ESTIMATION OF MICROBIAL BIOMASS NITROGEN IN SOME AUSTRALIAN FOREST SOILS

by

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A thesis submitted for the degree of Doctor of Philosophy at The Australian National University Canberra, Australia

November 1990
STATEMENT OF ORIGINALITY

I certify that this thesis contains no material which is being or has been used for the award of any degree or diploma in any University.

I also certify that the experimentation and analysis, interpretation and presentation of the results in this thesis are my own work, and any assistance received in preparing this thesis and all sources used, have been acknowledged.

November, 1990

(A.K.M. Afzal Hossain)
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SUMMARY

The soil microbial biomass plays an important role in the storage and turnover of carbon and the nutrient elements in ecosystems. Recognition of this has lead to an increased interest in measuring the C and nutrient contents of the soil microbial biomass. Review of the literature indicates that there is still much uncertainty associated with methodology for estimating biomass. Fumigation-extraction (FE) methods appear more promising than fumigation-incubation (FI) procedures, but both involve assumptions and limitations. The assessment of microbial biomass N in forest soils has received little attention, and presents additional problems associated with low pH and regular inputs of carbon in litterfall and fine root turnover.

This study has led to evaluation of a modified fumigation-extraction technique for estimation of microbial biomass N in acid forest soils. The method involves the use of hexanol as a fumigant rather than the more hazardous chloroform, the direct extraction of total soluble N in 0.5M K₂SO₄, and use of a different 'control'. Four acid forest soils of varying physical and chemical characteristics and with different management histories were employed for the studies.

Exposure of the different forest soils studied to liquid hexanol for 16 to 24 hours was adequate for complete fumigation in most cases, although longer fumigation (120 hours) yielded slightly (ca. 10%) higher FE-N flush (total N in fumigated extract - total N in unfumigated extract) compared to the 24-hour value in a highly acid (pH 3.8 in KCl) soil. The average FE-N flushes obtained after 1-day hexanol and 1-day chloroform fumigations were very similar (27.8 and 29.8 mg N kg⁻¹ soil, respectively). The effect of duration of chloroform fumigation on the FE-N flush varied with the seasons of soil collection. Overall, 1-day chloroform fumigation resulted in a 20% higher FE-N flush than the 5-day chloroform fumigation.
Repeated fumigations with hexanol or chloroform did not release additional microbial N into K$_2$SO$_4$-extracts. However, a single extraction did not remove all the microbial N into solution. A second extraction removed an additional 26 to 44% of total N in K$_2$SO$_4$ extracts. In unfumigated soils, extractable total N declined from the first to the second to the third extraction, and thereafter remained fairly constant. Microbial N appeared to be extracted from unfumigated soils by K$_2$SO$_4$. Subtraction of total N in extracts of unfumigated soils as a 'control' thus yielded low estimates (by about 55 to 81%) of microbial N. A curve splitting technique was employed to separate non-microbial and microbial fractions of total N in fumigated and unfumigated extracts utilizing data obtained from sequential extractions.

Microbial N calculated from curve-splitting procedures gives theoretically the best and most accurate estimate. However, this procedure is complicated and impractical for routine use. A simpler alternative method for calculating microbial N is proposed which gives very similar estimates to the curve-splitting method. In this alternative method, mineral N in the unfumigated soils is used as the 'control'. A soil specific factor can be determined to account for the incomplete extraction of microbial N and the contribution of non-microbial N.

Estimates of microbial N obtained by the proposed calculation procedure showed a reasonable correlation ($r^2 = 0.44$, $P<0.001$) with those obtained with the 1-day chloroform fumigation-extraction method, but a highly significant correlation ($r^2 = 0.86$, $P<0.001$) with those obtained with the 5-day chloroform fumigation-extraction method.

The duration of aerobic incubation after chloroform fumigation affected the subsequent mineral-N flush (Fl-N flush). Generally, higher Fl-N flush values were obtained after 10 days of incubation. Estimates of microbial N obtained from direct extraction methods were always higher (10 to 65%) than the Fl-N flush. There was,
However, a good agreement between microbial N estimates based on the 1-day hexanol fumigation-extraction and the FI method ($r^2 = 0.66$, $P<0.001$). The correlation between estimates based on the 5-day chloroform fumigation extraction and the FI method was also good ($r^2 = 0.64$, $P<0.001$), but the correlation for 1-day chloroform fumigation and FI was poor ($r^2 = 0.23$, $P<0.01$).

