane-block was limited by extraordinary events during this N-trial, the primary one being significant storm damage to the sugarcane mid-way through the trial resulting in cane stalk lodgement (cane fall) in previously sampled areas of the block. Sampling was subsequently shifted to another part of the cane-block (i.e. other plots) with the same treatment. As a result, sampling and analysis for soil ammonium and nitrate ions revealed some inconsistent and unusual results, shown in summary form in Figure 7-2 and Table 7-2.

Sample inconsistency manifested in two main ways. The **first** concerns apparent reversal of N-assay results for both ammonium and nitrate by treatment. That is, the lowest nitrogen fertiliser treatment initially resulted in the highest nitrogen concentration. The original design called for Plot 10 to be a Low-N treatment (5.3 kg N ha$^{-1}$), Plot 11 to be a Mid-N treatment (10.7 kg N ha$^{-1}$) and Plot 12 to be the High-N treatment (21.3 kg N ha$^{-1}$). The same treatment order held for Plots 14, 15 and 16 respectively. The **second** issue relates to the inconsistency of ammonium-N and nitrate-N concentrations across the three sampling dates. Ammonium- and nitrate-N levels declined between November 2003 and January 2004, but both N-species showed increases in concentration at depth at the third sampling date in June 2004.

Nitrogen-fertiliser soil amendment initially resulted in increased levels of ammonium ions in the topsoil and increased levels of nitrate ions deeper in the profile. Over the course of the sugarcane growing cycle the concentrations of both N-species generally declined but with some exceptions. Results indicated that background levels of ammonium-N in the cane soil are mostly $\leq 10 \mu g$ N g$^{-1}$ OD although June 2004 results show concentrations up to $25 \mu g$ N g$^{-1}$ OD in the cane soil AASS-PASS transition zone. Nitrate-N background levels were less predictable with the January 2004 results showing subsoil NO$_3^-$-N concentrations $\leq 25 \mu g$ N g$^{-1}$ OD. However five months later, June 2004 results showed nitrate-N subsoil soil levels at around 100 $\mu g$ N g$^{-1}$ OD.
Figure 7-2: Field N-Trial 1: Mean soil $\text{NH}_4^+$ and $\text{NO}_3^-$ levels in the cane-block profile
### Table 7-2: Field Trial 1 Result

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<tr>
<th>Nov03 Field Trial 1 Ammonium</th>
<th>Plot 10 mean NH4 Low-N (ug/g OD)</th>
<th>± s.e.</th>
<th>Plot 11 mean NH4 Mid-N (ug/g OD)</th>
<th>± s.e.</th>
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<th>± s.e.</th>
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<th>± s.e.</th>
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Following the September 2003 addition of the CK 44 fertiliser, soil nitrogen results from the **November 2003** soil sampling showed varying treatment responses. Relatively high levels of ammonium in the upper topsoil zone declined rapidly to low background levels (i.e. <10 μg NH$_4^+$-N/g OD soil) from 20 cm to 150 cm depth. The intended Low-N treatment created the highest surface soil ammonium-N levels (224 μg /g NH$_4^+$-N OD soil) in November. The Median-N and High-N treatments tested at 81 and 41 μg NH$_4^+$-N/g OD soil respectively. Soil nitrate levels showed anomalous and inconsistent results between treatments. The intended Low-N treatment produced the highest nitrate-N levels in the subsoil followed by the High-N treatment with the next highest. These Low-N and High-N treatment enhanced N-concentration responses appeared deep in the profile at the AASS-PASS transition zone around 80 to 100 cm soil depth. The medium-N treatment had its lowest nitrate-N results in this zone.

The **January 2004** plots show results for the Low and Median-N treatments. Ammonium-N concentrations were substantially lower relative to the November results. The Low-N and Mid-N treatment NH$_4^+$-N concentrations in the 0.1 m topsoil were 21 and 14.5 μg N g$^{-1}$ OD soil respectively. Low ammonium levels (<10 μg NH$_4^+$-N g$^{-1}$ OD soil) were typical for both treatments deeper in the profile to 150 cm. Soil nitrate results for both treatments showed relatively low nitrate levels of less than 20 μg NO$_3^-$-N g$^{-1}$ OD below 40 cm depth, with somewhat higher concentrations above 40 cm depth rising to 90 μg NO$_3^-$-N g$^{-1}$ OD soil in the upper topsoil.

**June 2004** testing of Low-N and High-N treatments was carried out on different plots to those used previously. Soil ammonium concentrations for both treatments were less than 25 μg NH$_4^+$-N g$^{-1}$ OD throughout the profile. Lower concentrations <13 μg NH$_4^+$-N g$^{-1}$ OD soil were observed in the upper soil layers. Ammonium ion concentrations were up to three times higher than those for January 2004 at all soil depths below 20 cm. Nitrate ion concentrations varied but were generally less than 100 μg NO$_3^-$-N g$^{-1}$ OD in the profile, with highest concentrations at 180 cm depth. Clear declines in nitrate-N levels were evident across the topsoil/AASS boundary and also the AASS/PASS boundary.

Harvest results

Whilst the principal aim here was to evaluate cane-soil nitrogen concentrations, harvest yield data was also collected to aid interpretation of other results. The cane harvest was assessed using tonnage per row amalgamated into three-row treatment plots, giving harvest figures based on nitrogen-fertiliser treatment. Results from this field trial also showed unexpected harvest yield response by treatment. Summary harvest yield results are shown in Table 7-3 and Figure 7-3.
Table 7-3: Field Trial 1 cane-block fertiliser treatments and harvest yields

<table>
<thead>
<tr>
<th>Plot</th>
<th>Fertiliser (kg CK44 / ha)</th>
<th>Fertiliser (kg NH4-N / ha)</th>
<th>Plot Cane Yield (tonnes ha⁻¹)</th>
<th>Plot Sugar Yield (tonnes ha⁻¹)</th>
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Cane yields show no evident response to nitrogen treatments. However substantial differences in cane yields between plots were evident. Plot cane yield increased in a curvilinear manner from north to south across the cane-block. From a low of 66.9 tonnes per hectare on the north side, the yield rose relatively uniformly to 107.6 tonnes per hectare on the southern side of cane-block. Sugar yields generally tracked the cane yields, rising from 9.0 tonnes (Plot 10) to 14.6 tonnes per hectare (Plot 16) across the block toward the south main drain.

In this field trial there was very weak correlation between plot N-treatment and cane yields per plot \( r = 0.16 \) but slightly stronger but still weak correlation between plot N-treatment and sugar yields \( r = 0.31 \). Conversely, cane yield and sugar yield were very strongly associated \( r = 0.95 \).

7.2.5 Field Trial 1 Discussion

Although some results here are anomalous, from a very general perspective this first field trial proved to be extremely valuable in evaluating the appropriateness of the methods used. This trial also gave some indication of levels of soil nitrogen to be found in these acid sulfate soils under sugarcane. The results also provide information on residual or background soil
nitrogen concentrations. Despite the somewhat inconsistent and variable results, they also give an indication of the temporal and spatial aspects of these N-species over the sugarcane crop cycles.

If nitrogen fertilisers such as ammonium nitrate or urea are used on these soils, it also indicated that initial high localised ammonium ion concentrations may be expected in topsoil near the fertiliser application point up to several months after fertilising. Increased nitrate-N concentrations could also be expected but only after the appearance of ammonium ions. There is some evidence indicating change in N-species concentrations at acid sulfate soil transitions zones. Although results here indicate increased levels of nitrate deeper in the profile, there is cause to regard these with some caution. These soil nitrate concentrations are so anomalous as to raise queries about the analysis methods used. This is further discussed later in this chapter.

Possibly the most puzzling anomaly in these results is the apparent reversal of treatment responses, with Low-N treatments apparently producing the highest soil concentrations of both ammonium and nitrate and vice versa. Thorough checking of all potential sources of error did not ultimately resolve this. One obvious gross error would be sampling of the wrong treatment plots, but as all three soil sampling field trips gave similar anomalies, this explanation was ruled out. Error by others in cane block fertiliser rate application is the most likely explanation for this outcome, but after the event there was no evidence to confirm this. This experience supported an intention to more clearly delineate the treatment plots in the second field trial.

Although not one of the objectives of the field trial, collection of cane harvest yields showed no treatment response. Discussions with the grower subsequently revealed the cane-block had recently undergone re-grading using laser levelling techniques to decrease water-ponding and enhance surface runoff after heavy rain. Some topsoil had been shifted from the north side and east end of the cane-block to the south side and west end respectively, resulting in relatively shallow topsoil on the north and east sections. This uneven topsoil distribution on the field trial block could have impacted on cane yields. However, the proximity of the headland drain within metres of the southern cane-block boundary could also have contributed substantial soil water to the south-side cane, thereby enhancing cane yields to the south of the block. This was discussed in section 6.2 and the view here is that this explanation is the most likely.

Methodology issues

Although not shown here, the unexpectedly large variation between individual replicated samples by treatment pointed to potential problems either with sampling technique and/or analysis. This lack of consistency in replicated samples only occurred with samples analysed for nitrate-N. This raises queries about the appropriateness and reliability of both soil nitrogen analysis methods, but particularly the Hach NitraVer 5 method used here. A further issue was associated with the unexpectedly high levels of nitrate ions found deeper in the cane-block profile. The Hach method is known to be subject to some degree of interference from raised
levels of other common soil ions such as chloride, so this apparent abnormal response could not
be ruled out. The outcome here was a resolve to find a more reliable and stable procedure for
nitrate evaluation.

Targeted further review of the literature revealed some shortcomings in the methodologies
initially adopted for ammonium and nitrate analysis. The Mulvaney ammonium analysis
procedure (Mulvaney, 1996) shows inference by some metal cations and many N-containing
organic compounds (Rhine et al., 1998). The fact that these interferences were both positive
and negative made it challenging to compensate for them. Rhine et al developed a modification
of the Mulvaney (1996) procedure, which reportedly gave more accurate recoveries, particularly
in the presence of soil amino acids. However, further investigation revealed that the steam
distillation methods (Bremner and Bundy, 1973) analysing for both ammonium and nitrate ions
give reliable and accurate recoveries. This steam distillation method was used for the second
field trial soil analysis.

7.3 Nitrogen Field Trial 2

Experience gained in the first field trial informed and assisted design of the second field trial
carried out in 2006-07. Finding a cane-block with a consistent soil profile and different
orientation to headland drains was a high priority given the poor treatment results of the first
field trial. Discussions with the same cane grower identified a suitable site. This sub-section
outlines aims and objectives, field trial design, methods used, N-trial results and discussion.

7.3.1 Aims and Objectives

Within the context of a multi-treatment nitrogen field trial the primary aim of the fieldwork
here was to evaluate ammonium- and nitrate-ion changes over the course of a sugarcane
planting-to-harvest cycle. A particular focus was to quantify any observed soil nitrate
movement down the cane-block soil profile. The intention was that this data would assist
evaluation of the primary aim in examining the potential for soil nitrate to react with ASS
pyrite.

Specific objectives here were:

- To carry out replicated soil sampling and N-species analysis at the McLeods Ck N-
  trial field site down to 1.7 m depth
- To collect bulk soil samples for use in the laboratory study and for soil carbon,
  mineralogy and clay analysis
7.3.2 Experimental Design

This second nitrogen fertiliser treatment trial used a single cane-block. Intensive replicated sampling of one cane-block could potentially give more consistent and useful results than less frequent sampling of more varied regional soils. It also could help provide greater understanding of general acid sulfate soil processes if consideration was given to choosing a field site that was representative of regional soils. A drawback of this approach was that it may not adequately represent and apply to soils outside the local field site area. Consultation with a McLeods Creek grower Robert Quirk led to choosing a 2.34 ha cane-block for the second N-treatment field trial. The location is shown in Figure 3-1 and Figure 7-4.

This cane-block had a duplex ASS profile with four distinct physical and geochemical soil horizons. These were an A layer topsoil, a B1 layer actual acid sulfate soil (AASS), a B2 AASS-PASS transition zone and a B3 layer of potential acid sulfate soil (PASS). The profile was relatively simple with no sand and/or shell layers or lenses. Extensive regional soil test information was available for total nitrogen in topsoils in the region (Reilly, 2001) but very little previous research information was available for nitrogen deeper in these soils.

The previous crop on the cane block had been ploughed out and fallowed and the block prepared for replanting. As the new plant cane was to be fertilised on two occasions with an initial Plant-mix and a later Side-dress application, a four treatment replicated field trial was designed to study soil nitrogen changes over the 12 month crop cycle. The Plant-mix fertiliser was applied at planting with the cane setts on 20 October 2006. Cane rows were fertilised seven weeks later on 6 December with a side-dressing to the growing cane. Previous cane farm practice in the region had been to add N-fertilisers to growing cane at rates up to 200 kg –N per hectare. Due to a policy of building up soil organic matter at the study site farm, significantly less artificial fertiliser was being used with experience of little or no reduction in crop yield.

The Plant-mix consisted of NPK fertiliser CK44 (Incitec, 2002) whereas the Side-dress application was solely urea fertiliser (Incitec, 2008). Specifications and treatment rates for these two soil amendments are shown in Table 7-4.

<table>
<thead>
<tr>
<th>Treatment name</th>
<th>Fertiliser Name</th>
<th>Chemical composition</th>
<th>Application Rate</th>
<th>N-Application rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant Mix (PM)</td>
<td>CK44(GrowForce, 2003)</td>
<td>8.5 % N, 9.4 % P, 26.5 % K, 0.8 % S.</td>
<td>200 kg / ha</td>
<td>17.0 kg N / ha</td>
</tr>
<tr>
<td>Side Dress (SD)</td>
<td>urea [(NH4)2CO] (Incitec, 2008)</td>
<td>46 % N</td>
<td>100 kg / ha</td>
<td>46.0 kg N / ha</td>
</tr>
</tbody>
</table>
Figure 7-4: Location of the nitrogen treatment field trial blocks at McLeods Creek
Total nitrogen added to the study cane-block was 63.0 kg N ha\(^{-1}\), or 6.3 g N/m\(^2\). This latter figure is relatively meaningless as the fertiliser is not applied uniformly over the cane-block but is added to the centre line of the cane row at the base of a narrow furrow approximately 10 cm deep and is therefore highly localised in depth and position.

If this fertiliser were uniformly distributed uniformly throughout the soil profile to a depth of 100 cm, the applied-N concentration would be in the order of 6.3 \(\mu\)g N/cm\(^3\), that is: 7 \(\mu\)g N g\(^{-1}\) moist soil, or around 12 \(\mu\)g N g\(^{-1}\) O.D. soil based on an average soil bulk density of 0.9 g cc\(^{-1}\) (adapted from (van Oploo, 2000) and an average soil moisture content of around 67% found in the current study. However, the fertiliser products are unlikely to spread uniformly through the soil profile. Given that hydraulic properties of the soil are likely to be substantially influenced by the proliferation of new and old vertical cane root canals, it is postulated that dispersal is more likely to be in the form a vertical plume emanating from the linear fertiliser band along the cane row. As a precaution against gross errors in the soil analysis procedures and to enable approximate checks to be made on soil nitrogen balance, a simple working assumption adopted was that this plume is a 0.5 m wide by 1.0 m deep rectangular section, equating to 0.5 m\(^3\) per row metre length.

The *Plant-mix* PM treatment CK-44 fertiliser contains 8.5 % (wt/wt) elemental nitrogen (N) in an ammonium compound form. At application rates of 200 kg/ha used here, this equates to 17.0 kg N ha\(^{-1}\) or approximately 16 \(\mu\)g N/cc of moist soil, or 30 \(\mu\)g N g\(^{-1}\) OD soil in the assumed infiltration / diffusion zone under the cane row. Similarly, the *Side-dress SD* treatment urea fertiliser contains 46 % N by weight which at the 100 kg/ha application rate equates to 46 kg N/ha, 40 \(\mu\)g N/ cc moist soil, or \(\sim\)75 \(\mu\)g N g\(^{-1}\) OD soil uniformly distributed restricted soil zone. Combined into a *PM+SD* treatment, this approximates to 105 \(\mu\)g N g\(^{-1}\) OD soil under the cane plant in the postulated diffusion zone. However, these calculations assume even distribution within this zone, and the suggestion here is that the nitrate will be dispersed in the soil below the application point, mainly through rainfall induced advective forces, in the form of a plume of decreasing nitrate concentration down the soil profile.

To give some sense of potential initial soil nitrate-N concentrations, calculations show that 1.008 \(\times\) 10\(^7\) \(\mu\)g N per cane-row lineal metre could potentially be supplied by the aforementioned 63 kg N ha\(^{-1}\) of Plant Mix and Side Dress fertiliser. This is equivalent to point source concentrations of 100 800 \(\mu\)g N per cm\(^3\) in the fertiliser band applied 0.1 m deep along the centreline of the cane row. Given the observed rapid vertical water infiltration characteristics of this cane block soil, assumed here to result from the presence of many vertically oriented decaying cane roots, the postulate here is that this nitrate-N will be dispersed mainly in a downwards direction, rather than sideways. The view is that this effect would be enhanced by compacted soil in the adjacent cane inter-row areas, compaction induced by wheeled farm machinery used to cultivate, form, and plant the cane rows as well as apply fertiliser.
In initial discussions the grower suggested the use of soybeans companion planting as a treatment and this was incorporated into the experimental design. However, during treatment application operations by the grower, the soybean treatment was not applied. This changed the experimental design at a very late stage but still allowed for slightly unbalanced randomised replicated treatments. It is acknowledged here that implementing a large sugarcane-block field trial where substantial portions of the crop have low or zero fertiliser application carries with it significant financial risks for the grower. The final trial design was thus shaped by the collaborative needs of both the cane grower and the researcher.

**Field Trial Design**

On-site design of the treatment plots had to accommodate the existing farm equipment so the cane-block (approx. 67 m wide by 360 m) was divided up into plots three cane rows wide by the length of the field to suit the fertiliser spreader width. Rows were approximately 160 cm apart and formed into rounded raised beds approximately 30 cm high, planted with a single row of cane. Allowing for a two and three row edge-effect zone on the eastern and western sides respectively, 12 separate treatment plots ran the full 354 m length of the cane-block beginning from the west side (Figure 7-5). Plots were identified with permanent numbered stakes at the ends of rows.

The final field trial design was not fully implemented at the time treatments were applied to the cane block. A companion planting soy-bean treatment was left out of the trial. This resulted in the design being somewhat unbalanced but this did not impact on results or conclusions. Final plot treatments comprised: (i) Control: no treatment [Control]; (ii) Plant mix only [PM], (iii) Side Dress only [SD], and (iv) combined Plant Mix and Side Dress treatments [PM+SD]. These treatments were assigned on the cane-block in a randomised block fashion using the R statistical software package (R-Project, 2006). All treatments were replicated three times across the cane-block although only two untreated plots were incorporated as nil-fertiliser Control treatments.

The highest nitrogen amendment rate in the study trial was relatively low compared with current industry recommendations and common usage in this region. The field N-trial PM+ SD treatment equates to 63 kg N / ha compared with up to 200 kg N commonly used on plant-cane crops (Meier et al., 2003). Both the Plant-mix and Side-dress granular fertiliser applications were added to the soil in a narrow 5 cm continuous band approximately 15 cm below soil level in the centre of the cane row. All augured holes for soil sampling were positioned 20 cm to the side of the row centre.
When it became obvious that field trial N-treatments may not provide substantial excess soil nitrate to unequivocally demonstrate nitrate movement down the soil profile, a separate ‘high-nitrogen’ treated cane-block (Blk 706) was chosen for sampling and testing later in the trial. The edge rows of this cane-block received residual fertiliser left in the spreader after applications on other cane-blocks. This section of the cane-block had received approximately 230-kg N/ha from applied urea fertiliser (Quirk, 2006).

Sampling and analysis was carried out a number of times over the cane growth-harvest cycle. After pre-plant soil sampling in September 2006 to establish baseline soil parameter data for the N-trial, field trips were carried out in January, April, June, September and October 2007 as detailed in the fieldwork schedule in Appendix 9.

### 7.3.3 Field and Laboratory Methods

Given the detailed previous investigative work carried out at this locale, full description of the cane-block profile including soil analysis was not carried out. However, many profile descriptions were undertaken throughout the field trial period to gather specific geochemical and biophysical data relevant to the current study. These data included measurement of horizon depth; general colour including presence of mottles and inclusions such as roots, sand layers and shell material; measurement of soil pH and Eh; and some temperature readings. These are shown in Appendix 12.

#### 7.3.3.a Soil sampling, extraction, storage and transport

Samples were collected from the study cane-block profile and processed for analysis as described in Section 7.2.3. Sampling depths were: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 0.9, 1.0, 1.2, 1.5, and 1.7 metres in the soil profile.
7.3.3.b Geochemical analysis

(i) \textit{N-species analysis}

The variable results from the first nitrogen field trial using colourmetric soil-analysis procedures prompted review of analytical procedures for ammonium and nitrate ion analysis. A more accurate and stable analytic procedure based on steam distillation, first described by Bremner in 1965 (Bremner and Keeney, 1965) was adopted. This method is unaffected by various organic and inorganic substances usually present in soil extracts (Mulvaney, 1996).

The procedure uses steam distillation of a 2M KCl soil extract with MgO and Devarda’s alloy (Mulvaney, 1996). Sequential steam distillation is carried out on a single 15 ml aliquot of sample extract using the same apparatus for both exchangeable ammonium and then nitrate. Quantification of ammonium and nitrate is achieved by titration of the distillate with 0.0025 M H$_2$SO$_4$ using a boric acid indicator solution. Given the known reactivity of nitrite, it was not tested for here. The full procedure is described in Appendix 5.

Results are presented as ammonium-N $\mu$g NH$_4^+$-N g$^{-1}$ 

\textit{Oven Dry (OD) soil}. These are equivalent to parts per million (ppm) concentrations. References to background levels in the text refer to the pre-existing ion concentrations found in the untreated fallow cane-block prior to the N-trial.

7.3.4 Field Trial 2 Results

Compiled summary data-plots showing results of this nitrogen trial are presented in Figure 7-6 and Figure 7-7. Results are presented by treatment in the following sections.

7.3.4.a Pre-plant soil N-species levels

\textbf{September 2006} pre-plant ammonium-N and nitrate-N levels were relatively low in the cane-block soil [Figure 7-6 (a) and (b)]. Ammonium-N levels were 2.4 to 9.0 $\mu$g NH$_4^+$-N g$^{-1}$ OD-soil down the profile with the highest concentration 1.2 m below the surface in the transition zone. Pre-plant nitrate-N levels were also relatively low, being highest in the topsoil layers (16.3 $\mu$g NO$_3^-$-N g$^{-1}$ OD-soil) and then staying within the range 4.1 to 7.6 $\mu$g NO$_3^-$-N g$^{-1}$ OD-soil down the profile to 1.7 m.

\textbf{In January 2007}, approximately seven weeks after the \textit{Side-dress (SD)} final fertiliser application, the \textit{Control} soil showed slightly increased ammonium-N and nitrate-N levels relative to the pre-plant soil concentrations. Soil ammonium shows an anomalous higher concentration ‘bulge’ between 0.7 m and 1.7 m with a high reading of 22 $\mu$g NH$_4^+$-N g$^{-1}$ OD-soil at 1.2 m depth. This is more than double the pre-plant NH$_4^+$-N concentrations at this depth. Soil nitrate-N concentrations were relatively higher (e.g. a high of 60 $\mu$g N g$^{-1}$ OD) in the
topsoil above 0.5 m depth, with levels then declining with depth to generally below 10 μg NO₃⁻-N g⁻¹ OD at 1.6 m depth.

The April 2007 Control soil results show ammonium-N and nitrate-N levels reverting to low background concentrations of the pre-plant fallow cane-block and this also held for the September 2007 assays of the nil-treatment plots.

7.3.4.b Plant-mix + Side-dress (PM + SD) treatment

At all the sampling depths across all sampling periods for this treatment, ammonium-ion concentrations were similar to the control soil [Figure 7-6 (c) and (d)]. It shows low concentrations in the topsoil with slightly higher levels at depth. Plotted here for comparison is the September 2007 High-N cane-block soil results showing negligible amounts of ammonium remaining in the soil at the end of the crop cycle.

Nitrate concentrations showed variable but substantial response to nitrogen inputs. Relative to background soil-N levels of < 10 μg N g⁻¹ OD soil, the January 2007 nitrate levels peaked at 89 μg N g⁻¹ OD, then declined to 71 μg N g⁻¹ OD in April and then further to 48 μg N g⁻¹ OD soil in September 2007. Nitrate in the Sept.07 High-N cane-block soil was low in the upper profile, and then increased to 27 μg N g⁻¹ OD at 0.7 m before declining uniformly to around zero at 1.0 m depth.

7.3.4.c Plant-mix (PM) treatment

Soil assay results for ammonium and nitrate revealed outcomes similar to the Control treatment [see Figure 7-7 (e) and (f)], but with slightly raised levels of nitrate evident in the upper soil horizon early in the crop cycle. At and below 0.3 m depth, nitrate-N levels showed no significant differences over the three sampling periods. At 0.1 m depth the low nitrogen PM treatment unexpectedly tested marginally higher for nitrate in January 2007 (at 95 μg N g⁻¹ OD) than did the much higher nitrogen input PM + SD treatment. PM+SD nitrate-N levels at this 0.1 m depth declined to 59 and 12 μg N g⁻¹ OD soil in April and September 2007 respectively.

7.3.4.d High-N cane-block

An adjacent block treated with a high application rate of urea fertiliser (High-N cane-block), was sampled only in September 2007. At this time ammonium levels were almost undetectable. Residual nitrate increased with depth from 8 to 27 μg NO₃⁻-N g⁻¹ OD at 0.1 and 0.7 m depth respectively in the profile. Nitrate concentrations then declined sharply to <1.5 μg NO₃⁻-N g⁻¹ OD at 1.0 m depth and below. Thus, there was a general nitrate-N concentration increase from 0.1 to 0.7 m, then a rapid decline to very low levels at 1.0 m and lower in the profile.
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Figure 7-6: Field N-Trial 2: Soil profile NH$_4^+$ and NO$_3^-$ over a sugarcane crop cycle. Note: Error bars have been omitted for clarity
Figure 7-7: Field N-Trial 2: Soil profile NH$_4^+$ and NO$_3^-$ over a sugarcane crop cycle
7.3.4.e Side-dress treatment

The Side-dress fertiliser treatment produced the highest soil response for both ammonium and nitrate concentrations in the current-study field trials [Figure 7-7 (g) and (h)]. In January 2007, ammonium ion concentrations were highly elevated in the topsoil zone where urea was applied. At 0.1 m depth, ammonium -N levels rose from 69 µg N to a maximum of 183 µg N at 0.2 m before declining to 74 µg NH$_4^+$-N g$^{-1}$ OD at 0.3 m depth. Below this in the profile, ammonium -N levels declined to levels similar to the control soil at depth (i.e. <21 µg NH$_4^+$-N g$^{-1}$ OD).

Nitrate-N concentrations for the SD treatment showed a similar pattern to the ammonium-N distribution in the soil profile. Nitrate-N concentration of 123 µg NO$_3^-$-N g$^{-1}$ OD at 0.1 m soil depth was evident in January 2007. This rose to a high of 183 µg N at 0.2 m before declining to 117 µg N (0.3 m) and 34 µg NO$_3^-$-N g$^{-1}$ OD soil at 0.5m profile depth. Below 0.5 m depth, nitrate-N concentrations declined reasonably uniformly to low background levels <2 µg NO$_3^-$-N g$^{-1}$ OD at 0.8m below the surface.

7.3.4.f Harvest results

As with field trial 1, collection of cane harvest data was not a field trial 2 primary objective but information was gathered to potentially allow more informed interpretation of other data. Results show cane yields varied by treatment. The order from highest to lowest yield (green cane tonnes ± s.e.) was: PM + SD > PM only > SD only > Control (Figure 7-8 and Table 7-5)

The PM+SD treatment plots had the highest cane plot yield at 15.87 tonnes (93.4 t ha$^{-1}$). This was significantly different from the other three treatments (t(4) = 5.17, p < .005).

At 13.73 tonnes per plot, the PM only treatment did not differ significantly from the SD only plots yield but was significantly different from the Control plot [ t(4) = 2.203, p < .05].

![Figure 7-8: Plot type cane yields by nitrogen treatment (bars = ± s.e.)](image)
Table 7-5: Field Trial 2 cane harvest yield by nitrogen treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Green-Cane Plot Yield (tonnes)</th>
<th>Mean Green Cane Yield (tonnes per hectare)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM + SD</td>
<td>15.87 ± 0.40 s.e.</td>
<td>93.4</td>
</tr>
<tr>
<td>PM only</td>
<td>13.73 ± 0.20 s.e.</td>
<td>80.8</td>
</tr>
<tr>
<td>SD only</td>
<td>13.06 ± 0.66 s.e.</td>
<td>76.9</td>
</tr>
<tr>
<td>Control (nil-N)</td>
<td>12.19 ± 0.67 s.e.</td>
<td>71.7</td>
</tr>
</tbody>
</table>

7.3.5 *Field Trial 2 Discussion*

Nitrogen trial results here comprise a mix of the expected and the atypical. In terms of expected outcomes, the lowest N-treatment returned the lowest cane tonnage and the highest N-treatment gave the largest amount of green cane per plot. From initial urea fertiliser application in a narrow band at 0.1 m soil depth, it is evident from the results that N-species moved to deeper layers in the cane-block profile. It was also evident that N-species concentration diminishes with depth, reducing to near zero at the redoxcline at around 1.0 m depth. It is postulated here that this N-species movement down the profile could occur through both diffusive and rainfall infiltration processes.

Unexpected results included

(i) the observed high 0.1 m depth nitrate-N concentration in the *Control* plots in January 2007;

(ii) the lower nitrate-N concentrations at 0.1 m depth in the *PM+SD* treatment plots relative to the higher-N input *SD* treatment plots;

(iii) the low cane yield of the high nitrogen input *Side-dress* treatment plots, and

(iv) the high cane yield of the low N-input *Plant-mix* treatment relative to the higher-N input of the *SD* treatment.

Nitrogen transformation pathways are not always one-way or sequentially linear. They may involve complex interactive recycling and reverse reaction pathways. Soil nitrogen literature leads one to expect a general soil biogeochemical transformational progression from inorganic fertiliser nitrogen forms to ammonification and thence, via various ‘loss’ pathways, to N₂ gaseous emission. These latter pathways include: assimilation into microorganisms; uptake by plant roots; denitrification processes; attachment to and/or absorption into soil materials; and leaching in the profile. Although some results here appear to fit the ammonification-denitrification linear transformation model, other data does not.

Raised levels of soil nitrogen species at the fertiliser deposition site of 0.1 m depth in the cane row mound are to be expected. In at least some of the treatments results soil ammonium does appear in the upper profile within 12 weeks of fertiliser being applied. This is accompanied across almost all N-treatments by relatively higher levels of nitrate in the topsoil.
The fact that ammonium did not appear in some treatment results on the dates sampled does not predicate a conclusion that it was not produced, only that the sampling intervals may not have detected its appearance. The prevailing soil ecology conditions could have supported its rapid assimilation or transformation as soon as it became available in the soil.

Preliminary estimates of likely fertiliser-N contribution to the soil, calculated earlier in Section 7.3.2, aid evaluation of these nitrogen treatment results. In summary, these estimates of soil-N concentration are based on uniform distribution of applied nitrogen-N into an assumed 0.5 m x 1 m deep plume space below the cane row (Table 7-6).

Table 7-6: Estimated soil nitrogen-N concentrations by fertiliser treatment

<table>
<thead>
<tr>
<th>Field Trial 2 Nitrogen Treatment</th>
<th>Estimated Nitrogen-N concentration μg N/g OD soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>nil</td>
</tr>
<tr>
<td>Plant-mix (PM)</td>
<td>30</td>
</tr>
<tr>
<td>Side-dress (SD)</td>
<td>75</td>
</tr>
<tr>
<td>Plant-mix and Side-dress (PM+SD)</td>
<td>105</td>
</tr>
</tbody>
</table>

Although these estimates are acknowledged to be a simplification of postulated nitrogen distribution in the form of a vertically oriented plume rather than being uniformly distributed across the whole cane block to depths of around one metre, there is some justification for this modelling. The cane soil here has, over the past 40 – 60 years of continuous cane growing, been hydraulically highly compromised vertically by the existence of a multitude of old cane roots penetrating up to 1.8 m into the profile. In addition, soil compaction of the inter-row soil by heavy farm machinery (Reilly, 2001) could be expected to limit movement of fertiliser chemical laterally in the upper profile through loss of soil pore space in this zone. Separate mathematical modelling of this postulated dispersal based on ion concentration as a logarithmic function of distance from application point gave highly validating results when compared with the field trial 2 data obtained in this study.

**Soil ammonium**

Using the **January 2007 Control soil** background ammonium-N levels of around 10 μg-N g⁻¹ OD soil, a **PM only** treatment (17 kg N ha⁻¹) could potentially induce up to a 300 % increase on background N-levels to around 40 μg NH₄⁺ -N g⁻¹ OD soil. This did not show up in the soil testing for the **PM** treatment at this time. Nor did this happen in the **PM + SD** treatment with its greater estimated 105 μg-N g⁻¹ OD soil input. This raises the issue of possible early losses of **Plant-mix** treatment nitrogen in the cane soil through either plant root uptake, or biotic assimilatory or dissimilatory processes. It could also indicate that the initial 0.5 m² per cane-row metre soil-volume assumptions for diffusion/leaching of the added-N could be revised somewhat.
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Another potential $\text{NH}_4^+$-N loss pathway involves ion absorption onto clays (Di and Cameron, 2002). Whilst ammonia is generally reported as being not very mobile in soils, the results here show early heightened ammonium concentrations at depth in all treatment plots. These declined over the cane growing cycle. The frequency of soil testing here and the absence of testing long before and after this field trial limits informed conjecture about the causes of this phenomenon. The consistency of this effect across different treatment plots indicates some mechanism for ammonium to appear in the AASSS transition zone before or just after cane planting.

Very little $\text{NH}_4^+$-N was detected in the PM+SD treatment in sharp contrast with the January 2007 SD treatment where high ammonium levels were found at 0.2 m depth. This raises the query as to why did the SD treatment ammonium survive in the soil until the January 2007 field testing when the PM+SD treatment with its greater total nitrogen loading did not. One possible explanation could relate to the more balanced nutrient input of the Plant-mix fertiliser [8.5 % N, 9.4 % P, 26.5 % K and 0.8 % S]. This is more likely to support the growth of soil-bacteria and the cane-plant than the SD treatment urea [CO(NH$_2$)$_2$] with its limited plant and microbial nutrients.

Thus it is possible that microbial assimilation and plant uptake accounted for the topsoil-N differences observed between the SD treatment, and both the PM+SD and PM treatments. If this postulate has any validity, the PM+SD and PM treatments would be expected to outperform the SD treatment in harvest yield, and this did occur. The PM+SD cane yields were significantly higher ($p < .005$) than all other treatments.

Less than 12 weeks later in April 2007, the previously elevated ammonium-N levels in these SD treatment plots had declined to near-zero. This invites a conclusion that ammonium is relatively transient in these subtropical sugarcane soils. Assuming a normal pathway of ammonification through nitrosification to nitrification, January 2007 results showing high concentrations of both ammonium and nitrate in surface layers indicate these two N-species have been produced concurrently for some weeks to build up to such levels in the soil. Whilst soil ammonium is seen to be relatively transient, results suggest nitrate is more persistent. However nitrate was still subject to 54 % decline from 184 to 84 $\mu$g g$^{-1}$ OD soil in the SD treatment over a six month period to harvest. Soil nitrate loss at 0.1 m soil depth for other treatments is shown in Table 7-7.

<table>
<thead>
<tr>
<th>Fertiliser Nitrogen Treatment</th>
<th>Jan07 NO$_3^-$-N $\mu$g/g OD Concentration (0.1 m depth)</th>
<th>Sept07 NO$_3^-$-N $\mu$g/g OD Concentration (0.1 m depth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>PM+SD</td>
<td>89</td>
<td>48</td>
</tr>
<tr>
<td>PM</td>
<td>95</td>
<td>12</td>
</tr>
<tr>
<td>SD</td>
<td>184</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 7-7: Cane-soil nitrate-N concentration at 0.1 m depth
Soil Nitrate

Some weeks after urea fertiliser application, soil nitrate appeared at depth in the soil profile at much higher concentrations than general background levels. It is postulated here that the agent for this dispersal to around 0.9 m depth was rainfall infiltration from the soil surface. Previously presented rainfall and watertable data showed that single rain events greater than 15 mm can infiltrate to at least one metre soil depth in around 10 hours if the soil is relatively dry, and in approximately 5.5 hours when at field capacity. At least 200 mm of rainfall occurred in the field trial region in the two months prior to the January 2007 soil sampling with around 130 mm of this occurring in January. Seven rainfall events > 15 mm occurred between fertiliser application and the late January 2007 soil sampling date. This amount is the minimum rainfall required to percolate to the watertable at least 1.4 m depth. Existing conditions thus supported distribution of soil nitrate found in the field trial 2 results.

Of other note here is the relatively high concentration of nitrate-N at 0.1 m depth in the Control soil in January 2007, a time of high nitrate-N in all other treatments at this depth (Table 7-6 and 7-7). However it was subsequently discovered that the grower applied a nitrogen folia spray to the cane crop very early in the growing season and this could have contaminated the control soil in this way.

Relative to Control soil low background concentrations of < 20 µg N g⁻¹ OD soil, N-treatment results here show increased nitrate concentrations not only in the upper profile but also to some depth following fertiliser application. An unusual nitrate spike was observed at 1.7 m in the Control and PM+SD plots in January 2007. Apart from this, significant levels of soil nitrate were not found below 1.0 m depth in the AASS / PASS transition zone. Nitrate levels generally diminished down the profile and decline to very low levels at and below 1 m depth. Whilst there may be many contributing factors to these phenomena, three primary explanations are suggested here.

The first relates to a possible dilution or gradient effect with distance from the source which in this case is the urea deposition site 0.10 to 0.15 m below the top of the row planting mound. The second relates to the potential for both dissolved and solid soil organic carbon to serve as a substrate for nitrate reduction as nitrate leaches down the soil profile. This could account for some of the apparent SOC depletion observed in the profile between 0.4 and 1.0 m depth (Figure 4-3). Thirdly, if nitrate is not consumed in any of the preceding soil processes, it is possible for nitrate to act as an oxidant of sulfide in the absence or lack of oxygen. Previously described XRD testing showed the presence of pyrite (iron sulfide) steadily increasing in the profile from 0.7 m to its peak at approximately 1.6 m soil depth. Above 0.7 m depth, sulfide was not detected in the soil.

Although all three of the above processes may occur, the results here – particularly the High-N cane-block results for September 2007 - implicate sulfide from pyrite in the disappearance of
nitrate in the AASS profile. High-N nitrate shows a clear and sharp transition at 0.7 m and a complete disappearance of soil nitrate at 1 m depth. It is around 0.9-1 m that sharp transitions are also observed in soil pH and redox measurements, clay mineralogy, and soil carbon, indicating this to be a zone of high geochemical activity.