Overall, the hexanol fumigation-extraction method appeared to be the most useful method for estimating microbial N in the acid forest soils studied. It is rapid, simpler, and less hazardous than alternative procedures.

The hexanol fumigation-extraction method developed for estimating microbial N was also used to estimate microbial C in some acid forest soils using the same K$_2$SO$_4$-extract. Residual hexanol-C must be removed prior to organic carbon measurements on the K$_2$SO$_4$-extract in order to avoid overestimates of microbial C. Hexanol-C can be removed by heating the fumigated extracts for 30 min at 70 °C. There was a highly significant correlation ($r^2 = 0.99$, $n = 12$) between the estimates of microbial C obtained by the hexanol and chloroform fumigation-extraction methods, indicating that the hexanol fumigation method may be a useful technique for simultaneous measurement of both microbial N and C using a single soil extract. This obviously will save time and labour. Suitable conditions under which soil microbial biomass C can be measured in the acid forest soils studied using the Substrate-Induced Respiration (SIR) technique were defined as: (a) a glucose concentration of either 5 or 10 mg g$^{-1}$ soil, and (b) measurement of respiration rates over 2 to 3 hours of incubation.

Season-of soil collection and forest management practices were found to affect the concentrations of soil microbial N. Soil microbial N in untreated yellow podzolic (YP), red podzolic (RP) and red earth (RE) soils were 19, 63 and 55% lower during dry summer months than during spring or winter. Generally, annual variation
in soil microbial N was not significant. Variously fertilized red earth soil, and burnt
and unburnt soils also showed similar seasonal patterns. A laboratory study of soil
drying and rewetting showed that the efficiency of fumigation is low (about 45%) in
air-dry soils. Rewetting prior to fumigation increased the estimates of microbial N
obtained.

In a yellow podzolic soil, microbial N was significantly higher (about 68%) after
sewage sludge treatment, but lower after N-fertilization. In a red earth soil, the
effects of fertilizer-N were very similar. Phosphorus (P) addition decreased microbial
N, but lime and P treatment caused a lesser reduction. Long unburnt soil contained
more (about 20%) microbial N than soil from regularly or very frequently burnt forest.
Microbial C estimated from SIR appeared to be erroneously high in the lime+P
treatment. It was significantly lower (by about 25%) in N-fertilized soil compared to
the unfertilized control soil. Microbial C was highest at the regularly burnt site, less
at the long unburnt site and least at the very frequently burnt site. The
corresponding values of soil microbial C for these three sites were 981, 646 and 406
mg C kg⁻¹ soil, respectively. Overall, the effects of various management practices on
microbial N and C, and seasonal changes in microbial N concentration in acid forest
soils are very difficult to explain.

Although microbial N and C did not always follow similar trends in differently
fertilized and burnt soils, they were highly significantly correlated ($r^2 = 0.78$, $P<0.001$)
when the lime+P treated and regularly burnt soils were excluded. In general, the C-
to-N ratios of the microbial biomass of fertilized soils (range 3.0 - 8.1, mean 5.0 with
the exclusion of soils from the lime+P treatment) were comparable (range 4.5 - 9.0,
mean 6.2) with those for forest soils found in the literature, but the ratios for the burnt
and unburnt sites varied more widely from 3.5 to 14.8 (mean 8.3). Both microbial N
and C decreased markedly with depth in the 0 - 10 cm soil profile.
Mineralization of soil N was much higher (96%, range 90 to 98%) in laboratory (disturbed soil, 25 °C) incubations than in in situ (undisturbed columns, fluctuating temperatures and moisture) incubations. Mineralization of soil N varied with season, being higher in spring and lower in autumn. Soil microbial N content was very poorly correlated with N mineralization in the acid forest soils studied. This indicates that N mineralization depends on the availability of substrates and the activity of soil microorganisms, not on the size of the pool of soil microbial N. Soil microbial N alone thus appears to offer little potential for assessing soil N availability or forest nutrition.
CHAPTER ONE

INTRODUCTION
CHAPTER ONE

INTRODUCTION

Nitrogen (N) is one of the key components of soil organic matter and is often the element required in largest amounts for plant growth. In forest ecosystems, N is generally a primary factor controlling above ground biomass productivity (Lea et al., 1980; Vitousek et al., 1982; Raison et al., 1990).