Largely due to the presence of the watertable and associated saturated capillary-rise zone, oxygen can become more limiting on approaching the PASS gel-clay zone. This is clearly demonstrated by the low Eh values at depth in the soil redox potential data (Figure 4-2 and Appendix 13) and the increasing presence of unoxidised pyritic gel-clay from 0.7 to 1.4 m profile depth. The soil is thus grading to being anaerobic. Where oxygen is absent, alternative electron acceptors may increasingly be preferred by assimilatory or dissimilatory microorganisms. Nitrate is one high energy oxidant that can serve as an electrochemically active electron acceptor under these sub-oxic/anoxic soil conditions.

Cane yields showed the PM+SD treatment significantly outperforming other treatments. Whilst the view here is that this was due to more efficient cycling of nitrogen by microbial populations, it is also possible that Potassium deficiency in the soil contributed to the higher performance of the PM+SD and PM treatments.

7.4 Concluding Comments

This nitrogen field trial investigation chapter provided substantial data on N-species concentrations in a cane-block over the course of a sugarcane crop cycle. Results also showed soil response to known inputs of nitrogen fertiliser and further provided information on background or residual nitrogen levels in these ASS cane soils.

Study results indicate nitrate moves down the soil profile. The suggestion here is that the relatively rapid infiltration of rainwater down these soil profiles, as shown in Section 6.2.4c, is primarily responsible for this nitrate translocation.

A finding of note here was the observed pronounced nitrate decline in the AASS transition zone from 0.7 to 1.0 m where pyrite concentration begins to increase from zero at 0.6 m. Associated with this decline were very low levels of soil nitrate below 1.0 m depth.

Given the observed close connection between increased nitrogen fertiliser inputs and greater nitrate leaching in the soil profile, the unanswered question here relates to the potential for this soil nitrate to act as an alternative electron acceptor in the redoxcline zone in these soils. The following chapter examines these potential interactions in the context of laboratory experiments specifically examining nitrate reduction coupled to pyrite oxidation in PASS soil under anoxic/sub-oxic conditions.
8 Nitrate Coupled Pyrite Oxidation

8.1 Introduction

Evaluating anaerobic nitrate reduction in the presence of pyrite oxidation at depth in an ASS profile is potentially problematic when soil structural heterogeneity, substrate complexity and non-specific reactivity of the main components are considered. From this experimental perspective, natural soil heterogeneity at the field site combined to make for a challenging environment in which to conduct in situ soil studies on rates of pyrite oxidation and electron donor contributors. This heterogeneity includes: natural variation in PASS pyrite concentration; observed geochemical variation in the transition zone; and variable depth of the ASS oxidation front. Consequently, a laboratory study was developed to provide specificity, to minimise the effects of unknown variables and to overcome some of the limitations of an in situ study, particularly addressing issues such as the effective extraction of small soil samples from specific depths in the profile without contamination and transformation. Despite the benefits offered by a more controlled environment, laboratory experimental work potentially introduces new issues – not least being the challenge of adequately recreating a field environment in a laboratory.

Any attempt to replicate field conditions in a laboratory setting runs the risk of oversimplifying of what is naturally a very complex biogeochemical system. However, as can occur in computer modelling (Floridi, 2007), iterative attempts at laboratory replication of a natural system can help us better understand complex field environments and this was the approach taken in this study. Staged experiments potentially provide enhanced understanding of the biogeochemical system of interest, allowing adaptive modifications to be made to experimental procedures.

This chapter follows the same form as the Field Study chapter 7 in outlining the laboratory study aims and objectives, followed by description of the experimental design, methods, results and a chapter-specific discussion. This chapter addresses another aspect of the study aim, that of investigating the impact of added nitrate on acid sulfate soil oxidation.

8.2 Experimental Study Aim

The aim of this chapter is to examine whether nitrate promotes increases rates of pyrite oxidation in unoxidised PASS gel-clay under sub-oxic/anoxic soil conditions. The specific objective of this laboratory test was to:

- Study the impact of added nitrate on pyrite oxidation in the laboratory using PASS gel-clay in replicated sub-oxic/anoxic incubations for periods up to 7 months.
The experimental work consisted of staged laboratory sub-oxic incubations of PASS gel-clay. Replicated untreated and nitrate-treated gel-clay samples were analysed for sulfide-S and nitrate-N after incubation periods of up to 215 days. This evaluation comprised three stages.

The **first stage** involved field-sampling and storing the un-oxidised PASS gel-clay. The **second procedure** comprised PASS gel-clay laboratory incubation with two treatments: (i) an *Untreated* deoxygenated incubation solution and (ii) an *N-treatment* deoxygenated nitrate-spiked incubation solution. The **third stage** involved analysis of sulfide-S and nitrate-N in incubation samples. The latter stage employed chemical digestion and sequential passive diffusion trap procedures for sulfide. Iodometric titration was used to determine the trapped sulfide.

### 8.3 Experimental Design

Any experiment involving complex and variable mixtures such as those found in soil necessarily needs to be designed in such a way as to limit the effects of that heterogeneity. Traditionally this is done through sample (or experimental unit) *replication*, as well as through *randomisation* in the trial layout order and/or in the way it is sampled (Mendenhall, 1979). In the study laboratory experiment, the implementation of *sample replication* as a design factor was reasonably simple to implement. The main concerns here were logistical considerations with respect to the number of samples processed to enable timely completion of the analysis. A minimum of three-fold replication was used in all instances of incubation, diffusion-trap procedures, standardisation and reagent strength checking and calibration.

Ideally, the replicated incubations would be set up using homogenous PASS gel-clay where the sub-sample soil properties and qualities were identical to each other. Normally this homogeneity is achieved with multiple soil samples from a field site which are then mixed thoroughly, with the aim that sub-samples would thereafter be very similar. Given the reactivity of the PASS soil, this method was possible but not advisable. The approach in this study was to use as small a bulk sample as possible on the understanding that its properties should then be reasonable uniform with preceding and following samples. Therefore, the primary requirement here was to obtain a bulk sample of unoxidised gel-clay with uniform pyrite content, and store and dispense this in such a way as to ensure sample uniformity and integrity over periods of months.

However, given the manner in which the iron sulfide was formed *in situ*, the concentration and physical form of the PASS gel-clay pyrite potentially varies over scales of centimetres to metres in the ASS profile (Dent, 1986b). There appears to have been little formal assessment or reporting of these properties to date. If valid, this micro- and macro-scale variability could compromise experimental work of this type using small 1 g gel-clay samples as the incubated experimental unit. One solution to this dilemma would be to use an artificial soil with added
pyrite. This was considered liable to introduce too many other variables and compromise effective replication of field conditions in the study soil system being examined.

The solution adopted was to nominally use one bulk sample and a variation on ‘matched-pairs’ sampling design to allow matched-pair incubation treatments. Bulk gel-clay samples (1.4 – 1.6 m depth) were collected from a relatively localised area in the centre of the cane-block and stored anaerobically in a small number of sealed 50 mm diameter polyethylene tubes. Sub-samples for the incubation experiment could be taken from these bulk-sample tubes by extrusion through a small eight mm diameter nipple at one end. Working on the assumption that 1 g sequentially extruded sub-samples could reasonably be expected to contain similar pyrite concentrations, the incubations were set up using extruded accurately weighed approximately 1 g gel-clay sub-samples in alternating untreated and treated incubation tubes. Thus, any change or drift in bulk soil pyrite concentration should theoretically be reflected in both paired results.

8.4 Methods and Procedures

This experimental work drew on many published sources to inform and integrate the methodologies and procedures used here. While a number of procedures were used unchanged, many methods and items of equipment were specifically developed to serve the challenges of this work. Variations on standard methods are explicitly stated, and ambiguous information in standard methods will be highlighted along with specific procedures adopted here to overcome these limitations.

Methodologies for the four stages of the experimental work are described in this section. PASS gel-clay sampling and storage is discussed first, followed by description of the incubation procedures, the digestion and diffusion method and finally the iodometric titration methods conclude this section.

8.4.1 PASS Sampling and Storage

Subsoil potential acid sulfate soil at the study site exists below 1.3 m profile depth in an anoxic soil environment. This moist, mostly saturated, pyritic gel-clay material is relatively chemically stable within the soil profile when kept below the watertable, but is liable to biochemical degradation when exposed to oxidants. To meet the analytical requirement for minimally undisturbed bulk soil samples, an initial objective was to retrieve PASS gel-clay from around 1.5 m deep within the soil profile and store this so as to minimise any physical or biochemical transformation.
Previous methods used by researchers to collect PASS gel-clay for later analysis focussed on two main approaches: (i) bulk or block sampling: that is, collecting a large integral mass of soil, wrapping and cold-storing, and then using the un-degraded inner part after discarding outer layers, and (ii) freezing smaller bulk samples in polypropylene or polyethylene sealable bags to stabilise them during long term storage (Kinsela and Melville, 2004a). Specific requirements here negated use of either of these methods. Large bulk sampling volumes present challenges in maintaining geochemical integrity of the bulk soil over periods of months. Freezing was not an option due to potential impacts on soil flora populations. Freezing can also affect soil nitrogen species (Clough et al., 2001), induce potential changes to gel-clay soil structure and present challenges in extracting small samples without partially unfreezing and risking chemical change to the whole bulk sample.

Any storage medium ideally needed be: (i) highly gas impermeable (i.e. thick-walled), and (ii) capable of being totally enclosed and sealed from the atmosphere yet allowing simple but controlled access to small gel-clay sample volumes without loss of anaerobic integrity of the container or the sample. It also needed to have properties of toughness and high chemical inertness. For its technical properties, cost effectiveness and ready availability, polyethylene (PE) plastic tubes were chosen here.

8.4.1.a  Bulk sampling procedure

High density polyethylene (HDPE) tubes (50 mm diameter by 200 mm long) were used to both collect and store the gel-clay samples. This method avoided double-handling and allowed samples to be minimally disturbed whilst in storage. The selected tubes were closed at one end and incorporated a 12 mm diameter threaded nipple. The other end of the tube was fitted with a gas tight plunger cap that sealed the open-end effectively when in place. A purpose made 2 m long hollow rod assembly that screwed to the collection tube allowed insertion of the whole assembly into the soil up to 2 m depth.

A 100 mm bucket auger was used to bore down to the chosen collection depth. The sampling tube plus its extension tube was then inserted to this fresh gel-clay surface. After the collection tube system was flushed with high purity Argon gas (BOC, 2008) to expel residual air and any soil water in the tubing, a vacuum of 0.7 bar was applied to the apparatus allowing atmospheric pressure to assist the tube’s insertion into the very moist clay. Once filled, the tube was withdrawn and sealed. Bulk sample tubes were packed in a tough resealable polythene bag, evacuated, and stored in a cooler at around 1-3 degrees C. Five tubes of gel-clay were collected from adjacent separately augered holes into the PASS soil zone 1.4 -1.6 m below ground level.
Bulk soil samples were kept refrigerated at 2-3 °C until required. Incubation and analysis subsamples were taken from individual tubes by applying pressure to the internal plunger which resulted in a small cylindrical plug of moist clay (approx. 7 mm diameter) being expelled from the tube nipple end. Soil moisture content was determined gravimetrically by triple-replicated drying of 6 g. sub-samples at 105 °C for a minimum of 24hrs until constant mass was achieved.

### 8.4.2 Reagent Preparation and Quality Control

Aside from the reagents used for sulfide analysis, sulfide standards were required in the study to standardise the sulfide analytical procedure. In addition, nitrate standards were used in the evaluation of nitrate in the incubation solution. Primary chemicals and reagents used were generally *Analytical Reagent* (AR) grade and secondary reagents made up from these as required following quality control procedures set out in Methods of Soil Analysis – Chemical Methods (Klesta and Bartz, 1996).

Sulfide compounds, even in dried inorganic reagent form, are unstable over time and present challenges when used as calibration standards. Initial testing with a synthetic pyrite sample and sodium sulfide nonahydrate gave variable. Finely ground high purity Zinc Sulfide (Chem-Supply, 2009b) was acquired and ultimately used for calibration standards in the analyses with suitably consistent results (e.g. 94 ± 2.6 % to 98 ± 0.6 % recovery).

Use of AR grade Potassium Nitrate allowed standard nitrate solutions to be made up stoichiometrically for nitrate spiking of the pyrite incubation tubes. This standard solution was prepared in two litre batches in brown glass reagent bottles to 2000 μg NO$_3^-$ -N ml$^{-1}$ concentration and stored in the dark at 3 °C. Nitrate ion concentration checking and testing was carried out on a Horiba C-141 selective ion nitrate meter (Horiba, 2006a). This was calibrated before every use with both proprietary standards and in-house potassium nitrate calibration standards made up every few weeks and kept in dark bottles at 3 °C. Comparison of new and old standards showed nitrate standard deterioration to be negligible over these time periods.

Acidity and alkalinity was checked and controlled generally with a regularly calibrated *Horiba D-53* multifunction pH meter (Horiba, 2006b) with an *Ionode IJ44* double-junction pH probe (Ionode, 2007). Oxidation-reduction potential (ORP) was measured using the same Horiba meter with an Ionode IJ64 ORP electrode (Ionode, 2007). All Horiba meter pH and ORP readings were temperature compensated to 25 °C and the redox potential and pH sensors calibrated before use using proprietary solutions. An *Orion 5-Star Plus* multifunction meter with DO and Electrical Conductivity (EC) electrodes (Thermo, 2008) allowed checking of oxygen saturation and conductivity. Both probes were re-calibrated prior to each daily use according to the Orion instruction manual (Thermo, 2007). Titration reagents for iodometric sulfide analysis were created from analytical grade reagents including the Standard 0.1 M Thiosulfate solution acquired to enable accurate standardisation of the iodine titration solution.
8.4.2.1 Deoxygenation

Effective anaerobic incubation and analysis procedures require rigorous attention to minimising inappropriate sources of oxidative degradation of the analytes and reagents. One of the most reactive oxidant sources in these systems is oxygen.

Solution deoxygenation methods range from traditional methods such as boiling; low-pressure de-aeration methods; purging with inert gases (e.g., Venetti and Orfe, 1981, Rollie et al., 1987, Butler et al., 1994); chemical reduction methods and membrane techniques (Moscin et al., 1995); to more modern methods using electrochemistry (Tamminen et al., 1996). As the lowest limit of deoxygenation using inert gas bubbling methods is stated to be in the order of 200 ppb (Butler et al., 1994), cited in (Moscin et al., 1995) the use of inert gas sparging was considered adequate here to eliminate the bulk of dissolved oxygen from solutions used in this laboratory work.

The current study experimental procedures involved a number of sub-oxic redox reactions during both the incubation and analytical phases of this work which could potentially be affected by unwanted oxidants. Rogue oxidation risks were perceived to fall into five main categories: (i) chemical redox reactions from inappropriate reagent preparation, (ii) gaseous and dissolved oxygen degradation of reagents and reaction products, (iii) degradation of the gel-clay samples by gaseous oxygen and/or dissolved oxygen in reagents, (iv) reagent combination and sequencing issues, and (v) brief oxygen exposure by the gel-clay sample during incubation tube setup.

The first three of the above risks largely involves dissolved oxygen in solution, and two approaches were adopted to minimise this. The first approach involved reagent preparation with deoxygenated Ultra-Pure (UP) Millipore water, and the second technique involved final deoxygenation of reagents after they were mixed.

Using the nitrogen sparging method (Rollie et al., 1987, Butler et al., 1994), 15 litre batches of Millipore UP water (18.2 MΩ per cm) were deoxygenated to <0.7% oxygen saturation using high purity nitrogen (99.99%) at a flow rate of approximately two litres per minute for one hour. Oxygen concentration was monitored using an Orion 5-Star Plus meter with a 08305MD dissolved oxygen (DO) probe (Thermo, 2008).

Each 15 litre deoxygenated UP water batch was subject to the same procedure and kept oxygen free during water extraction through the use of a nitrogen-filled headspace supplemented by a nitrogen-filled expansion / contraction bag attached to a stop-cocked cap fitting. These expansion/contraction bags were constructed from laminated gas-impermeable material to minimise contamination of their inert gas contents with oxygen. As water was withdrawn, inert gas was drawn into the headspace to maintain the anoxic headspace atmosphere. A similar treatment was applied to each reagent for incubation, diffusion, and iodometric titration procedures using the same purging techniques. Mixed reagents were subject to final oxygen purging in 1 and 2.5 litre glass reagent bottles fitted with O-ringed caps, stopcocks and internal Teflon tubes.
The fourth risk, that of reagent combination and sequencing issue, arose in the context of iodometric titration, and the procedure adopted to minimise this risk is discussed later in this section. The fifth issue involved exposure of the gel-clay sample to the open air for less than 30 seconds during extrusion and weighing prior to insertion into the incubation tube and purging with inert gas. Given the known relatively slow oxidation rate of these pyritic gel-clays, the assumption here was that significant deterioration of the gel-clay would not occur in this period.

8.4.2.b Anoxic transfer procedures

Transferring samples and reagents from one container to another poses risks of contaminating the reagent and reagent containers with gaseous oxygen. This was avoided using three main techniques. The first involved the use of stop-cocked deoxygenated reagent containers with an inert gas headspace. Secondly, transfer of reagent solution was carried out using gas-tight syringes after first injecting a similar volume of inert gas into the source container headspace. The third technique, used when the receiving solution / vessel was open to the air, made use of under-surface liquid dispensing to avoid contact with oxygen in the transfer.

8.4.3 Gel-clay Incubation

In this context, incubation refers to the procedure of maintaining a soil sample under specific controlled environmental and geochemical conditions in order to evaluate a response to those conditions. Incubations used approximately one gram sub-samples of moist PASS gel-clay in small polyethylene (PE) cups within flooded 25 ml polypropylene (PP) tubes sealed at both ends by two way stop cocks (Figure 8-1). Incubation tubes were uniquely and permanently numbered (1 to 74) and stored on matching numbered clips on a timber and steel frame. This frame was designed to fit into a lightproof box of approximately 100 W x 100 H x 60 D cm dimensions. All the-study gel-clay anaerobic incubations were carried out in the absence of light to exclude the possibility of reactions by phototrophic microorganisms.

Procedures

Approximately one gram sub-samples of PASS gel-clay were extruded from the bulk soil tube into a pre-weighed small purpose-made polypropylene cup and accurately weighed again to determine the mass of soil. A stopcock-fitted plug sealed the incubation tube after the cup was inserted and purged of oxygen using high-purity nitrogen. This whole procedure took less than 30 seconds, minimising pyrite exposure to oxygen. Using a transfer syringe with Luer fittings
the incubation tube was then filled completely from the base with the appropriate deoxygenated treatment solution. Incubation of these samples occurred in the dark for the selected period.

Incubation enclosure temperature was monitored using an iButton temperature logger previously described in field work section 6.1. As the incubation enclosure was kept in a temperature-mediated (not temperature-controlled) room, incubation temperature varied inside the lightproof enclosure, but variations closely matched the previously ascertained temperature range of the cane-block subsoil (Section 6.1.3). Maximum temperature reached in the enclosure was 25.0 °C, minimum temperature 14.5 °C with the mean temperature being 20.4 °C (see Appendix 11).

To minimise sub-sampling errors and errors due to soil heterogeneity in the collected bulk gel-clay, incubation tubes were loaded with PASS gel-clay in sequential alternating pairs with Untreated and N-treated incubation solution. Incubation batches typically contained 24 incubation tubes of which 12 were untreated and the other 12 treated. Three paired (i.e. six) incubation tubes were withdrawn from the incubation box at each sampling / analysis date. These were analysed for sulfide-S and nitrate-N content along with three fresh gel-clay Controls and three sulfide-S calibration standards derived from high purity KNO₃. Purpose designed laboratory booking sheets allowed accurate tracking of incubation samples and procedures.

8.4.3.b Incubation program

Due to initial lack of information regarding the kinetics of the postulated reaction, an iterative or adaptive approach was adopted to develop the incubation procedures. Incubation experiments here were run in consecutive batches using only two treatments, as follows:

1. A Nil-nitrogen-treatment consisting only of deoxygenated incubation solution (See Table 8-1 and Appendix 6), and

2. A Nitrate-N treatment consisting of the identical incubation solution as in the Nil-nitrogen treatment, plus added nitrate. See Table 8-1 for specifications of each batch.

Whilst it is acknowledged that ‘normal’ soil nitrate concentrations in these soils are likely to be in the order of < 20 μg NO₃-N ml⁻¹, the postulate here is that the banded fertiliser would disperse downward in a relatively narrow plume under the cane row. Therefore, it was considered feasible that study nitrate concentrations actually found in the upper soil layers (i.e. approx. 200 μg NO₃-N g⁻¹ OD soil) could potentially leach down and react with pyrite lower in the soil profile. In these soils in the AASS zone this equates to around 80 μg NO₃-N ml⁻¹, the N-treatment concentration used for initial incubations. Due to large initial sulfide losses in both the treated and untreated incubation samples in Incubation 2 giving ambiguous results, a ten-fold more concentrated N-treatment in Incubation 3 provided a greater reserve of nitrate-N to allow the incubation to run longer and potentially provide a clearer outcome.

Both treatments were incubated in a light-proof box for varying periods before six incubated samples (i.e. 3 Untreated, and 3 N-treated) were withdrawn for analysis and testing.
Table 8.1: Gel-clay incubation parameters

<table>
<thead>
<tr>
<th>Batch</th>
<th>Untreated Incubation solution</th>
<th>N-treated Incubation solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation 1</td>
<td>Deoxygenated UltraPure (UP) Millipore water</td>
<td>Deoxygenated UP Millipore water with 80 μg NO$_3^-$ - N ml$^{-1}$</td>
</tr>
<tr>
<td>Incubation 2</td>
<td>Deoxygenated UP Millipore water made up into a synthetic groundwater solution</td>
<td>Deoxygenated UP Millipore water made up into a synthetic groundwater solution with 80 μg NO$_3^-$ - N ml$^{-1}$</td>
</tr>
<tr>
<td>Incubation 3</td>
<td>Deoxygenated UP Millipore water made up into a synthetic groundwater solution</td>
<td>Deoxygenated UP Millipore water made up into a synthetic groundwater solution with 800 μg NO$_3^-$ - N ml$^{-1}$</td>
</tr>
</tbody>
</table>

A preliminary gel-clay incubation (Incubation 1) was run using deoxygenated high-purity de-ionised water. Results drew attention to the need to incubate PASS samples in a salt solution to avoid dispersion of the gel-clay in the untreated sample. A synthetic groundwater solution was therefore created to act as the incubation solution. By necessity this was a simplified version of the actual groundwater as reported by van Oploo (van Oploo, 2000) but the electrical conductivity (EC) and main cation and anion proportions were nominally similar to the natural groundwater solution. See Appendix 6 for details of this solution.

A second incubation experiment (Incubation 2) using this synthetic groundwater gave relatively more consistent results enabling reaction kinetics to be evaluated along with oxidising substrate availability factors.

The third incubation (Incubation 3) with a higher nitrate-N concentration treatment provided a more effective non-limiting biogeochemical environment in which to clarify the core aim of this part of the study. This higher nitrate-N treatment was implemented to compensate for the postulated ‘capture’ and transformation of incubation solution nitrate by PASS gel-clay organic matter (soil carbon), observed during the second incubation trial.

8.4.4 Digestion and Diffusion Analytical Procedures

Following withdrawal from the incubation enclosure, incubated gel-clay samples were analysed for sulfide concentration. Suitable analytical methods would ideally allow for use of wet gel-clay samples, use readily available chemicals and reagents, and use obtainable and reasonably simply laboratory apparatus. The only standard sulfide analysis procedure available at the time was the chromium reducible sulfur (CRS) purge and trap method of Sullivan et al. (Sullivan et al., 2000) which requires expensive apparatus and considerable time to process large sample numbers. Another method more suited to the current study was found and developed.

8.4.4.a Sulfide determination

The method adopted and adapted here in 2006-07 was purportedly first published by Hsieh and Yang (Hsieh and Yang, 1989), and later refined by Hsieh and Shieh (Hsieh and Shieh, 1997). However, this method appears remarkably similar to an earlier unacknowledged procedure
described by Sugino (Sugino, 1983). The Hsieh and Shieh method was chosen for its capability to analytically discriminate monosulfide from disulfide and elemental sulfur species, and to use moist whole soil samples without a distillation step as required in the Sullivan (Sullivan et al., 1998) procedure. After the current laboratory study was substantially complete, a paper by Burton et al. (Burton et al., 2008) touted further refinements to the Hsieh digestion / diffusion method as a replacement for the Sullivan et al. (2000) CRS procedure.

The full Hsieh and Shieh (1997) sequential digestion-diffusion procedure first tests for acid volatile sulfur (AVS) detecting acid-soluble sulfide and monosulfides, then chromium reducible sulfur (CRS) analysing for acid-insoluble metal disulfide forms such as pyrite. The third step was analyses for elemental sulfur (ES). The sulfur species are released from the soil sample as hydrogen sulfide (H₂S) that is subsequently diffusively trapped in an alkaline zinc acetate solution in the form of zinc sulfide, an insoluble white precipitate in alkaline solutions. Using iodometric titration, the trapping solution containing zinc sulfide is transferred into an acidic 0.0250N iodine solution where the zinc sulfide is solubilised by the acid and reacts with the iodine. Residual iodine is then volumetrically determined by back-titration with standard 0.0250N sodium thiosulfate solution. The modified iodometric titration procedure is detailed in Appendix 7.

Extensive initial testing of the procedure using field pyritic gel-clay samples showed that acid volatile sulfur (i.e. monosulfides) and elemental sulfur were at undetectable concentrations in these samples. As a consequence, the full sequence of digestions using different reagents was not carried out. A combined AVS/CRS procedure was the only procedure used to test for pyrite concentration in the gel-clay sample. Separate sequential digestions were carried out towards the end of the experimental work to again check for the presence of monosulfides in a new bulk sample of PASS gel-clay. These were found not to be present.

Incubated gel-clay sample replicates were transferred into 125 ml Erlenmeyer flasks into which an open glass vial containing 15 ml alkaline zinc trap-solution was placed (Figure 8-2). The flask was then sealed with an O-ringed polyethylene cap fitted with dual stop-cocks and one internal Teflon tube extending to the flask base. A Teflon tube fitted to the second stopcock extended part way into the flask. Each flask was purged of oxygen using high purity argon gas for five minutes, then depressurised to around 35 cm of mercury (0.45 bar) to allow for addition of digestion reagents by syringe and expansion of resulting digestion gases. After digestion reagents were added, the flasks were agitated for several hours on a shaker then stored in the light-proof incubation
enclosure for the duration. After the nominated digestion period (48 hrs for CRS), flasks were withdrawn from the incubation box and the trap solution vial removed and capped with the appropriately numbered screw cap.

Trap solutions were then analysed for sulfide-S using a modified iodometric titration procedure. Sulfide-S analyte is a highly reactive reduced substance which, in the words of one commercial laboratory manager, is “always a tricky task” to analyse. Numerous methods are available, including colourmetric, potentiometric, titrametric, chromatographic, and mass spectrometry (if no other S-species are present).

8.4.4.b Iodometric titration

The Hsieh and Shieh method adopted here nominated iodometric titration to quantify sample sulfide. Rather than describe this procedure, their paper simply referenced the APHA method cited below. However, there are several widely cited and used ‘standard’ iodometric methods, including: Vogel (Vogel, 1961), APHA (Franson, 1985), and USEPA (EPA, 1996). Given the ambiguity, inaccuracy and contradiction evident in and between these methods, the Hsieh and Shieh ((Hsieh and Shieh, 1997)) procedure was modified in several ways to correct these, as described in Section 2.3.3 of the review of literature. In 1999, Pawlak and Pawlak reviewed iodometric titration methods and after extensive testing, these researchers highlighted several key problems and offered modifications to techniques to resolve these. These are, described below in the first and second points. Other procedural issues identified and modified in the current study are detailed as items three and four here.

The first potential problem identified relates to timing of the acidification of the zinc sulfide precipitate in the trap solution. If this is done in the highly alkaline trapping solution by addition of concentrated hydrochloric acid (HCl), then hydrogen sulfide release (from the zinc sulfide) is likely to occur and be lost to the analysis. The zinc sulfate precipitate must therefore not be acidified with HCl before being transferred into the acidified iodine solution.

The second issue relates to the high alkalinity of the zinc acetate solution and the neutralising effect of this when and how it is introduced into the acidified iodine solution, potentially creating conditions for a sulfate side reaction to occur. A procedure modification involves use of glacial acetic acid to decrease the alkalinity of the zinc sulfide trap solution from pH 13 to between pH 5 and 6 to maintain the acid integrity of the iodine solution when the trap solution is added to it. Acetic acid is used here as zinc sulfide remains insoluble only in this acid.

A third issue identified in the current work relates to the difficulties in transferring all of the insoluble zinc sulfide precipitate into the acidified iodine solution when significant portions of it are stuck to the sides of the glass trapping vial. Another procedural modification here was to add a measured small volume of iodine solution (say 5 ml) plus 5 ml of 2N HCl acid (in that order) into the empty vial to solubilise the ZnS precipitate and allow it to react with the iodine. This
additional iodine solution volume is simply incorporated into the titration solution (and calculations) to obtain the correct amount of sulfide-S in the gel-clay incubation sample.

A fourth issue, highlighted in Vogel (Vogel, 1961), relates to the use of more dilute iodine solution than the 0.0250N specified in the Hsieh (1989) APHA procedure. Vogel suggested iodine concentration of < 0.2N in the titration would be more appropriate "for reasonably satisfactory results", and even advocated dilutions as low as 0.01N. As a consequence, the 0.0250N iodine solution here was diluted 1:1 with DI water after it was accurately dispensed into the titration flask, giving a final iodine dilution of 0.0125N.

The full modified procedure used in the current study is detailed in Appendix 7.

8.4.5 Measuring equipment

A temperature compensated multifunction meter (Horiba, 2006b) with Ionde pH and ORP electrodes (Ionde, 2007) was used to measure acidity and oxidation-reduction potential (ORP). Validation of incubation solution initial nitrate-N concentration and testing for incubation solutions residual nitrate was done using a Horiba nitrate-specific ion meter (Horiba, 2006a). This specific ion meter was calibrated using a made-up primary nitrate standard (10 mg Nitrate-N ml⁻¹) from A.R. grade potassium nitrate, along with two secondary working standards (100 μg Nitrate-N ml⁻¹ and 1000 μg Nitrate-N ml⁻¹). These calibration standards were checked against proprietary nitrate-N standards (Horiba, 2006a). Working N-standards were freshly created from the refrigerated primary standard on each use of the nitrate-ion electrode. Primary standards were regularly checked against new primary standards, generally at three monthly intervals, and replaced as required.

Nitrogen-spiked incubation solutions of 80 μg and 800 μg Nitrate-N ml⁻¹ were mixed from the primary N-standard for use in each incubation experiment. On being pulled from the experiment at the end of each incubation period, tube solutions were transferred into a numbered 35 ml centrifuge tube and re-checked for nitrate concentration using the pre-calibrated specific nitrate ion meter. Redox potential and pH was also assessed at this time. The condition of the gel-clay was also noted in terms of colour, texture and dispersion.

8.5 Results and Discussion

8.5.1 Incubation 1

This preliminary incubation was set up using two treatments: (i) untreated deoxygenated Ultra Pure Millipore DI water, and (ii) deoxygenated Ultra Pure Millipore DI water spiked with nitrate to create an 80 μg Nitrate-N ml⁻¹ solution. In this instance, six incubation tubes were used for each treatment. This incubation was terminated after 38 days due to substantial dispersion occurring in the untreated gel-clay sample. Over this incubation period these two treatments showed no significant sulfide-S decline within or between treatments, although the results did
show a non-significant sulfide-S decline in the dispersed untreated sample relative to the undispersed \textit{N-treated} one. Results for Incubation 1 are shown in Table 8-2.

Sulfide-S values have been standardised to show sulfide-S (mg) per gram of oven dry soil, and do not indicate the actual individual or mean sulfide-S mass in incubation samples. This value will be calculated and reported for later incubation results.

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Incubation Treatment</th>
<th>Soil type</th>
<th>Sulfide-S (mg g$^{-1}$ OD soil)</th>
<th>Standard Error (s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Untreated DI</td>
<td>PASS gel-clay</td>
<td>21.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0</td>
<td>Nitrate-spiked DI (80 \mu g N ml$^{-1}$)</td>
<td>PASS gel-clay</td>
<td>21.5</td>
<td>0.5</td>
</tr>
<tr>
<td>38</td>
<td>Untreated DI</td>
<td>PASS gel-clay</td>
<td>20.8</td>
<td>0.7</td>
</tr>
<tr>
<td>38</td>
<td>Nitrate-spiked DI (80 \mu g N ml$^{-1}$)</td>
<td>PASS gel-clay</td>
<td>21.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Some brown residue was evident in both the untreated and treated sample cups at the end of the incubation period. Incubation 2 was set up using a salt solution resembling the groundwater at the field site.

Although inconclusive in terms of amount of sample sulfide loss, the Incubation 1 experiment over 38 days highlighted the importance of ionic soil water solutions in stabilising subsoil gel-clays. \textit{Untreated} incubation samples had dispersed completely into constituent mineral particles by day 38, whereas the \textit{N-treated} gel-clay largely maintained its original extruded cylindrical shape. It was concluded that the 80 \mu g N ml$^{-1}$ KNO$_3$ salt content in the N-treatment incubation solution was sufficient to maintain gel-clay form and structure.

Both sets of replicated treatment samples displayed evidence of mid-brown floc discolouration. In the dispersed clay \textit{Untreated} samples this was uniformly distributed throughout the sample. Brown colouration in minerals is indicative of iron oxidation products such as iron oxides (hematite Fe$_2$O$_3$ [moderate reddish-brown]) and iron oxyhydroxides (goethite $\alpha$-FeO(OH) [strong yellowish-brown] Lepidocrocite, $\gamma$-FeO(OH) [moderate orange], ferrihydrite Fe$_3$H$_4$O$_8$·4H$_2$O [brownish-orange]) (Scheinost and Schertmann, 1999). Thus, on colour alone, the oxidation products observed here could have been hematite, goethite, and/or ferrihydrite. Luther \textit{et al}. (Luther \textit{et al}., 1982) suggested that oxyhydroxides such as goethite and ferrihydrite are common oxidation products of pyrite.
8.5.2 Incubation 2

The second incubation experiment used PASS gel-clay subjected to two treatments: (untreated and treated with 80 µg nitrate-N ml⁻¹) with incubation periods of 27, 60, 88 and 133 days. All incubation tubes contained 25ml of deoxygenated water adjusted to 1.35 dS m⁻¹ (Appendix 6). At the end of each incubation period, sulfide-S, nitrate-N, pH and redox values were measured in incubation solution. At the start and at the end of each incubation period samples of freshly extruded PASS gel-clay (Control samples) were also analysed for sulfide-S. Given the natural chemical heterogeneity in soils, these control samples were expected to vary slightly in sulfide content, and this was evident in the results.

Results showed a decline in PASS gel-clay sulfide in the nitrate treated samples relative to the untreated incubations. This response was non-significant at all tested intervals. Onset of this treatment response occurred between day 27 and 60.

<table>
<thead>
<tr>
<th>Incubation Period (days)</th>
<th>Sulfide-S (mg g⁻¹) in Nil Nitrate-N Treatment (± s.e)</th>
<th>Sulfide-S (mg g⁻¹) in 80 µg g⁻¹ Nitrate-N treated Incub'n (± s.e)</th>
<th>Treatment Difference (Sulfide-S mg g⁻¹ OD soil) (± s.e)</th>
<th>Mean pH Untreated / Treated</th>
<th>Mean Eh [SHE] (mV) Untreated / Treated</th>
<th>Mean Nitrate-N (µg/ml) in incubation solution Untreated / Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>6.3 / 6.3</td>
<td>400 / 400</td>
<td>4 / 80</td>
</tr>
<tr>
<td>27</td>
<td>24.9 ± 0.8</td>
<td>25.3 ± 1.0</td>
<td>0.4 ± 0.9</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>60</td>
<td>19.5 ± 0.8</td>
<td>18.7 ± 0.6</td>
<td>0.8 ± 0.7</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>88</td>
<td>19.1 ± 0.7</td>
<td>17.8 ± 0.6</td>
<td>1.3 ± 0.6</td>
<td>4.8 / 4.8</td>
<td>416 / 419</td>
<td>5 / 60</td>
</tr>
<tr>
<td>133</td>
<td>18.5 ± 0.6</td>
<td>18.0 ± 0.8</td>
<td>0.5 ± 0.7</td>
<td>4.4 / 4.6</td>
<td>415 / 413</td>
<td>3 / 46</td>
</tr>
</tbody>
</table>

A summary of the Incubation 2 sulfide-S results is shown graphically (Figure 8-3) and numerically with other data (Table 8-3). Sulfide-S results shown here are reported in sulfide-S mg g⁻¹ OD soil. Exceptions to this are noted. Sample sulfide-S concentrations of around 25 mg S g⁻¹ OD were unexpectedly found at day 27, the first analysis date. These values are high relative to the previously tested sulfide-S concentration in this PASS gel-clay of between 19 - 21 mg S g⁻¹ OD. On day 27, these two treatments were not significantly different from each other.
At and after day 60 the *N-treated* incubations gave non-significant but consistently lower sulfur-S assay results relative to *Untreated* incubation samples. Solution pH, Eh, and nitrate concentration of the incubation solutions were recorded at day 0, and for the last two incubation periods (day 88 and 133). Incubation solution pH decreased over the incubation period, from around pH 6.3 at the start to approximately pH 4.5 after 133 days. On day 133, the untreated solution pH was 0.2 pH units lower than the nitrate treated solution (pH 4.4 and 4.6 respectively).

Redox potential of the incubation solutions increased during the incubation, but by a relatively small amount. The initial Eh reading of the deoxygenated incubation solutions was around 400 mV. The 88 day and 133 day incubation period solutions were measured at approximately 417 and 414 mV respectively, with relatively small differences between treated and untreated solutions.

Incubation solution nitrate-N declined over the 133 day period of this second incubation experiment. From an initial high of 80 μg nitrate-N ml⁻¹ (2.00 mg N per incubation tube) the concentration of nitrate-N ions fell to 60 and 46 μg N ml⁻¹ at 88 and 133 days respectively.

This equates to a net nitrate loss of 20 and 44 μg nitrate-N ml⁻¹ at 88 and 133 days, or 0.50 and 0.85 mg N respectively, shown in Figure 8-4. Although both sulfide and nitrate declined, the nitrate loss does not appear to be closely coupled to sulfide-S decline after day 60.

Using mean incubation-tube sulfide-S mass calculations, Figure 8-4 shows an overall decline of 2.1 mg (*Untreated*) and 2.2 mg (*N-treated*) with most of this occurring between day 0 and 27. Although nitrate was not tested at day 27 and 60, it had declined by 0.5 mg / tube by day 88 from an initial 2.0 mg N / tube. It declined a further 0.36 mg between day 88 and 133. Brown discoloration was evident on all gel-clay incubation samples.

This Incubation 2 experiment with synthetic groundwater solution used the same 80 μg N ml⁻¹ nitrate treatment as Incubation 1. Over 133 days both treatments again showed evidence of iron oxidation in the gel-clay soil sample. Substantial decline in sample sulfide-S was observed in both treatment samples, but with no significant difference between treatments. The *N-treated* samples tested consistently lower for sulfide-S after the first sampling period. Three key aspects were noted in these results.
1) No incubation samples were subject to dispersion. The synthetic groundwater solution used here had a similar EC value to the field site groundwater and effectively served to stabilise the gel-clay samples.