Many forest soils are highly acid, and have a high organic matter content in the surface horizons because of impeded decomposition rates. Most of the N in forest ecosystems is contained within the litter layer and the soil organic fraction (Gosz et al., 1973; Rosswall, 1976; Tate, 1987). The rate of net mineralization of this N is usually less than 1% per year. Much of the organic N can thus be described as 'passive' (Jansson, 1958) in terms of the turnover process. The more 'active' fraction is made up of the living microbial N and non-microbial active-N, consisting of microbial metabolites and/or recently stabilized microbial residues (Paul and Juma, 1981). Although microbial biomass comprises only a small fraction of soil organic matter, usually 1 to 5% of the total soil C or N, the measurement of this labile pool and its dynamics is important in understanding transformations of N, P and S (Jenkinson and Ladd, 1981).

Microbial biomass is considered as the agent of biochemical change in soil (Jenkinson, 1988). It serves as both a sink and a source of plant nutrients, especially N, P and S (Jenkinson and Ladd, 1981; Carter and Rennie, 1984). For example, Okano et al. (1987) calculated that 21 kg N ha\(^{-1}\) yr\(^{-1}\) was released through the microbial biomass in the root mat layer of a pastoral soil and concluded that the soil microbial biomass played an important role as the source of available N.
Recently, Smith and Paul (1990) discussed the significance of microbial biomass estimations in a global perspective and concluded that the rapid turnover of microbial biomass could cause major fluxes of important plant nutrients. Measurement of microbial biomass can also reveal management-induced changes in soil fertility before they become apparent in total soil organic C and N reserves (e.g. Schnurer et al., 1985; Powlson et al., 1987). Methods for early detection of change in soil fertility are especially important in forestry because alteration to growth of trees occurs over time scales of a decade or more. Early detection of long-term adverse trends can allow changes to management practices and thus lessen soil damage in the long-run.

Different methods for measuring soil microbial biomass N have been developed in recent years. Among them, the chloroform fumigation-incubation or FI method (Jenkinson and Powlson, 1976b; Shen et al., 1984) and chloroform fumigation-extraction or FE method (Brookes et al., 1985b) are used widely. Other available methods are based on the measurement of ninhydrin-reactive N (Amato and Ladd, 1988) and chloroform containing K2SO4-extractable soil N (Azam et al., 1989a). Most of these methods have been reviewed by several workers (see Sparling, 1985; Vance and Brookes, 1987; Jenkinson, 1988). The FI method gives apparently low estimates of microbial biomass N in strongly acid soils, or on soils with recent additions of fresh organic material (Jenkinson and Powlson, 1976b; Powlson and Jenkinson, 1976). These two situations are common in forest ecosystems where large annual inputs of C come from litterfall and fine root turnover. Direct fumigation-extraction (FE) procedures overcome many of the problems associated with the FI method. But the FE method has mostly been tested on near neutral soils, using chloroform as the fumigant. The few tests on forest soils have given poor results for microbial biomass N estimates (Brookes et al., 1985d; Davidson et al., 1989). Brookes et al. (1985b) cautioned that further testing was needed before the FE method was adopted for soils which have received fresh
substrate, and acid soils. They suggested that verification of the method was required in acidic forest soils where microbial growth is slow.

There have been numerous studies of factors (i.e. management practices such as different fertilizer additions, liming, crop rotation, tillage and straw retention as well as temporal and seasonal changes) affecting microbial biomass N in agricultural soils (see Chapter 2). In forests, many management practices, for example addition of fertilizer, sewage sludge amendment, liming, and fire of varying frequency and intensity, affect the nutrient capital of forest soils and alter the rates of mineralization of organically bound nutrients. These practices are also expected to influence microbial biomass C and N. An understanding of the microbial biomass C and N dynamics and their link with nutrient cycling processes may lead to enlightened management of forest soil fertility.