2) In actual mass terms, the total sulfide loss over the 133 day incubation was 2.1 and 2.2 mg sulfide-S g\(^{-1}\) OD in the Untreated and N-treated incubation tubes respectively. From Figure 8-4 it is apparent that most of this decline occurred between day 27 and day 60. Due to missing data, it is conservatively estimated that a loss of around 0.4 mg nitrate-N occurred over this same 33 day period. Of the three potential equations suggested by Rivett et al. (Rivett et al., 2008), the first one involving nitrate reduction of sulfide appears to be the more feasible (Equation 8-1):

\[
5\text{FeS}_2 + 14\text{NO}_3^- + 4\text{H}^+ \rightarrow 7\text{N}_2 + 10\text{SO}_4^{2-} + 5\text{Fe}^{2+} + 2\text{H}_2\text{O}
\]

By this reaction, one mole of sulfide-S reacts with 1.4 mole of nitrate-N. If all the consumed nitrate-N here (0.85 mg) were to redox couple with the incubation sample sulfide-S, then it would react with 1.39 mg of sulfide-S by day 133 in the N-treated sample. In fact, 2.2 mg sulfide-S was missing from N-treated samples including 2.1 mg S in the Untreated soils with no added nitrate. Nitrate declined along with sulfide in the N-treated incubation but sulfide also declined in the Untreated incubation, therefore nitrate is not the oxidant in the latter case.

Clearly other transformations are occurring here, one of which is appears to be pyrite oxidation. Therefore other electron acceptors must be active in this system. Based on reported interactions in previous work, possibilities must include oxygen, manganese, ferric iron, and sulfate, interacting singly or jointly. Given the thickness of the polypropylene incubation tubes, the medical grade stopcocks, and the tight O-ring seal at the plugged end, significant diffusion of oxygen into tubes was initially discounted, but in tests of polycarbonate (PC) film [125\(\mu\)m], polyethylene terephthalate (PET) film [25\(\mu\)m], polypropylene (PP) film [200\(\mu\)m], polyvinyl dichloride (PVDC) film [30\(\mu\)m] and aluminium foil [100\(\mu\)m], Labthink (Labthink, 2010) found that at 20 \(^\circ\)C, the PP film had an oxygen transfer rate (OTR) around 3 times higher that PET and PVDC film despite is greater thickness. In the Labthink tests, PC film had the highest gas permeability with an OTR nearly 3 times that of PP film at 20 \(^\circ\)C.

Although the incubation containers used in the current study were sterile medical grade polypropylene syringe tubes with 1 mm (1 000 \(\mu\)m) wall thickness, the view here is that over the incubation periods of this study, oxygen and other gaseous diffusion cannot be ignored as a possible factor in the observed responses.

It is suggested here that cyclic processes may also be occurring, where an initial transformation starts and feeds secondary reactions which then feed tertiary transformations, and so on. Not only may these reactions be sequenced, but multiple abiotic and bio-catalysed oxidation and reduction reactions may branch off and occur concurrently, including those
whereby some original reactants are regenerated. These issues will be discussed further in the next section on Incubation 3 results.

3) The third point relates to the high initial levels of sample sulfide-S in the treated and untreated gel-clay samples, around 25 mg sulfide-S g⁻¹ OD soil, observed at the initial day 27 testing period. This is nominally 5 mg sulfide-S g⁻¹ OD higher than the fresh gel-clay Control samples, equating to a 25 % increase over previously ascertained ‘background’ levels of gel-clay sulfide. That all six samples (i.e. three replicates in two treatments) tested around this value (with standard errors < ± 1.0 mg) raises queries about the uniformity of pyrite distribution in the gel-clay used as the bulk sample source. Analytical errors are ruled out due to the use of replicated sulfide standards and gel-clay ‘controls’ during the analysis procedures. This anomaly does highlight the issue of obtaining, storing and using a uniform anoxic pyritic-clay ‘standard’.

Pyrite exists in two main forms in soils and sediments (i) framboidal structures associated with bacterial colonies around sites of large organic matter fragment decomposition, and (ii) euhehedral micron-sized particulate pyrite (0.5 – 5 µm) distributed reasonable uniformly throughout sediments. Framboids have been reported in the 15 – 50 µm range and given their reported mode of creation it is possible these could be distributed unevenly throughout sediments to create the effects seen here. Given the limited and indeterminate response here, a third incubation experiment was set up using freshly sampled bulk gel-clay and a more concentrated 800 µg NO₃⁻-N ml⁻¹ incubation solution for the N-treatment incubation.

8.5.3 Incubation 3

This third Incubation used pyritic gel-clay samples from deeper in the PASS subsoil (1.5 versus 1.2 m depth), and a higher concentration of nitrate-spiked incubation solution treatment (800 µg nitrate-N/ ml). Incubation 3 ran for 215 days with four sub-sample assays in this period at days 14, 21, 76, and 215. At each assay period, incubation samples showed increasing evidence of discolouration from grey to mid-brown, as well as some structural slumping. N-treated samples displayed these properties the most and Untreated samples the least. Results showed greater sulfide-S decline than previous incubations. At day 76 and 215 significant differences between the two treatments were evident (Table 8-4 and 8-5 and Figure 8-5)

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Sulfide-S (mg S g⁻¹) in Control gel-clay (± s.e)</th>
<th>Sulfide-S (mg S g⁻¹) in Nil-N Treatment (± s.e)</th>
<th>Sulfide-S (mg S g⁻¹) in 800 µg ml⁻¹ Nitrate-N Treatment (± s.e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>20.4 ± 0.0</td>
<td>20.1 ± 0.1</td>
<td>20.1 ± 0.1</td>
</tr>
<tr>
<td>14 days</td>
<td>21.0 ± 0.1</td>
<td>18.0 ± 0.3</td>
<td>16.7 ± 0.8</td>
</tr>
<tr>
<td>21 days</td>
<td>19.8 ± 0.5</td>
<td>16.9 ± 0.3</td>
<td>16.1 ± 0.4</td>
</tr>
<tr>
<td>76 days</td>
<td>19.6 ± 0.2</td>
<td>17.1 ± 0.1</td>
<td>15.0 ± 0.5</td>
</tr>
<tr>
<td>215 days</td>
<td>19.6 ± 0.2</td>
<td>17.3 ± 0.1</td>
<td>13.3 ± 0.5</td>
</tr>
</tbody>
</table>
Three conspicuous aspects are noted in these results. The first concerns the raised sulfide concentration in the Control gel-clay sample at 14 days. Although this increase is relatively small at around 1 mg sulfide-S g⁻¹ OD, it is irregular compared with most of the Control gel-clay sulfide assays. The second issue relates to the significant and rapid sulfide-S decline in both treatment incubations in the first 21 days. The Untreated and N-treated incubation samples declined around 3 mg and 3.9 mg sulfide-S g⁻¹ OD gel-clay respectively in this period. The third point relates to the greater sulfide-S decline observed in N-treated samples relative to the Untreated incubations. This is obvious from day 14 onwards. At day 14 and 21 these differences are non-significant, but they are significant at day 76 and 215, indicating greater treated incubation sulfide loss of around 2 and 4 mg sulfide-S g⁻¹ OD respectively.

Solution pH, Eh and nitrate-N data was collected at each incubation sampling period and are shown in Table 8-5 and Figure 8-6. Incubation solution Eh rose significantly over the 215 day incubation period, from around 310 to 590 mV. Although the N-treated solution recorded slightly higher Eh readings than Untreated samples over the duration, these differences were not significant.
Untreated solution Eh showed little response in the first 14 days. From day 14 to 21 Eh in both treatments increased relatively rapidly, then rose more slowly to day 215. Solution redox potential values show a similar response to that seen in the nitrate-N concentrations in having three apparent stages of increase. A 1.9 mV day$^{-1}$ rise for the first 14 days contrasts with an 8.1 mV day$^{-1}$ increased rate over the next 9 days to day 21, with a tapering-off to a 0.9 mV day$^{-1}$ rise over the final 194 days.

Incubation solution pH generally declined over the 215 day period from pH 6.8 to 3.6 (N-treated) and to 4.0 (Untreated). The treatment pH difference at day 215 was significant [ $t(4) = 4.22, p<.01$]. Untreated solution pH changed relatively slowly to day 14, but thereafter declined at much the same rate as the N-treated solution although always consistently less than the Untreated solution. Mirroring the Eh record, the N-treated incubation solution declined 1.9 pH units in 24 days but then took another 194 days to decline a further 1.3 units to eventually reach pH 3.6 at day 215.

Figure 8-7 shows a data compilation of actual sulfide-S in incubation tubes compared with nitrate-N loss in the same tubes. Although sample standard errors are shown for the N-treated sulfide-S incubations, standard errors for the Untreated sulfide-S are so small (<0.17 mg) as to not register at this scale. Differences between Untreated and N-treated sulfide-S determinations are significant at day 75 (0.93 mg S) and at day 215 (1.78 mg S).
Two main modes of biogeochemical functioning were initially indicated in the data (Figure 8-7). The first mode appeared to run from day 0 to day 21, and the second from day 21 to day 215.

However calculation of incubation parameter rates of change, particularly that for sulfide-S decline and nitrate-N loss, indicated that the incubation system operated in two quite distinct biogeochemical modes from day 0 to 14, and day 14 to 215. These rate calculations are shown in an integrated table for Incubation 3 (Table 8-6). Darker tones indicate increased rate of activity or change.

Table 8-6: Rates of change of incubation system parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Rate of loss / gain per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0-14</td>
</tr>
<tr>
<td>Sulfide-S (mg)</td>
<td>Untreated</td>
<td>-0.351</td>
</tr>
<tr>
<td></td>
<td>N-treated</td>
<td>-0.350</td>
</tr>
<tr>
<td>Nitrate-N (mg)</td>
<td>Untreated</td>
<td>-0.048</td>
</tr>
<tr>
<td></td>
<td>N-treated</td>
<td>-0.049</td>
</tr>
<tr>
<td>Solution pH</td>
<td>Untreated</td>
<td>-0.020</td>
</tr>
<tr>
<td>Solution Eh (mV)</td>
<td>Untreated</td>
<td>0.214</td>
</tr>
<tr>
<td></td>
<td>N-treated</td>
<td>2.214</td>
</tr>
</tbody>
</table>

Assessing Table 8-6 from a general system activity perspective, it is obvious that the highest rate of sulfide-S loss occurred in both treatments during Period 1 from day 0 to 14. The rate of loss here in each treatment was virtually identical. This was accompanied in the N-treated solution by a moderate rate rise in solution Eh and a moderate rate rise in solution pH relative to the Untreated incubation solution.
Highest activity rates for Untreated and N-treated incubation solution pH and Eh and for N-treated solution nitrate-N were recorded during Period 2 (day 14 to 21). The rate of sulfide-S loss for both Untreated and N-treated incubations declined to around a third of that for the previous period. Incubation solution pH showed the highest rate of decrease (-0.17 pH units day\(^{-1}\)) in both treatments. In this same period the incubation solution Eh rate increased to +11.57 and +9.86 mV day\(^{-1}\) for Untreated and N-treated samples respectively. This was accompanied by the highest loss rate for nitrate-N of 0.58 mg day\(^{-1}\).

System activity generally slowed during the Period 3 (day 21 to 76). Sulfide concentrations changed with the N-treated solution sulfide-S rate falling to -0.004 mg day\(^{-1}\) but the Untreated solution sulfide-S change rate rose to +0.012 mg day\(^{-1}\) during this period indicating creation of sulfide. Period 4 (day 76 to 215) was also characterised by diminished rates of change in most parameters apart from the N-treated sulfide-S.

Comparison of activity rate ratios of Incubation 3 parameters can assist evaluation of system functioning (Table 8-7). This comparison table confirms the assessment of incubation Period 1 as having a quite different mode of activity compared with the following three periods from day 14 to 215. Colour coding generally shows ratios of the same order of magnitude. Lighter tones denote divergent values. The exception to this occurs in the ratios for N loss : S decline rate (N-treated) where the darker colour indicates N:S ratios closer to the 1.64 stoichiometric nitrate-N:sulfide-S ratio for nitrate coupled pyrite oxidation by Equation 2-24.

### Table 8-7: Incubation 3 activity-rate comparison

<table>
<thead>
<tr>
<th>Rate Ratio</th>
<th>Treatment</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Period 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0 to 14</td>
<td>Day 14 to 21</td>
<td>Day 21 to 76</td>
<td>Day 76 to 215</td>
</tr>
<tr>
<td>1 N loss : S decline</td>
<td>Untreated</td>
<td>0.14</td>
<td>4.90</td>
<td>10.25</td>
<td>2.88</td>
</tr>
<tr>
<td></td>
<td>N-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 S decline : pH decline</td>
<td>Untreated</td>
<td>17.55</td>
<td>0.63</td>
<td>-0.75</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>N-treated</td>
<td>7.14</td>
<td>0.70</td>
<td>0.33</td>
<td>1.60</td>
</tr>
<tr>
<td>3 N loss : pH decline</td>
<td>Untreated</td>
<td>0.98</td>
<td>3.43</td>
<td>3.42</td>
<td>4.60</td>
</tr>
<tr>
<td></td>
<td>N-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 S decline : Eh rise</td>
<td>Untreated</td>
<td>-1.640</td>
<td>-0.009</td>
<td>0.009</td>
<td>-0.003</td>
</tr>
<tr>
<td></td>
<td>N-treated</td>
<td>-0.158</td>
<td>-0.012</td>
<td>-0.004</td>
<td>-0.009</td>
</tr>
<tr>
<td>5 N loss : Eh rise</td>
<td>Untreated</td>
<td>-0.02</td>
<td>-0.06</td>
<td>-0.04</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>N-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

With the exception of the last row, it is clear when comparing activity-rate ratios that Period 1 incubation functioning was distinctly different compared with Periods 2 to 4.

In row 1 in Table 8-7, the stoichiometric ratio, nitrate-N:sulfide-S (N/S), for nitrate reduction coupled to pyrite oxidation (Equation 2-25) is 1.64. It is clear that this reaction was not (or not fully) operating during Period 1 with its N/S loss ratio of 0.14. Conversely, it is fully possible that this reaction was occurring in incubation Periods 2 to 4 with their N/S loss ratios well above
1.64. Further, in Periods 2 to 4 it is inferred that other nitrate consuming reactions must also have been occurring.

Row 3 in this table shows a moderately consistent relationship between pH decline and nitrate-N loss across all four incubation periods. However, less acidity is being created (relative to unit nitrate-N loss) as the incubation progresses. This could occur if other acid generating reactions, such as pyrite oxidation by O$_2$ (Equation 2-13) or Fe$^{3+}$ (Equation 2-29), become less active. It could also occur if the nitrate oxidation of pyrite reaction (Equation 2-25) was becoming more dominant. This latter reaction consumes acidity. However, Figure 8-6 shows that pH continues to decline over the whole incubation period, but at a decreased rate after day 21. This shows that the nitrate coupled pyrite oxidation reaction (Equation 25) never becomes the dominant reaction otherwise incubation solution pH would have risen in these two latter periods.

The observed pH decline is interesting in the sense that the commonly reported nitrate coupled pyrite oxidation reaction (Equation 2-24) does not produce acidity, but consumes it. Only one reaction reported in this study produces acidity. This is the second equation in Table 2-19, viz:

\[
2 \text{FeS}_2 + 6 \text{NO}_3^- + 4 \text{H}_2\text{O} \rightarrow 2 \text{Fe(OH)}_3 + 4 \text{SO}_4^{2-} + 3 \text{N}_2 + 2 \text{H}^+
\]

\underline{Equation 8-2: Nitrate oxidation of pyrite – ferric hydroxide product}

The observed difference of 0.4 pH units between Untreated and N-treated incubations (Figure 8-6) actually occurred in Period 1 to day 14. Here the N-treated incubation solution declined 0.7 pH units in association with loss of 0.67 mg nitrate-N, whereas the Untreated solutions declined 0.3 pH units without nitrate-N loss. Thereafter, pH decline was very similar for both treatments leading to the conclusion that pH cannot be used here as a diagnostic tool to distinguish between specific nitrate reduction reactions.

Even though Incubation 3 results very clearly show from day 21 that sulfide is declining only in the N-treated incubations associated with nitrate decline, it is not clear which nitrate reduction reaction is taking place. There is always the possibility that both known reactions, Equation 2-24 and Equation 8-2, were occurring simultaneously. This would tend to have a more pH neutral impact as Equation 2-25 consumes acidity and Equation 8-1 creates it in almost equal molar ratios. However, an additional fact is that both treatment incubation solutions show near identical pH decline in Periods 2, 3 and 4. Clearly, some other acid forming processes are occurring in both treatments. The available study data does not provide enough information to determine whether this acidity is created by a similar reaction or set of reactions in both treatments, or whether different process with similar pH impacts are occurring separately in the Untreated and N-treated incubations.

From the data here, initial sulfide-S decline in both treatments cannot be strongly linked to nitrate reduction. As there was no nitrate in the Untreated incubation replicates, some other oxidant(s) must be acting on the pyrite in these incubation samples. There are only two proven
oxidants of pyrite: molecular oxygen, and ferric iron. Although manganese(IV) has been associated with FeS₂ oxidation (Schippers and Jorgensen, 2001), the current understanding is that Mn(IV) plays a secondary role as an electron acceptor in the regeneration of Fe(III) from ferrous ions. It is noted here that current study testing showed that the only significant sulfur species present was FeS₂ with virtually undetectable levels of iron monosulfides or elemental sulfur in these gel-clay samples.

Delaune and Reddy (DeLaune and Reddy, 2005) reported electron donors (organic matter, organic compounds, and reduced inorganic compounds such as NH₄⁺, Fe²⁺, Mn²⁺, S²⁻, CH₄, and H₂); and electron acceptors (O₂, NO₃⁻, Mn(IV)O₂, Fe(III)OOH, SO₄²⁻, and HCO₃⁻) in wetland soils. They stated that saturated sub-oxic soils are usually limited by electron acceptors and have an abundant supply of electron donors, which is generally the opposite of that for aerated soils.

Possible electron acceptors here are: oxygen, nitrate, manganese(IV) oxide, and iron(III) oxyhydroxide under positive redox-potential conditions, and sulfate ions and carbon dioxide under negative redox-potential conditions. As the measured Eh redox potential was not observed to fall below 300 mV during the incubation experiment, the assumption here was that the sulfate – HS⁻ couple (around -200 mV redox potential in a system under standard conditions) did not operate. The carbon dioxide-methane couple (-250 mV) may also be disregarded for now, as may nitrate for this part of the discussion. This leaves O₂, Mn(IV)O₂ and, FeOOH as possible inorganic oxidant contenders.

There is a possibility of some oxygen gas diffusion through the incubation tubes as noted in the Incubation 2 discussion. However, given the observed reaction kinetics between the active initial 14 day Period 1, and slower 201 day Periods 2 to 4, it is reasoned here that if oxygen diffusion were a significant factor it would exert more effect over longer time periods. Very clearly this is not the case in the Untreated samples after day 21, so oxygen diffusion is discounted here as a significant factor.

Oxygen concentrations in the incubation solutions were low, but not zero. These solutions were deoxygenated by nitrogen-gas sparging to a sub-oxic level of < 0.7 mg O₂ L⁻¹. At the altitude and temperature of the study laboratory, this equates to < 8 % oxygen saturation. Thus up to 17.5 μg dissolved O₂ is potentially available for reaction in the 25 ml incubation tubes.