Broadly, the objectives of the present study were:

(a) to develop and evaluate in a range of acid forest soils, a direct fumigation-extraction (FE) procedure for estimating microbial biomass N based on the use of hexanol as the fumigant. This method was compared with existing procedures for estimating microbial biomass N where chloroform is used to kill soil microorganisms,
(b) to compare microbial biomass N estimates based on the fumigation-extraction methods with those obtained by the chloroform fumigation-incubation (FI) method,
(c) to assess the changes in soil microbial biomass N caused by various forest management practices, particularly fertilization, liming and low-intensity prescribed burning,
(d) to observe in detail the seasonal changes of microbial biomass N in three soils, one soil subjected to a range of treatments, and
(e) to examine linkages between estimates of soil microbial biomass N and the net mineralization of organic N in a range of differently treated acid forest soils.
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CHAPTER TWO

REVIEW OF METHODOLOGY FOR MEASUREMENT OF MICROBIAL BIOMASS NITROGEN AND CARBON IN SOILS

2.1 Introduction

The microbial biomass is considered to be the total weight of living microorganisms in the soil (Waid, 1984). Jenkinson and Ladd (1981) defined it as the living portion of the soil organic matter with the exclusion of plant roots and soil organisms larger than about $5 \times 10^3$ μm$^3$ in size. Many workers (e.g. Brookes et al., 1985c; Douglas, 1987 and Vance and Brookes, 1987) have reported that the soil microbial biomass consists of a large number of bacterial and fungal species, together with other soil organisms such as algae and protozoa.

The soil microbial biomass accounts for 1 to 5% of total soil C or N (Jenkinson and Ladd, 1981; Smith and Paul, 1990). Although small in size compared to other soil organic matter fractions, the microbial biomass is a potentially important source of plant nutrients, such as N, P, and S because of its rapid turnover. The turnover time of the soil microbial biomass varies markedly depending upon its degree of protection by soil colloids (van Veen et al., 1984), but as a whole, turnover has been estimated to range from <1 to about 3 years (Jenkinson and Ladd, 1981; Schnurer et al., 1985; Smith and Paul, 1990). Due to the rather large pool size, [for example, 374 kg C ha$^{-1}$ and 68 kg N ha$^{-1}$ to a depth of 25 cm from a soil where stubble had been incorporated for eighteen years (Powlson et al., 1987)], the microbial biomass can act as a major nutrient sink during immobilization and as a source during mineralization (Carter and Rennie, 1984; Juma and Paul, 1984; Steele and Vallis, 1988). The microbial biomass plays a dual role in the soil: as an agent of decomposition thus determining the release of organically-bound nutrients, and as a
labile reserve of plant available nutrients (Jenkinson and Ladd, 1981). As pointed out by Sparling (1985), the soil microbial biomass also affects root physiology and soil structure.

Because of the importance of the microbial biomass in the metabolism of organic substances in soil, it is essential to be able to accurately estimate the size and activity of this component of the ecosystem. The quantitative determination of microbial biomass in soil has been actively researched in recent years with the advent of promising biochemical techniques. Microbial biomass estimations have proved useful in a variety of studies of different ecosystems, but seasonal fluctuations in natural (Ross et al., 1984; Lynch and Panting, 1982) or perturbed (Sparling et al., 1981; West et al., 1986c) systems have complicated the picture. The change in microbial biomass estimates due to factors, such as soil type, climate, tillage; cropping history, application of fertilizers and biocides has been the focus of much research in recent years (e.g. Doran, 1987; Ladd et al., 1985; Granatstein et al., 1987; Woods and Schuman, 1988; Follett and Schimel, 1989; Saffigna et al., 1989). Soil microbial biomass measurements have also been used in studies of degradation of added organic chemicals (Anderson, 1984; Domsch, 1984; Ou and Street, 1987), residue decomposition (Adams and Laughlin, 1981; Chaussood et al., 1986), and effects of pollution (Brookes and McGrath, 1984; Boyle and Paul, 1988) or other soil disturbance (Sundman et al., 1978; Baath, 1980). Microbial biomass also appears to be a good early indicator of soil changes under different management systems (Powlson and Jenkinson, 1981; Powlson et al., 1987; Wu and Brookes, 1988). Recently, Smith and Paul (1990) discussed the significance of soil microbial biomass measurements in a global perspective. They considered soil biomass as an ecological marker for perturbed systems, such as clear-cutting of forests, acid rain pollution and agricultural soil disturbance.

This review concentrates on methods for measurement of soil microbial
biomass nitrogen and carbon in soils. Limitations and uncertainties associated with currently available methods are identified. Emphasis is given to methods applicable to the measurement of microbial biomass N in acid forest soils where problems in applying methods developed for agricultural soils have been identified (Brookes et al., 1985d; Jenkinson, 1988; Davidson et al., 1989).

In this thesis, soil microbial N or C refers to the N or C derived from live microbial biomass. The term 'microbial biomass' refers to an estimate of biomass based on the use of a k-factor to account for the incomplete measurement of N or C derived from killed biomass. Thus soil microbial N and C are considered to be indices of soil microbial biomass N and C. Nitrogen is dealt with first.

2.2 Methods for determining microbial biomass N in soil

The most commonly used methods for measuring microbial N in soils are the fumigation-incubation (FI) method (Jenkinson and Powlson, 1976b; Shen et al., 1984; Voroney and Paul, 1984) and the fumigation-extraction (FE) method (Brookes et al., 1985b). Chloroform is usually used as the fumigant for both methods to lyse microbial cells, causing the release of protoplasmic constituents within the soil, but the techniques differ in how the released microbial N is measured. Microbial N is estimated by the FI method from the mineral N flush extracted by 2M KCl after a 10-day incubation of fumigated and unfumigated soils at 25 °C (Shen et al., 1984) and by the FE method from the NH₄⁺-N flush in 0.5M K₂SO₄ extracts of fumigated and unfumigated soils, using a Kjeldahl digestion to convert organic N (Brookes et al., 1985b).

2.2.1 Fumigation-Incubation (FI) method

Fumigation kills soil microflora, and inorganic N is released from biomass,
both directly and during subsequent aerobic incubation (Russell and Hutchinson, 1909; Waksman and Starkey, 1923). Jenkinson and Powlson (1976a) found a small immediate increase in K$_2$SO$_4$-extractable NH$_4$-N due to fumigation with chloroform which they ascribed to the decomposition of organisms killed during fumigation. In the original FI method (Jenkinson and Powlson, 1976b), portions of soil are fumigated with ethanol-free chloroform for 1 day and left unfumigated. After fumigation, soils are inoculated with a small amount (usually 0.4%) of fresh soil. Both fumigated-reinoculated and unfumigated control soils are incubated aerobically for 10 days. After incubation, soil inorganic N contents are measured and microbial N is determined from the flush of mineral-N ($F_N$) caused by fumigation (Ayanaba et al., 1976; Jenkinson and Powlson, 1976b; Shen et al., 1984).

The amount of microbial biomass N ($B_N$) is calculated from the following expression:

$$B_N = \frac{F_N}{k_N}$$

where $F_N$ is the flush of N mineralized after fumigation less that released by the control (i.e. mineral N flush) and $k_N$ is the fraction of the N in the killed biomass which is mineralized during incubation.

A number of workers have used different approaches to calculate microbial N in soil. Anderson and Domsch (1980) used the C content of pure microbial material as a measure of microbial N, and found a conversion factor of 0.15. Several workers (e.g. Marumoto, 1984; Marumoto et al., 1982b; Kelly and Stevenson, 1985; Azam et al., 1986) have subsequently used this conversion factor. Nicolardot et al. (1986) calculated biomass N from the following relationship:

$$\text{Biomass N} = \frac{\text{Biomass C}}{(\text{Biomass C-to-N ratio})}$$

where Biomass C-to-N ratio is the C-flush-to-N flush of the microbial materials labelled with $^{14}$C and $^{15}$N.
A number of methods for calculating $F_N$ are available, each of which is based on the use of a different control (Table 2.1). The $F_N$ is generally considered as the difference between the net mineralization of N by a fumigated soil and by an unfumigated control soil during a 10-day aerobic incubation (Jenkinson and Powlson, 1976b; Shen et al., 1984; Voroney and Paul, 1984). In this method, freshly sampled soils are pre-incubated before fumigation to stabilize microbial activity after sample preparation. In a second method, Shen et al. (1989) used the N mineralization by an unfumigated control during 10 to 20 day period of incubation for soils that were not pre-incubated. These workers showed that pre-incubation (i.e. method 1) gave 10 to 20% lower results than those obtained without pre-incubation (i.e. method 2). They attributed the difference to be due to that part of biomass which decomposed during the pre-incubation. In a third approach, the $F_N$ is calculated as the NH$_4^+$-N mineralized by the fumigated soil over the 0 - 7 day period, less that mineralized by the same fumigated soil over the 7 - 14 day period. In a fourth method, Voroney and Paul (1984) did not use any control. According to them, almost all the N mineralized after fumigation is derived from microbial tissue. They found that less than 2 mg N

### Table 2.1 Methods of calculation the mineral N flush (FI-N flush) after chloroform fumigation-incubation using different controls.