Given what is known about the dynamic nature of the cane-block watertable and the rapid percolation of rainfall to the groundwater surface, it is highly likely that some dissolved oxygen is always present near the groundwater surface zones in the profile. Using Equation 2-13 for the molecular oxygen oxidation of pyrite, stoichiometric calculations show potential reactant concentrations:

\[ \text{FeS}_2 + 2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{SO}_4^{2-} + \text{S}^0 \]  
\text{Equation 2-13}

showing 4 moles of oxygen react with 1 mole of pyrite (or 2 moles sulfur), so that: 1 μg O₂ reacts with 1.87 μg FeS₂ (or 1 μg sulfide-S)
Given the possible 17.5 μg O₂ in each incubation tube, this reaction could potentially induce 17.5 μg sulfide-S declines in each tube. Compared with the mean incubation sulfide loss to day 21 of 5.7 mg sulfide-S, the actual experimental sulfide loss is between 2 and 3 orders of magnitude larger. This reaction stoichiometry indicates that dissolved oxygen could account for only <0.3 % of the sulfide-S loss in incubation tubes to day 21. However, this dissolved oxygen could have been sufficient to initiate a chain of reactions in conjunction with other alternative electron acceptors.

Subsoil pore-water analysis by van Oploo (2000) at 1.3 m depth showed the presence of iron (31.3 μg ml⁻¹, or 19.4 μg g⁻¹ OD), manganese (30.1 μg ml⁻¹, or 12.5 μg g⁻¹ OD), and sulfate (1371 μg ml⁻¹, or 850 μg g⁻¹ OD). These were soluble species around neutral pH so the iron would be Fe(II), and the manganese mainly soluble MnCl₂ and MnSO₄ (i.e. Mn(II), forms), although some MnO₂ was also reported in soil sample analysis (van Oploo, 2000). Additionally, the synthetic groundwater incubation solution contained sulfate at concentrations of 432.5 μg ml⁻¹ or 10.8 mg per incubation tube (Appendix 6). Thus, sulfate is at relatively high concentration in these incubation tubes. Whilst the view here is that a sulfate oxidant reaction is unlikely given the Eh readings above +300 mV, the high concentration of this sulfate could have induced some partial sulfate reduction, especially early in the incubation cycle.

Manganese(IV) may be present in small quantities and has been indirectly associated with pyrite oxidation in anaerobic sediments, but is understood to act as an oxidant to regenerate ferrous iron to Fe(III), thus will be disregarded here for now. This leaves the iron oxyhydroxide FeOOH. Goethite (α-FeOOH) is commonly associated with pyritic sediments (Luther et al., 1982), but study results along with mineral and clay analysis indicates that there should not be significant quantities of iron oxyhydroxides initially in the incubation samples unless it was in amorphous form. This can often occur as coatings on other mineral surfaces and, if present, would help account for the relatively high amorphous material found in the soil mineral analysis in the study soil.

The analysed soil amorphous content increased with depth from around 11 to 12 % at 0.6 to 0.9 m depth, to over 30 % at 1.7 min the soil profile. One explanation for this diminished amount in the AASS zone could be through reactive depletion at the redoxcline – the pyrite oxidation front zone. This is supported by the mineral analysis data indicating an increase in amorphous content from 0.9 m depth downwards, below the redoxcline. Thus there is some support here for a mechanism whereby amorphous iron oxides are actively coupled to pyrite oxidation. Iron oxyhydroxides are a source of ferric iron, a key oxidant of pyrite, yet have low solubility at circum-neutral pHs and are not known to directly oxidise pyrite (Schippers and Jorgensen, 2001).
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However, Luther et al. (Luther et al., 1992) proposed novel iron cycling mechanisms based on formation of soluble iron(III) organic complexes in saturated sediments which they postulated were able to react with soluble and insoluble sulfide minerals (including FeS₂) and even regenerate Fe³⁺ ions. Thus, iron oxyhydroxides cannot be excluded from the list of potential reactants here. The observed brown and red colouration of the incubation end-samples suggests iron oxide production, thus indicating its availability as a reactant.

Incubation nitrate loss may be accounted for in many ways. Apart from nitrate coupled pyrite oxidation, nitrate can participate in the oxidation of ferrous to ferric ions. This may occur via Equation 2-22 or Equation 2-23.

Nitrate also reacts with organic matter in the denitrification pathway, and with gel-clay samples assaying at around 3.1 % SOC, this potentially offers another mechanism of nitrate removal. With an average incubation gel-clay OD sample mass of 600 mg, total organic carbon would approximate 18.6 mg SOC. If only 10 % of this were labile, this still leaves 1.86 mg SOC available to potentially react with nitrate. By the greatly simplified Equation 2-21, this equates to 3.47 mg nitrate-N being potentially consumed. From day 21 to 215 in Incubation 3, 5.42 mg nitrate-N loss is associated with 1.31 mg sulfide-S decline. By Equation 2-24, 1.31 mg sulfide-S would react with only 0.8 mg nitrate-N leaving 4.62 mg residual nitrate-N. Therefore the soil organic matter-nitrate reaction pathway can help to explain Incubation 3 nitrate-N losses. Whilst all these calculations are hypothetical, at minimum they do confirm that the potential reactants and products are of the same order of magnitude. Additionally, this postulated SOC denitrification pathway could also explain part or all of the nitrate loss during the first stage of Incubation 3.

Incubation solution ionic strength varied for untreated and N-treated incubations, with untreated solutions containing 950 mg L⁻¹ salts and the N-treated solutions having a total of 1750 mg L⁻¹. Although the ionic strength is raised relative to the untreated solution, it is still relatively low compared with seawater at around 35000 mg L⁻¹, and is here considered to have had no effect on pyrite oxidation in this experiment.

It is useful to keep in mind that more than one agent or factor may be involved and working in synergistic ways. In the study incubation experiment it was obvious that other oxidants were present and active up to day 21 as significant quantities of sulfide disappeared from both the Untreated and N-treated gel-clay samples during this period. These initial oxidants were not identified here. Logistical issues, specifically time, prevented further experiments to determine these oxidation pathways. However, from day 21 onwards in the incubation experiment, sulfide in the N-treated samples consistently and significantly declined further relative to the Untreated samples through until day 215.
8.6 Concluding Comments

Outcomes of this chapter substantially met the study aim and objective of investigation of the impact of nitrate on pyrite oxidation. Many aspects of this experimental work resulted in effective and useful outcomes. The bulk sampling and storage techniques developed here for pyritic gel-clay collection over periods of months allowed delivery of consistent relatively undisturbed gel-clay samples for the incubation experiments. Additionally, the sulfide analysis methods modified and used here allowed accurate and consistent measurement of sample sulfide.

Results of laboratory work provide data that demonstrate the possible role of nitrate in pyrite oxidation under these experimental conditions. PASS gel-clay oxidation did not occur in the untreated samples over the latter 90% of the 215 day incubation period when sulfide was being oxidised and nitrate reduced in the nitrate-treated samples. The incubation solution chemistry was similar to field site groundwater and pH or Eh were not constrained over the incubation period. What was less clear was how nitrate was contributing to pyrite oxidation. Whilst stoichiometry provides insight into what is possible in terms of specific reactions, it does not provide information as to what is probable in this case. Therefore, although some reactions or pathways may be inferred, the view here is that none may be specifically asserted. One clear inference that can be drawn from these results is the need for caution when using nitrogenous fertilisers on ASS.

The following general discussion chapter provides summary discussion of this whole study.
9 General Discussion

9.1 Overview

A study aim was to examine fertiliser-derived nitrogen changes in an agricultural acid sulfate soil. Although it was carried out at one field site in northern NSW, it was envisaged that results may have broader application to similar soils in the region and beyond. Impetus for this work came from the fact that nitrate is a powerful oxidant with the potential to oxidise reduced substances in saturated subsoils. The study aim was to clarify the role of fertiliser-derived nitrate in contributing to pyrite oxidation in agricultural acid sulfate soil. The following general discussion summarises study outcomes in the context of this aim and offers a final conclusion.

Soil characterisation of the study site generally validated earlier descriptions of a typical floodplain ASS in having a layer of alluvial topsoil over a band of acidic oxidised AASS zone largely devoid of pyrite. Below this is partially oxidised acidic AASS transition zone grading to an unoxidised deep pyritic gel-clay subsoil zone (PASS). Soil redox measurements confirmed the oxic nature of the upper profile, and the anoxic state of the PASS zone at depth. Combined pH and Eh analysis allowed nomination of soil in the 0.9 to 1.0 m depth zone as the redoxcline, the point at which the most active oxidation-reduction processes are occurring.

Soil organic carbon (SOC) ranging from over 6% in the topsoil to 3% in the PASS provided ample substrate for oxidising reaction pathways. It could be inferred from the results that a zone of low SOC between 0.4 and 1.0 m depth (the oxidised AASS zone) was depleted through reaction either with sulfuric acid generated by pyrite oxidation and/or with soil nitrate leached from above. Interestingly, the fully oxidised AASS zone, verified by the soil mineralogy and clay analysis, corresponded to the zone of greatest SOC depletion, leading to conjecture about relationship between these two phenomena.

Clay analysis showed high levels of smectite with lesser amount of illite and kaolinite. Also evident were unexpectedly high levels of amorphous material in the study soil profile, varying from 12.5% (at 0.6 m) to 31% at 1.7 m depth with a low of 11.4% at 0.9 m depth, the redoxcline zone. Soil amorphous matter may comprise many different materials, including: non-crystalline mineral matter such as some ferric iron oxides, (oxy)hydroxides including ferrihydrite, siliceous material, carbonaceous matter, vitrified volcanics, and humic substances. The importance here of this material has to do with the capability of some of these (e.g. the iron oxides group and humic substances) to bind, complex, adsorb, and react with other soil substances, particularly in acidic environments in the AASS zones where iron oxides may become soluble, releasing ferric irons which are a powerful oxidant of pyrite.

Knowledge about clay types in the study soils assisted with interpretation of the study gel-clay micromorphology investigation. Meeting the aim of examining gel-clay microstructure led
to development of novel techniques for the preparation of saturated soil samples and subsequent SEM imaging of these. Obtained images confirmed the general colloid/clay size-range (i.e. <2 μm) of the gel-clay material and also showed the pore space size and shape as being suitable habit for microorganisms. It also clearly showed for the first time the open ‘card-house’ structure of this saturated estuarine floodplain sedimentary material. As smectites have the most active electrochemical surface activity of the three clays identified here, it was not unexpected that the card-house microstructure would be present, but also evident were the laminar flat-stacked sub-structures that are more normal for, say, the kaolinite clays. One striking aspect of this SEM investigation was the lack of evidence for the presence of framboidal pyrite in this gel-clay. In only one instance out of approximately one hundred was a framboidal pyrite form observed. This leads to the conclusion that soil pyrite, at least in study soils, is present mainly in micro-crystalline euhedral particulate form. This information assisted interpretation of laboratory incubation experiment results.

Soil temperature monitoring showed the soil to be operating in the microorganism mesophilic range with mean soil temperatures ranging from approximately 12.5 to 24°C at 0.3 m, 15 to 24 °C at 0.8 m, and 17 to 24 °C at 1.3 m depth. This data, although not unexpected, indicated that no temperature limitations should exist with respect to biological functioning in this soil. Diurnal and seasonal variations were evident in the recorded data. Thus the soil winter minimum temperature at 1.3 m depth lags the mean air-temperature winter minimum by around 45 days. This has implications for the seasonal peaks in soil geochemical transformations. The range between soil temperature diurnal and seasonal maxima and minima decreased with soil depth.

Soil hydrology data gave a comprehensive picture of the water relations in the study soil. The study period was somewhat atypical in that site rainfall was 57% of the long term regional average over the same period. This long term regional average is 1725 mm per year based on records from 1887 to 1972. More recent trends since then indicate significantly less annual rainfall in the region (1590 mm, a decline of 135 mm). It is noted that the lower rainfall at the study site in 2006-07 could significantly impact on water dynamics of the study site, and this appeared to manifest in the data.

In the 2006-07 cane-growing season, groundwater logging results showed the groundwater initially receding to around 1.5 m soil depth, and then rising under the influence of changing precipitation – evapotranspiration over the sugar-cane cycle. It is self-evident that this groundwater drawdown continues to contribute substantially to pyrite oxidation in the transition AASS and the upper PASS gel-clay through allowing oxygen into these zones. No surface flooding occurred during the study period although the watertable rose close to the soil surface on two occasions under the influence of moderately high rainfall events relative to the historical averages for this region. The historical rainfall record shows 1725 mm annual rainfall equating
to 144 mm per month but due to seasonal influences, the historical record shows monthly highs of over 250 mm (March) and lows of 60 mm around September in early Spring. The highest monthly rainfall at the study site was 215 mm in August 2007, with the lowest (11.6 mm) recorded in the preceding month of July 2007. This high variability impacts substantially on soil moisture and groundwater dynamics.

Logged data shows groundwater rapid response to rainfall with the response varying with prevailing soil moisture and watertable depth. Generally the watertable responded rapidly, within hours, to rainfall events greater than 15 mm per day. This response was faster and greater if the soil was already moist from recent rain, and also if the watertable was higher in the profile – validating similar findings by Wilson in the same locale (Wilson, 1995). Permanent logging soil moisture probes showed that rainfall less than 10 to 15 mm per day appeared to be taken up by sugarcane plant roots in the surface horizons to 0.6 m depth.

One of the main objectives of the investigation into cane-block water dynamics was to identify and quantify water movement into subsoil zones so as to better evaluate the movement of near-surface applied fertiliser-derived nitrate. The data repeatedly and unequivocally demonstrated that rainfall in excess of 10 – 15 mm over 1-2 days could rapidly penetrate the soil profile up to 1.3 m depth (past the redoxcline at 1.0 m) in less than 10 hours and initiate groundwater rise. During the field study investigation period of 8 months, this type of rainfall event occurred nine times. Under conditions where soil was at or close to field capacity, the groundwater at times showed response to rainfall in less than 3 hours. From this and other data it was concluded that soil nitrate could be effectively and rapidly leached into the redoxcline, zone of this ASS.

It was inferred from logged watertable data that cane-block mole drains and field-drains significantly mediate drainage from the soil block under conditions where groundwater has risen sharply but surface flooding has not yet occurred. Under conditions of relatively high rainfall (i.e. > 60 – 70 mm per day) resulting in rapid groundwater rise close to the soil surface, the data indicates that a large proportion of the precipitation may be directly and rapidly exported off-site through near-surface lateral flow, potentially taking with it acid and nutrient products from within the soil zone at and below the depth of the mole drains. These results support those of Wilson et al. (Wilson, 1995). Kinsela and Melville reported that diffusion and surface runoff accounted for most of the exported acid from cane-block soils, but it is postulated here that much of this may occur via the mole and field drain pathways (Kinsela and Melville, 2004a).

Other study results showed that headland drainage channels within the cane farm, those channels emptying the cane-block mole and field-drain network, can markedly impact on cane-block soil moisture and groundwater properties. The data demonstrated this in two ways. Logged groundwater data at times showed a sloping equipotential groundwater surface from the
relatively higher drain channels to well into the adjacent cane-block. The recorded height differences between drain and the cane-block watertable ranged up to 1.0 m in height, with the sloping equipotential surface extending up to 50 m into the cane-block. Sloping equipotential surfaces normally demonstrate the existence of lateral flow (Hillel, 1998) therefore the study data indicates that water is inflowing up to 50 m into the cane-block from the higher adjacent drain. In the second instance, treatment plots cane yields in Field Trial 1 increased significantly right across the 60 m block toward the main drain immediately adjacent, and parallel, to cane rows. This postulated soil moisture factor completely masked any nitrogen treatment effect.

Cane-block soil nitrogen changes in the context of a cane planting-harvest cycle were investigated in this study. Two nitrogen treatment field trials were conducted. The first trial was exploratory to gather initial data and test various methods and procedures. Much was learnt that supported the effective design and implementation of the second nitrogen field trial. Field trial 2 was set up using 3 nitrogen treatments plus a control. Nitrogen treatments consisted mainly of an initial Plant-mix (17kg N /ha) and a Side-dress (46 kg N /ha) in various combinations. Results showed that any ammonia/ammonium mineralised was transformed within weeks to nitrate, which generally disappeared more slowly from the profile over months. In some treatments a significant amount of soil nitrate appeared lower down the profile, diminishing with depth to disappear at the redoxcline around 1.0 m. Repeated instances of this effect across treatments indicate that nitrate reduction could be occurring within the cane-block profile, and more importantly that this reduction is closely associated with pyrite appearance at the top of the AASS transition zone at approximately 0.7 m depth. Pyrite concentration increased with depth from this point.

Laboratory experimental work to investigate the impacts of nitrate on acid sulfate soil oxidation yielded interesting and useful results. Some unusual experimental outcomes were not able to be investigated further during the study due to time constraints. The experimental work was conducted using anaerobic incubation of pyritic gel-clay taken from the study site soil profile. After running some preliminary incubation batches to gather information on reaction kinetics and to test procedures, a final replicated incubation (Incubation 3) was run for 215 days with sub-sampling and assay at days 14, 21, 76 and 215. Significant and near identical sulfide-S loss occurred in both Untreated and N-treated incubations over the first 21 days. During this time substantial nitrate-N was lost from the N-treated incubations. From day 21, the incubation system functioning underwent a major shift, evident in no further loss of sulfide-S in Untreated batches but with further sulfide-S decline in the N-treated incubation tubes accompanied by further loss of nitrate-N in these N-treated incubations. Stoichiometric calculations for nitrate oxidation of pyrite revealed that more nitrate was being consumed than required by the sulfide moiety in pyrite oxidation. However many other electron donor substances may readily redox

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couple with nitrate in these saturated systems. These include: intermediate sulfur species, ferrous iron, ferric iron, and organic matter.

Whilst there is considerable circumstantial evidence that pyrite oxidation occurred coupled with nitrate reduction in these laboratory incubations from day 14 to day 215, there is perhaps less definitive proof. Resources were not available at the time in the study to explore and resolve these study outcomes, particularly those relating to early sulfide loss is both treatments. Significant sulfide-S decline occurred only in the \textit{N treated} incubation samples after day 21 and this outcome provides support for the involvement of nitrate in ASS pyrite oxidation.

Stoichiometric comparisons indicated that multiple reactions involving nitrate loss were potentially occurring in the \textit{N-treated} incubations. Although these calculations showed nitrate reduction coupled pyrite oxidation was feasible in this experiment, the available data did not support specific reaction pathways being identified. However the experimental work clearly linked reduction of added nitrate with increased rates of pyrite oxidation in ASS. Given the high nitrate concentration (800 \( \mu g \) NO\(_3\) \(-N\) ml\(^{-1}\) [from KNO\(_3\)]) used in the final incubation experiment, any inferences drawn from this result would have to demonstrate similar concentrations from nitrogen fertiliser inputs in the field sugarcane crops. Whilst calculations theoretically allow for extremely high soil nitrate-N concentrations at the fertiliser application point (i.e. up to 100 000 \( \mu g \) N cm\(^{-3}\)), further research may be necessary to further clarify exactly how this may be dispersed in the soil below the cane row under the influence of diffusion and rainfall induced advection. However, what is clear is that the nitrate-N concentrations found in the cane block soil are not inconsistent with these high point source nitrogen-N concentrations. Apart from the Field Trial results, more detailed information on this localised distribution of nitrate in the cane-block soil profile was not acquired in this study due to time and other resources constraints.

9.2 Study Implications for Agricultural ASS

In a very general sense, the study outcomes confirm the biogeochemical complexity of these estuarine floodplain soils and demonstrate that there is still much to understand about them. From an agricultural perspective, the study results bring attention to the use of soil amendments generally, and use of nitrogenous fertilisers on the pyritic soil specifically. If these results implicating fertiliser-derived nitrate in increased rates of pyrite oxidation can be taken at face value, then there is sufficient cause for any agricultural enterprises operating on these estuarine floodplain pyritic soils to review their soil amendment practices.