<table>
<thead>
<tr>
<th>Approach</th>
<th>Method of calculation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$N_F(0-10) - N_{UF}(0-10)$</td>
<td>Jenkinson and Powlson (1976b)</td>
</tr>
<tr>
<td>2</td>
<td>$N_F(0-10) - N_{UF}(10-20)$</td>
<td>Jenkinson and Powlson (1976b), Shen et al. (1989)</td>
</tr>
<tr>
<td>3</td>
<td>$N_F(0-7) - N_F(7-14)$</td>
<td>Nicolardot and Chaussod (1986)</td>
</tr>
<tr>
<td>4</td>
<td>$N_F(0-10)$</td>
<td>Voroney and Paul (1984)</td>
</tr>
</tbody>
</table>

Note: $N_F$ and $N_{UF}$ represent the N mineralized from chloroform fumigated and unfumigated soils during incubation, respectively. Inside brackets, figures represent the period (days) of incubation.
kg\(^{-1}\) soil was mineralized in the unfumigated control during 10 days of incubation and concluded that this did not significantly affect estimate of microbial N.

Overall, the use of different controls depends largely upon whether pre-incubation is used. Errors associated with the use of an inappropriate control are often minor, because the ratio of N mineralized by the fumigated soil to that mineralized by the unfumigated soil is usually high (Jenkinson, 1988).

The main problem in calculating microbial biomass N by the FI method is the determination of an appropriate \(k_N\) value. Jenkinson and Ladd (1981) highlighted some of the likely problems that are associated with the conversion of \(F_N\) values to microbial biomass N by the use of a single \(k_N\) value. These include the variable N content of microorganisms, and the different sections of the soil population which cover a much wider range for N than for C concentration. The \(k_N\) cannot be obtained simply by growing organisms in vitro, adding them to soil, fumigating and determining how much of their N is mineralized to NH\(_4\) (Jenkinson, 1988). The \(k_N\) will vary considerably due to variable N contents of soil organisms and changes in their relative proportions in soils (McGill et al., 1986). In addition, Voroney (1983) demonstrated that the \(k_N\) and the C-flush to N-flush followed a non-linear relationship for a group of organisms with C : N ratios varying from 3.7 to 11.3. A wide range of \(k_N\) values varying from 0.28 (Paul and Voroney, 1984) to 0.68 (Shen et al., 1984) have been used for converting the flush of mineral N to microbial biomass N. This large variation is probably due to the nature of the microorganisms which are mineralized and the nature of microbial habitats and protective capacity of soils. A number of workers (e.g. Carter, 1986; Powlson et al., 1987; Robertson et al., 1988) have assumed a \(k_N\) value of 0.68 applies to different soil types.

Jenkinson (1976) determined \(k_N\) factors for bacterial N varied from 0.45 to 0.59, but observed no net mineralization of fungal N for the species studied. Adams
and Laughlin (1981) calculated a mean $k_N$ of 0.32 and a range from 0.30 to 0.58, for bacteria and yeast, and also found no net mineralization of fungal N. Amato and Ladd (see Jenkinson and Ladd, 1981) determined an average $k_N$ of 0.46 and 0.28 for two bacterial and two fungal species, respectively. Voroney et al. (1981) studied the response of microbial biomass to additions of glucose and nitrate, and suggested a $k_N$ of 0.28 for the determination of biomass N in soil. Marumoto et al. (1982a) obtained a $k_N$ value of 0.37 from studies with dead biomass in arable soils, and a value of 0.24 was found for paddy soils (Marumoto, 1984). Inubushi and Watanabe (1987) found a $k_N$ factor of 0.43 for an anaerobic soil. Nicolardot et al. (1986) estimated a $k_N$ between 0.36 and 0.42 for three French soils. Voroney and Paul (1984) suggested a $k_N$ factor of 0.30 for soils where most of the soil population represents a long-term steady state condition with adequate available N. Work by Juma and Paul (1984) supported this value. Azam et al. (1988) obtained $k_N$ values ranged from 0.19 to 0.42 for microbial biomass produced in situ and mineralized under anaerobic conditions. They also reported that the value increased with increasing levels of amendment (N + glucose). Nicolardot et al. (1989) calculated a $k_N$ value of 0.37 for soils that had not received recent additions of decomposable organic matter. Jenkinson (1988) reviewing available data concluded that a $k_N$ value of 0.57 is appropriate where the C : N ratio of the soil microbial biomass is less than 6.7.