It should be noted that this is not a 'problem' which has just emerged. If correct, the study conclusions only draw attention to soil processes that are likely to have been occurring for many decades since nitrogenous fertilisers were first used on these regional agricultural soils.
The primary issue arising from this study concerns not the use of chemical fertilisers *per se*, but specific agricultural management practices that support the creation of excess free nitrate in the crop soil profile. These include (i) use of high fertiliser amendment rates, (ii) the type of fertiliser used, and (iii) the manner and timing of fertiliser application. Soil nitrogen cycles through soils from nitrogen gas, through ammonia, ammonium ions, nitrate ions, nitrite ions, nitric oxide, nitrous oxide, to nitrogen gas. While plants, and specifically sugarcane, may take in soil ammonium, it is reported that they mainly take up nitrate for their nitrogen requirements (Crawford and Glass, 1998). Excluding the option of supplying N in nitrate form directly to the crop, it would appear that *nitrification* (the bio-production of nitrate from ammonia), is an essential N-cycle step for viable commercial-production sugarcane systems. It would seem that one effective approach to managing nitrate impacts on ASS is to manage the *denitrification* N-cycle step in agricultural acid sulfate soils.

Effective nitrate management in ASS could involve several separate or combined strategies. Broadly, these focus on preventing excess nitrate in the cane soil and, failing that, preventing it from reaching the AASS transition zone. The subsequent denitrification and other nitrate reduction pathways in this transition zone that create the nitrate coupled transformations described in this study. But the view here is that it is unrealistic and impractical to consider suppression of nitrate reduction reactions after nitrate has moved away from the cane-plant root zone.

The first strategy relates to supplying the cane crop with only the nitrate it requires through multiple small applications throughout the cane growing cycle. Some existing cane planting practices go some way towards achieving this by splitting the fertiliser application into two applications (i) a planting mix of, say, N:P:K:S fertiliser, and (ii) a post-planting (e.g. 6-7 weeks later) side-dressing fertiliser of urea. The urea can take weeks to hydrolyse to ammonia and then be fully nitrified to nitrate. In the study Field Trail 2, a similar two-part treatment resulted in the highest cane yields per treatment plot. However, machinery access to the cane-block is normally limited to less than two months after planting, so this strategy is impractical unless a variable slow-release version of urea is used in the second side-dressing application.

The second strategy could involve enabling a consistent natural supply of nitrate to cane roots through symbiotic N-fixing bacteria associated with cane roots. This is a strategy adopted many decades ago in the Brazilian sugar industry where poor crop response to nitrogen inputs is common (Oliveira *et al.*, 2002). The N-fixing bacteria have been identified in other countries as well as on sugarcane plants in Australia (Li and Macrae, 1991, Li and Macrae, 1992), and later studies have found evidence of large natural population of these microorganisms across the cane growing regions of Queensland (Robertson, 2006).

The third option is to build up soil organic matter and biodiversity in the upper soil layers to bind, assimilate, trap and transform the mobile nitrate before it leaches down to the AASS
transition zone. Nitrate assimilated by bacteria and incorporated into soil organic nitrogen stores is potentially more consistently available to cane plants over the whole sugarcane growth cycle. Natural cycling of soil organic nitrogen, even that assimilated by microorganisms, means that a portion of this is always becoming available to plants.

The question of how to prevent this key nitrate reduction reaction occurring is complex. In similar ways to the natural and anthropogenic disturbance of pyritic soils, possibly little can prevent the reaction from occurring. However management strategies may minimise the impacts through limiting the contact between the reactants in the first place.

9.3 Methodological Issues

The multi-decade long debate over specific pathways and (bio)chemical mechanisms of aerobic pyrite oxidation finally appears to have been settled with the Crundwell (2003) paper. Beginning with the work of Luther (1987) (Luther, 1987), the issue of how pyrite can be attacked seems to be resolved in favour of the purely oxidative pathway. Thus it appeared that the role of microbes must be relegated to side-reaction roles such as assisting with the regeneration of ferric iron from ferrous ions. However, very recent work seems to be blurring the division between direct and indirect mechanisms of pyrite oxidation. These report on several novel and biochemically sophisticated mechanisms employed by microorganisms to obtain energy from pyrite dissolution (Rojas-Chapana and Tributsch, 2004, Pisapia et al., 2008, Zeigler et al., 2009).

Despite all this resolution, it is clear from the present study outcomes that many potential pathways exist for further transformation of pyrite oxidation products, including those involving both the iron and sulfur moieties. Potential electron acceptors for the anaerobic oxidation of intermediate sulfur compounds have been reported generally, but knowledge about specific conditions required for the various reaction pathways is still unclear. There are many potential pathways for anaerobic iron oxidation coupled to nitrate reduction in saturated ASS systems, and further research could usefully be directed to investigating iron cyclic transformation pathways in anoxic ASS systems.
10 Conclusion

10.1 Key Findings

Apart from study site soil characterisation, this study essentially set out to (i) examine cane-block soil hydrology to evaluate leaching processes involving nitrate migration to depth, (ii) evaluate the fate of fertiliser-derived nitrate in sugarcane cropping acid sulfate soils, and (iii) to evaluate the effect of nitrate ions on the iron sulfide in cane-block gel-clay subsoil.

Gel-clay micromorphology investigation revealed this material to possess an open 'card-house' type structure with continuous pores in the 0.1 - 3 μm range. This provides clear evidence for the observed relatively low hydraulic conductivity of the undisturbed natural PASS clay material below around 1.1 m depth. However, the PASS soil profile to around 1.8 m depth has been penetrated by deep sugarcane roots which greatly aid the infiltration of surface. The hydraulic conductivity of this and the upper soil profile including both AASS horizons was found to be relatively high. Rainfall greater than 15 mm per day could impact on the ground watertable at 1.0 - 1.3 m depth in less than 10 hours. This occurs more rapidly if the soil profile is already near field capacity and the watertable is closer to the surface. These data demonstrate the potential for soil amended nitrate to leach quickly down the soil profile to the reductocline. Study rainfall data showed precipitation greater than 15 mm per day occurred

Sugarcane-block nitrogen treatment trials generally showed relatively fast transformation of 0.2 m depth soil-amended nitrogenous fertiliser to ammonium, then to nitrate ions. Nitrate ions subsequently appeared lower in the soil profile, but not below 1.0 m depth. In the sampled High-N cane-block soil profile, nitrate concentrations increased to 0.7 m then declined sharply in a linear fashion to barely detectable levels at 1.0 m depth. This and other N-trial data provides much support for a nitrate reduction-pyrite oxidation pathway occurring in the biochemically active AASS transition zone from 0.7 to 1.0 m depth.

Laboratory pyritic gel-clay incubation experiments conducted to clarify the study aim produced results supporting the involvement of nitrate in pyrite oxidation. Accompanying this were indications of other potential biogeochemical transformations simultaneously occurring which were not able to be identified due to resources constraints. The decisive test was based on the use of incubation solution with nitrate-N concentration of 800 μg N ml⁻¹. Whilst nitrate concentrations quite this high were not found in the cane soil at the times it was sampled, point source concentration calculations show that concentrations of over 100 000 μg N cm⁻³ at the banded fertiliser application point are theoretically possible using the historically relatively low fertiliser application rate of 63 kg N ha⁻¹ applied in this study. Given the demonstrated vertically oriented anisotropic property of the soil in allowing rapid water infiltration to PASS zone plus the demonstrated high rainfall events in this region, it is suggested that it is entirely
feasible that relatively high nitrate concentration soil solution (i.e. 800 µg N ml⁻¹) could rapidly move directly down to around 0.7 m and deeper where pyrite becomes more evident in the cane-block profile.

Although direct (bio)chemical coupling of nitrate reduction with pyrite oxidation was not demonstrated here, it is very clear from the laboratory data that pyrite oxidation was significantly enhanced in the presence of nitrate in long term pyritic subsoil incubations relative to the nitrate free untreated incubation.

10.2 Study outcomes

The study aim, posed in Chapter 1, stated: the underlying aim of this research was to examine initial transformations of near-surface applied fertiliser nitrogen in a sugarcane agricultural ASS system and to evaluate its potential impact on increasing the rate of pyrite oxidation in this soil. The following provides some commentary on whether or not this aim was met in this study.

Soil characterisation allowed benchmarking of selected soil geochemical factors, and also revealed no factors likely to inhibit any postulated nitrate reduction-pyrite oxidation redox reactions in the partly oxidised AASS-PASS transition zone. Significant concentrations of unoxidised pyrite were found in the soil profile from around 0.7 m depth, and pyrite levels increased with soil depth to a maximum of 4.9 % pyrite at 1.7 m depth. The soil profile zone of interest here (0.7 to 1.3 m) had mesophilic properties in the 15 - 25 °C range and thus provided an acceptable though slightly sub-optimal temperature environment for the known mesophile microorganisms in these soil types. Subsoil soil moisture was consistently in the range able to support microbial activity. Additionally, the AASS-PASS transition zone was shown to be saturated for long periods due to capillary rise effects from groundwater. This would help sustain a low-oxygen environment essential for nitrate reduction reactions to occur. Investigation of cane-block hydrology also provided information supporting the rapid movement of nitrate down the soil profile.

Soil hydraulic properties were found to allow precipitation greater than approximately 15 mm per day to penetrate the soil profile to the watertable up to 1.3 m below ground in less than 10 hours. It is suggested that this rapid infiltration is capable of directly moving nitrate down the can-block profile. Soil profile testing for ammonium and nitrate ions clearly showed nitrate being moved down the profile but not beyond the redoxcline at 1.0 m depth.

Ironically, some aspects of the laboratory experimental work provided outcomes that were at times inconsistent with the overall experimental result, indicating that complex biogeochemical processes were possibly occurring in this setting. One of these outcomes was the significant sulfide loss (i.e. pyrite oxidation) in nitrate-free Untreated incubation samples in the first 21
days of a 215 day sub-oxic/anoxic incubation experiment. After day 21, sulfide loss in the Untreated samples ceased, but continued in the N-treated soil incubations. The sulfide-S difference between the Untreated and N-treated samples was significant at day 76 [ t(4) = 3.55, p<.025], and very significant at day 215 [ t(4) = 7.49, p<.005]. This latter data clearly demonstrates nitrate involvement in anoxic pyrite oxidation in the study site gel-clay soils.

Despite some results inconsistencies, the view is that integrated study results provide considerable support for reaction pathway(s) involving nitrate reduction associated with pyrite oxidation in the study soil. Although nitrate valence band and Gibbs free energy considerations make nitrate a strong contender for the direct oxidation of pyrite in the manner of the Fe(III) reaction, the data here did not support a definitive determination being made with respect to this reaction pathway. Whilst not discounting a direct reaction pathway, it is possible that nitrate is also involved indirectly in the bio-catalysed regeneration of Fe\(^{3+}\) ions from Fe\(^{2+}\) as well as participating in the sequential oxidation of intermediate sulfur products and gel-clay organic matter. These nitrate coupled reactions could easily account for the observed losses of nitrate in this incubation experiment.

### 10.3 Future Research Directions

It is evident from the literature review in this study that sub-oxic / anoxic processes, particularly in soils with highly variable watertable, and specifically in low lying coastal ASS, are not well understood. Whilst there appears to be widespread awareness of the groundwater polluting effects of leached nitrate, it is obvious from the literature that this awareness is not so developed with respect to the impact of nitrate as an oxidant in soils. A similar comment could be made about the role of microorganisms in major geochemical cycles. Therefore, the view is that much work remains to be done in identifying and describing processes in anoxic acidophilic soils.

The importance and influence of soil organic matter in soil biogeochemical transformations in cane-block acid sulfate soils cannot be over emphasised here due to its role as a highly preferred electron donor. Whilst organic matter has numerous other benefits for agricultural cropping soils, it also has very high biochemical activity potential including bio-catalysed redox transformations driving primary geochemical cycles. SOM component analysis is developing into a mature field of science. Current and new analytical techniques could productively allow evaluation of key substrates for, and drivers of, these soil transformations. Characterisation of organic matter fractions in acid sulfate soils could assist interpretation of the role of this soil carbon as an electron donor for redox reactions.

Hydrology results here highlighted the hidden but seemingly substantial effect that mole and field drains have in exporting excess soil water from the cane-block. Despite the proven
efficacy of these drains in preventing soil surface flooding, study data showed rapid water removal from 0.5 - 0.6 m in the profile where there is little organic matter to buffer acidity or complex ASS oxidation products. Further research work could usefully investigate the physical and geochemical changes of these drainage systems.

The current study experimental outcome apparently showing initial rapid pyrite oxidation occurring in a nominally anoxic soil system devoid of nitrate oxidant is interesting and unusual but also presents excellent opportunities for scientific investigation of these phenomena.

10.4 Recommendations for Industry Management

Despite the existence of some ambiguity in the field and laboratory outcomes, the combined results provide considerable support for adoption of a policy of caution with regard to nitrogenous fertiliser use on coastal floodplain acid sulfate soils used for sugarcane agriculture. Recommendations evolving from this study are framed within a context of maintaining the functional ecology of these estuarine floodplain soils.

This study highlighted the mobility and reactivity of fertiliser-derived nitrate and also demonstrated the risk of increased rates of pyrite oxidation should this nitrate move to the redoxcline, the oxidation front in ASS. Although there are potentially many ways to deal with this issue, two primary strategies are: (i) limiting nitrogen fertiliser inputs to the soil, and (ii) managing nitrogen fertiliser concentration and activity in the soil. Whilst recognising the efficacy of the first option, discussion here will focus on point (ii) above dealing with soil nitrogen management.

If nitrogen is to be managed in these soils, it is suggested that the optimal soil zone to manage nitrate transformation is in the upper oxic zone rather than attempting to influence reactions in the lower sub-oxic/anoxic horizons. As highlighted in this study, the deeper soil anoxic zones support nitrate reduction, the key type of reaction to be avoided in the presence of pyritic ASS due to its positive impact on ASS oxidation rates. It is further suggested that adoption of agricultural management strategies to control and limit excess fertiliser-derived nitrate in the upper soil profile and to contain nitrate within the cane plant root zone (i.e. <0.6 m depth) could do much to reduce nitrate leaching to AASS anoxic zones deeper in the soil profile. On the basis of this, three industry recommendations are presented here for consideration.

1. Limiting high localised concentrations of free nitrogen ions in pyritic cane soils could significantly minimise nitrate leaching to unoxidised pyrite zones in the soil profile, thereby diminishing the potential for increased pyrite oxidation to occur. The recommendation is therefore to adopt strategies to decrease the amount of
nitrogenous fertiliser applied to these ASS soils, to apply this more evenly over the cane crop cycle, and/or to supply this in a slow release form to enable cane-plant roots to preferentially take this up as it is released. Options to achieve this include: slow release artificial fertilisers; use of ‘natural’ fertilisers; inter-row and companion planting using legumes; urea treatment to slow its hydrolysis in the soil; and inoculation of plant cane with symbiotic nitrifying bacteria.

2. Adoption of strategies to build and maintain soil organic matter and soil micro-flora within the upper profile could provide a reserve of potentially mineralisable nitrogen capable of providing a more uniform supply of plant-N over the cane growing cycle. In addition, this living and decomposing biomass can sequester and bind not only carbon and nitrogen, but also a range of other nutrients for use by the cane crop.

3. Given the strong indications here linking fertiliser-derived nitrate with increased rates of pyritic oxidation, the final recommendation is to employ soil management strategies to build and maintain high soil biota populations in the upper soil horizons. This can help ‘trap’ in the upper soil profile any free nitrate not immediately used by plants and thereby limit leaching of excess nitrate to the anaerobic subsoil zone. Continuous biological N-recycling can then slowly mineralise this biological-N, thus making it available to the growing cane. This strategy can be achieved through preserving and building up soil organic matter in the topsoil to around 0.5 m depth. If fertilisers are used as a soil amendment, results from this study indicate that a multi-nutrient fertiliser as a low-rate planting mix can help achieve higher cane yields as well as supporting soil biota populations.

It is suggested that soil systems have ‘inertia’ in the sense that contemporary biogeochemical functioning may not respond quickly to different practices in the short term (e.g. months to one year) but experience at the study field site cane farm shows that significantly degraded acid soils can be rehabilitated over periods of 5 to 10 years to produce viable cane crops. General approaches could include:

(a) a commitment to building up soil organic matter

(b) commitment to protecting and enhancing microorganism communities in the cropping soil and gradually withdrawing farm practices likely to harm these biota
(c) implementing practices to increase soil nitrogen inputs through natural means such as:

- using soil fauna populations as natural stores of nitrogen
- using cane plant root inoculation with nitrifying bacteria to ensure an ongoing supply of nitrogen to the cane, and
- use of short lived legume inter-row and companion planting to provide added nitrogen during the cane crop high-growth phase.

Maintaining the quality of cane soils and the surrounding environment is a key component in protecting the substantial social and economic investment in the infrastructure of regional sugar production. The environmental benefits and social amenity flowing from these strategies can benefit not only sugarcane growers and local communities in the short term, but can also help enhance and protect large regional interests such as agriculture, industry and tourism in the longer term.
Appendices

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Appendix 1: Soil Organic Carbon analysis method

This SOC determination procedure is based on the Walkley and Black methods (Walkley and Black, 1934, Walkley, 1947) with a Heanes modification (Heanes, 1984). A mixture of dichromate and sulfuric acid is added to the soil and heated to oxidise the carbon to CO$_2$. Residual dichromate is determined by back titration with ferrous sulfate solution.

Depending on the amount of SOC in the sample, between 0.2 and 1 g of dried soil (ground to pass a 0.42 mm sieve) was accurately weighed into a 250 ml beaker and 10 ml 1N potassium dichromate solution added. A subsample of each identified soil sample was concurrently dried at 105$^\circ$ C for 24 hrs to ascertain moisture content and allow reporting of organic carbon results on an oven-dry (OD) basis.

Reagents
- Concentrated H$_2$SO$_4$
- 1N Potassium dichromate solution
- 0.4N Ferrous sulfate solution
- Ferroin indicator

The dichromate-soil mixture was shaken to mix the contents and 20 ml of concentrated sulfuric acid added to each sample. Whilst being swirled by hand, the solution was heated to 135$^\circ$ C, and then set aside for 30 minutes. On cooling, the solution was diluted to 200ml with distilled water and titrated manually with 0.4N Ferrous sulfate solution using 'Ferroin' as the indicator. Percentage SOC can be determined using Equation 11.1.

\[ \text{SOC} \text{ (%)} = \frac{0.003g \times 1N \times 10ml \times (1-T/S) \times 100}{W} \]

Where:  
N = normality K$_2$Cr$_2$O$_7$ solution  
T = volume FeSO$_4$ used in sample titration (ml)  
S = volume FeSO$_4$ used in blank titration (ml)  
W = soil oven-dry weight (g)
Appendix 2: Soil Organic Carbon analysis data

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<th>Sample No</th>
<th>I.D.</th>
<th>% Org.C (calc)</th>
<th>Depth (m)</th>
<th>Mean SOC%</th>
<th>s.e. (n=3)</th>
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Appendix 3: Colourmetric procedure for ammonium-ion analysis

Colourmetric procedure for ammonium-ion analysis

**Ammonium-ion analysis procedure** (adapted from (Mulvaney, 1996))

Reagent preparation:

- **EDTA reagent** – Dissolve 6 g of the disodium salt of ethylenediaminetetraacetic acid reagent in 100 ml of deionised water in a volumetric flask.
- **Salicylate sodium nitroprusside** – Dissolve 7.813 g of sodium salicylate and 0.125 g sodium nitroprusside pentahydrate in approximately 80 ml of deionised water in a 100 ml volumetric flask, and bring the solution to volume with deionised water. Mix thoroughly, and then transfer the solution to an amber bottle for protection from light, store in a refrigerator.
- **Buffered hypochlorite agent** – Dissolve 2.96 g of sodium hydroxide and then 9.96 g of sodium monohydrogen phosphate heptahydrate in approximately 60 ml of deionised water in a 100 ml volumetric flask. Add 10 ml of sodium hypochlorite solution. Adjust the pH to 13.0 with sodium hydroxide, and dilute to 100 ml with deionised water.
- **Standard ammonium solution** – Dissolve 0.4717 g of ammonium sulfate in deionised water, and dilute the solution of a volume of 1 L in a volumetric flask. If dry and pure reagent is used, the solution will contain 100 μg NH₄⁺ / ml. Store the solution in a refrigerator. Prepare working standards from this primary standard as needed.

Procedure

In a screw-topped borosilicate glass test tube, add the following reagents in sequence to make up a final 10 ml solution:

1 ml soil sample extract
0.4 ml EDTA solution
1.6 ml salicylate nitroprusside
5 ml Millipore UltraPure (UP) DI water
0.8 ml buffered hypochlorite
1.2 ml Millipore UP water

Tubes were sealed with their screw caps, incubated for 30 minutes at 37 degrees C and then allowed to cool to room temperature before spectrophotometric evaluation. Pre-prepared ammonium-N standards of 0.08, 0.16, 0.24, 0.4, 0.8, 2.0, and 4.0 (g NH4⁺-N / ml) were used to calibrate the Hach spectrophotometer prior to running any batches of soil analyses. Additionally, these same refrigerated ammonium-N standards, freshly made weekly, were run with each soil sample batch of twenty samples, along with two tubes 2 M KCl solution as controls. A control tube plus two different ammonium-N standards were run through at the beginning and end of each batch as a quality control measure. On a regular basis (i.e. every 3 to 4 batches) a minimum of three samples from previous batches were repeated to ensure overall integrity of the reagents, standards and procedure.

After the nominated 30 minute heating period, colour development in the analyte was assumed to be complete and tubes were inserted directly into the Hach spectrophotometer using the supplied adapter and read for ammonium ion concentration in mg NH₄⁺-N/L at a wavelength of 667 nanometres (nm). Colour development in the tubes was found to be stable for at least 24 hours, but in all instances in the current study the tubes were evaluated for ammonium-N concentrations within one hour of incubation completion.

Raw spectrophotometer readings were normalised for any N-standards errors, and mathematically adjusted for extract dilution including soil moisture, and for oven-dried (OD) soil weight to give a final readout of μg NH₄⁺-N g⁻¹ OD soil.
Appendix 4: Colourmetric procedure for nitrate-ion analysis

Reagents
- 2M Potassium Chloride
- Laboratory grade Potassium nitrate to make up nitrate-N standards
- Hach Company proprietary NitraVer 5 powder pillow sachels for nitrate analysis
- High purity deionised water

Equipment
- Spectrophotometer measuring in the visible light region
- 10 ml cuvette containers with caps

The procedure was carried out as follows:

Nitrate standards of 0, 2, 4, 10, 20, 30, and 50 µg NO₃⁻·N/ml were made up from laboratory grade Potassium nitrate reagent in 2M KCl prior to running the analysis and these were used to calibrate the Hach DR/2010 spectrophotometer. Fresh nitrate standards were prepared bi-weekly and stored in dark bottles in a refrigerator. Spectrophotometer zero calibration was achieved using a cuvette filled with high purity deionised water.

A 10 ml soil sample extract was introduced into a 10 ml square cuvette bottle and the NitraVer 5 dry reagent added. On capping the container was vigorously shaken by hand for one minute and then set aside for five minutes of reaction time and development of colour. After wiping with a lint-free cloth, the cuvette was placed into the Hach 2010 spectrophotometer and read for µg NO₃⁻·N/ml at a wavelength of 500 nm. Previous soil moisture and 2M KCl extract solution dilution calculations allowed reporting of nitrate in µg NO₃⁻·N/g OD soil.

Investigation during the initial stages of procedure testing revealed that colour development was not stable with time and that the five minute reaction time needed to be adhered to closely for accurate results. In a similar fashion to the ammonium analysis described above, a 2m KCl blank extract and nitrate standards covering the expected range were run through at the beginning and end of each 20 soil sample batch. At times, previous extracts were repeated in later batch analysis to check the precision and ongoing integrity of the procedure.
Appendix 5: Steam distillation method for NH$_4^+$ and NO$_3^-$ ion analysis

Reagents

- Magnesium oxide [heavy] (MgO)
  - Heat heavy MgO in an electric muffle furnace at 600 – 700$^\circ$ C. for 2 hours.
  - Cool in a desiccator and store in a tightly stoppered bottle.
- Boric acid indicator solution
  - Add 40 g of reagent grade boric acid (H$_2$BO$_3$) to 1.8L of deionised water in a 2 litre volumetric flask
  - Add 40 ml of indicator solution made up in a 2 L batch as follows
    - Dissolve 0.0495g bromocresol green and 0.0330g methyl red in 50 ml of ethanol, and bring to 2 L volume with deionised water
    - With continuous stirring, adjust the pH to 4.8 to 5.0, or until the solution assumes a reddish purple tint, by cautiously adding 1 M sodium hydroxide (NaOH). If excess NaOH is added, the pH can reduced by adding dilute HCl.
- Devarda’s alloy – finely ground
- Sulfamic acid 0.2 M. Dissolve 2 g of sulfamic acid in 100 ml of deionised water. Store in a refrigerator.
- Sulfuric acid (H$_2$SO$_4$) 0.0025 M standard
- Standard NH$_4^+$ and NO$_3^-$ solutions
  - NH$_4^+$ Standard – dissolve 0.4716 g of ammonium sulfate in deionised water and bring up to 1 L in a volumetric flask. If pure dry reagent is used, this solution will contain 100 µg NH$_4^+$ - N per ml. Store in a dark bottle in a refrigerator.
  - NO$_3^-$ Standard. Dissolve 0.7218 g of ammonium sulfate in deionised water and bring up to 1 L in a volumetric flask. If pure dry reagent is used, this solution will contain 100 µg NO$_3^-$ - N per ml. Store in a dark bottle in a refrigerator.

Ammonium nitrogen procedure

- Add 5 ml of Boric acid indicator solution to a 50 ml beaker marked to indicate a volume of 35 ml, and position the beaker under the condenser of the distillation apparatus with the tip of the outlet touching the beaker side about 1 cm below the top
- Pipette a 15 ml aliquot of soil extract into the distillation flask, and add 0.2 g of MgO using a long-handled micro-spoon to reach the bottom of the round-bottomed flask.
- Immediately attach the flask to the distillation apparatus and commence steam distillation. When the beaker volume reaches 35 ml, stop the distillation and rinse the condenser tip into the beaker.
- Titrate the distillate with 0.0025 M H$_2$SO$_4$. At the end-point the colour changes from green to permanent faint-pink.

Nitrate nitrogen procedure

- Following steam distillation with MgO, remove the distillation flask from the apparatus and add 0.2 g of Devarda’s alloy to the bottom of the flask using a long-handed micro-spoon, rapidly replacing the distillation flask onto the apparatus.
- Carry out the steam distillation and titration as described above for the ammonium procedure
Calculations

Calculate the amount of N liberated by steam distillation from the expression:

\[ \text{N- concentration} \mu\text{g (NH}_4^+ \text{ or NO}_3^-) \text{ per ml} = (S - C) \times T \]

Where \( S \) = volume of 0.0025 \( M \) H\(_2\)SO\(_4\)
\( C \) = volume used in titration of 2 \( M \) KCl control
\( T \) = titer of 0.0025 \( M \) H\(_2\)SO\(_4\) tiritant = 70 \( \mu\)g per ml

Using soil moisture and extract volume recorded data, final reporting of ionic concentrations was made in \( \mu\)g / g OD soil by calculation of sample oven-dried soil mass and extract dilution.
## Appendix 6: Synthetic Groundwater Solution

### Synthetic Groundwater Solution

Laboratory incubations

<table>
<thead>
<tr>
<th>Ion</th>
<th>Compound</th>
<th>Initial Mineral Solution (g / L)</th>
<th>1/37.9 dilution of Mineral Solution (g / L)</th>
<th>Chloride ion (g)</th>
<th>Sulfate ion (g)</th>
<th>Final dilution of Salts in Mineral Solution (mg / L)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>NaCl</td>
<td>9.972</td>
<td>0.263</td>
<td>0.160</td>
<td></td>
<td>263.1</td>
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<tr>
<td>K</td>
<td>KCl</td>
<td>1.272</td>
<td>0.034</td>
<td>0.016</td>
<td></td>
<td>33.6</td>
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</tr>
<tr>
<td>Ca</td>
<td>CaCl</td>
<td>4.229</td>
<td>0.112</td>
<td>0.052</td>
<td></td>
<td>111.6</td>
<td>Same ratio as the McLeods Ck groundwater (van Oploo, 2000)</td>
</tr>
<tr>
<td>Mg</td>
<td>MgSO₄</td>
<td>20.538</td>
<td>0.542</td>
<td>0.432</td>
<td></td>
<td>541.9</td>
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<tr>
<td>SO₄</td>
<td></td>
<td></td>
<td>0.432</td>
<td></td>
<td></td>
<td>432.5</td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td></td>
<td></td>
<td>0.228</td>
<td></td>
<td></td>
<td>227.9</td>
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</table>

<table>
<thead>
<tr>
<th>Dissolv'd Salts g/L</th>
<th>Dissolv'd Salts g/L</th>
<th>Dissolved Salts mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.010</td>
<td>0.950</td>
<td>950</td>
</tr>
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</table>

### Field site Total Salts (measured)

<table>
<thead>
<tr>
<th>Tweed Groundwater</th>
<th>1 - 1.35 dS/m</th>
<th>700mg - 950mg / L</th>
<th>low-mod. Saline</th>
<th>FAO (1992)</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Oploo, 2000</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Appendix 7: Iodometric Titration Procedure

The method used in the current study trapped the hydrogen sulfide from soil pyrite in a 15 ml glass scintillation vial inserted inside a 200 ml Pyrex Erlenmeyer digestion flask. This scintillation vial contained the alkaline zinc trapping solution. At the end of digestion/diffusion stage, the numbered glass vials containing the insoluble zinc sulfide precipitate were removed from the flasks and sealed with their screwed caps. These were stored until ready to be iodometrically analysed for sulfide. The iodometric procedure requires some reagents to be pre-prepared and calibrated. These are the titration solutions: 0.0250N iodine solution, and the 0.0250N Thiosulfate solution.

The full procedure is as follows:

Equipment

- Magnetic stirrer plate, and Teflon coated stirrer-bar
- 200 ml glass Erlenmeyer flasks for titration
- Transfer Pipette
- Micro-burette of at least 15ml capacity

Reagents:

- Hydrochloric acid, HCL, 2N
- Glacial acetic acid
- High purity deionised water
- Standard Iodine solution, 0.0250N
- Dissolve 20 to 25g KI in DI water and add 3.2g Iodine and dissolve
- Dilute to 1000ml and standardise against the standard 0.0250N thiosulfate sol’n using starch solution or Vitex as the indicator
- Standard Sodium thiosulfate solution, 0.0250N, here prepared from off-the-shelf 0.1N Sodium thiosulfate standard.
- Starch solution, or Vitex indicator

Procedure

- Measure 0.0250N iodine solution from a burette or Pipette into a 200 ml magnetically stirred a volume of the iodine estimated to be in excess of the sulfide present – add an equal amount of distilled water to dilute the iodine solution to <0.02N
- Add 5ml 2N HCl
- To the trapping vial, add 1.5ml of glacial acetic acid to neutralise the alkalinity to around pH 5.5
- Pipette all of the trap vial sample into the stirred flask, discharging under the solution surface
- If iodine colour begins to disappears, add more measured amounts of iodine solution so that colour remains
- To the trapping vial, add 5 ml of iodine solution, followed by 5 ml of 2N HCl. Recap the vial and shake gently to dissolve any precipitate stuck to the glass, and transfer to the titration flask. Rinse with 5 ml of DI water and transfer this also to the titration flask
- Back-titrate with 0.0250N thiosulfate , adding the indicator (three drops of starch solution or approx. 0.1g of Vitex indicator) as the end point is approached (indicated by a light straw colour). Continuing back-titrating until the blue colour disappears
Calculation

One ml of 0.0250N iodine solution reacts with 0.4mg S²⁻:

\[
\text{mg } S^2^- / L = [(A \times B) - (C \times D)] \times 16 000 / \text{ml sample}
\]

Where
A = ml iodine solution
B = normality of iodine solution
C = ml thiosulfate solution
D = normality of thiosulfate solution
Appendix 8: X-Ray Diffraction analysis of cane-block soil

<table>
<thead>
<tr>
<th>Sample</th>
<th>R6 (0.6m) wt.%</th>
<th>R6 (0.6m) sd</th>
<th>R8 (0.9m) wt.%</th>
<th>R8 (0.9m) sd</th>
<th>R9 (1.0m) wt.%</th>
<th>R9 (1.0m) sd</th>
<th>R12 (1.7m) wt.%</th>
<th>R12 (1.7m) sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amorphous quartz kaolinite plagioclase smectite illite pyrite</td>
<td>0.6</td>
<td>2.1</td>
<td>11.4</td>
<td>2.1</td>
<td>16.1</td>
<td>2.4</td>
<td>31.1</td>
<td>2.5</td>
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</tbody>
</table>

See following pages for XRD mineral and clay analysis sheets
R12 clays
### Appendix 9: Field Trial 2 Fieldwork Schedule

<table>
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<tr>
<th>Field trip date</th>
<th>Purpose</th>
<th>Notes</th>
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<tr>
<td>5-9 Sept. 2006</td>
<td>Field trial set up with numbered plot stakes</td>
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</tr>
<tr>
<td></td>
<td>Preplant soil sampling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soil description</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Side-drain sampling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DGPS survey of cane-block</td>
<td></td>
</tr>
<tr>
<td>20 Oct 2006</td>
<td>Cane planting</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amendment with CK44 Plant-Mix fertiliser</td>
<td>Fertiliser rate: 200 kg / ha (17.0 kg N / ha)</td>
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<tr>
<td>6 Dec 2006</td>
<td>Cane-row soil amendment with urea fertiliser</td>
<td>Fertiliser rate: 100 kg / ha (46.0 kg N / ha)</td>
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<tr>
<td>27-31 Jan. 2007</td>
<td>Soil-N sampling / extraction</td>
<td>All temperature loggers checked for</td>
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<tr>
<td></td>
<td>Soil Description</td>
<td>operation and accuracy</td>
</tr>
<tr>
<td></td>
<td>Side-drain sampling</td>
<td>Groundwater level loggers checked and</td>
</tr>
<tr>
<td></td>
<td>Creek water sampling</td>
<td>calibrated</td>
</tr>
<tr>
<td></td>
<td>DGPS survey of logger height and position</td>
<td>Moisture logger checked and calibrated</td>
</tr>
<tr>
<td></td>
<td>Installation of loggers</td>
<td>Rain gauge checked and calibrated</td>
</tr>
<tr>
<td></td>
<td>• Rain gauge</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Soil moisture</td>
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</tr>
<tr>
<td></td>
<td>• Groundwater level</td>
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<td></td>
<td>• Soil temperature</td>
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</tr>
<tr>
<td></td>
<td>• Air temperature</td>
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<tr>
<td>20-24 Apr. 2007</td>
<td>Soil-N sampling / extraction</td>
<td>30cm soil temperature logger found not</td>
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<tr>
<td></td>
<td>Soil Description</td>
<td>working</td>
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<tr>
<td></td>
<td>Logger data download</td>
<td>Soil moisture logger found not working –</td>
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<tr>
<td></td>
<td>DGPS survey of Survey Bench Marks (AHD) – connect to local field site</td>
<td>repaired sensor wire connection</td>
</tr>
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<td></td>
<td>survey</td>
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<td>12-15 Jun. 2007</td>
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<td></td>
<td>Soil moisture logger calibration check</td>
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<td>Gel-clay bulk sampling</td>
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<tr>
<td></td>
<td>Temperature logger calibration check</td>
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<td>17-19 Oct. 2007</td>
<td>Harvest data collection</td>
<td>Gel-clay bulk samples for use in the</td>
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<td>Gel-clay bulk sampling</td>
<td>Laboratory study</td>
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### Appendix 10: Soil analysis data – Field Trail 2

**Cane-block soil profile pH and Eh**

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<th>Depth (m)</th>
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<th>pH s.e.</th>
<th>Eh (Sep06)</th>
<th>Eh s.e.</th>
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<td>1.5</td>
<td>5.87</td>
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<td>242</td>
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<td>1.7</td>
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<td>225</td>
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<table>
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<th>Depth (m)</th>
<th>pH (Jan07)</th>
<th>pH s.e.</th>
<th>Eh (Jan07)</th>
<th>Eh s.e.</th>
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</thead>
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<th>pH s.e.</th>
<th>Eh (Sep07)</th>
<th>Eh s.e.</th>
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<tr>
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<tr>
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<td>3.97</td>
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### Incubation 1

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<tr>
<th>Day</th>
<th>Sulfide-S (mg / g OD)</th>
<th>s.e.</th>
<th>Mean Sulfide-S (mg)</th>
<th>s.e.</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21.0</td>
<td>0.5</td>
<td>19.9</td>
<td>0.3</td>
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</tr>
<tr>
<td>38</td>
<td>20.8</td>
<td>0.7</td>
<td>12.9</td>
<td>0.4</td>
<td>N-treated</td>
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</tbody>
</table>

### Incubation 2

<table>
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<tr>
<th>Day</th>
<th>pH (Untreated)</th>
<th>s.e.</th>
<th>Eh (Untreated)</th>
<th>s.e.</th>
<th>Mean Sulfide-S (mg / g OD)</th>
<th>s.e.</th>
<th>Untreated Sulfide-S (mg)</th>
<th>s.e.</th>
<th>Mean nitrate-N / tube (mg)</th>
<th>s.e.</th>
<th>Nitrate-N (ug/ml)</th>
<th>s.e.</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>n.d.</td>
<td></td>
<td>n.d.</td>
<td></td>
<td>20.0</td>
<td>0.8</td>
<td>11.93</td>
<td>0.35</td>
<td>0.00</td>
<td>0.00</td>
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<td>0</td>
<td>Start</td>
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<tr>
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<td>n.d.</td>
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<td>0.52</td>
<td>n.d</td>
<td>n.d</td>
<td>T1</td>
<td></td>
<td></td>
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<tr>
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<td>206</td>
<td>10</td>
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<td>0.03</td>
<td>4</td>
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<td>T1</td>
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<td>0.01</td>
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<td>0.3</td>
<td>T1</td>
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<table>
<thead>
<tr>
<th>Day</th>
<th>pH (N-treated)</th>
<th>s.e.</th>
<th>Eh (N-treated)</th>
<th>s.e.</th>
<th>N-treated Sulfide-S (mg / g OD)</th>
<th>s.e.</th>
<th>Mean nitrate-N / tube (mg)</th>
<th>s.e.</th>
<th>Nitrate-N (ug/ml)</th>
<th>s.e.</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
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<td>n.d.</td>
<td></td>
<td>n.d.</td>
<td></td>
<td>20.0</td>
<td>0.8</td>
<td>11.93</td>
<td>0.35</td>
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<td>0.6</td>
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Appendices

Appendix 11: Laboratory incubation analysis data
Incubation 3 light-proof enclosure temperature
Appendices

### Appendix 12: Field Trial 2 soil descriptions

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### Quirk Blnk 704 (8B) 26Jan07

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## Appendices

### Quirk Block 704 (11B) – 21Sep07 (relatively high watertable, recent heavy rainfall periods)

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### Appendix 13: Mean soil pH and redox measurements

R. Quirk Cane-block 704

Note: Standard errors shown as s.e. (n = 3)

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Appendices

Appendix 14: Fertiliser Specifications

**CK44 Fertiliser** (Incitec-Pivot, 2009)

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**urea** (Incitec, 2008)

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ns.


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