To overcome the problem of use of a single $k_N$, several workers (e.g. Ross et al., 1980b; Adams and Laughlin, 1981) have decided not to convert the flush of mineral-N to microbial N. Carter and Rennie (1984) and McGill et al. (1986) decided not to convert the flush of NH$_4$-N released after fumigation to estimate biomass N because of variability in published methods of calculating biomass N (Ladd et al., 1981; Voroney and Paul, 1984). West et al. (1988a) also did not convert the flush to biomass N, because of the lack of valid $k_N$ factors for their soils. They presented their data as microbial N-flush, assuming that the N flushes were linearly related to
biomass N content. Ayanaba et al. (1976) also avoided the use of \( k_N \) to calculate biomass N, but proposed a direct relationship between the mineral-N flush and biomass C. They obtained the equation: \( \text{Biomass C} = 8 F_N \), using a \( k_C \) value of 0.5, but when a \( k_C \) value of 0.45 was used, the relationship becomes: \( \text{Biomass C} = 9 F_N \) (Jenkinson and Ladd, 1981).

The FI method does not provide useful data for soils which have received recent organic substrate additions, and in strongly acid soils (pH <4.5) (Jenkinson and Powlson, 1976b). In these situations, mineralization of soil N from the unfumigated control soil frequently exceeds that from the fumigated samples (Jenkinson et al., 1979; Sparling, 1981a; Williams and Sparling, 1984). This method can not be used with very wet soils because there is a restriction to the rapid permeation of chloroform in the waterlogged soil (Jenkinson, 1988). However, Inubushi et al. (1984) overcome this problem by spreading the soil on a Petri dish to form a thin layer and fumigating with chloroform vapour using five evacuation steps to allow the chloroform to permeate into the soil.

Soils must not be air-dried, because air-drying solubilizes native organic matter, rendering it easily decomposable, and kills a portion of the biomass (Powlson and Jenkinson, 1976). The FI method can be less reliable for estimating microbial N than microbial C, because N content of microbial cells is much more variable than C content and some of the N mineralized during the incubation of fumigated soil is reimmobilized (Jenkinson, 1976; Marumoto et al., 1982a). Brookes et al. (1985a) showed that either denitrification or immobilization of N by the soil organisms during incubation could mask the fumigant induced release of N. Shen et al. (1984) and Voroney and Paul (1984) attempted to overcome the problem of N immobilization during incubation. Shen et al (1984) assumed that a constant fraction of the N released from the killed population was reimmobilized. Using this assumption, they calculated \( k_N \) of 0.68 from the ratio of C-flush to N-flush, taking the mean C-to-N
ratio of microorganisms to be 6.7 and a $k_C$ value of 0.45. On the other hand, Voroney and Paul (1984) labelled the microbial biomass \textit{in situ} by adding $^{14}$C-glucose and $^{15}$N-KNO$_3$ to soil, incubating and measuring C and N mineralization after fumigation. From these studies, they proposed a variable $k_N$, using an adjustment factor calculated from the ratio of CO$_2$-C evolved to N mineralized during incubation to correct for N immobilization. It is thus necessary to be cautious when the FI method is used to estimate microbial N in soil because the reimmobilization of NH$_4$-N depends on the C : N ratio of the soil microbial population and of the soil.

Despite the various limitations, the FI method has still been used widely to estimate the quantity of microbial N in a wide range of soils (Azam \textit{et al.}, 1986; Mallouhi and Jacquin, 1985; Marumoto, 1984; Williams and Sparling, 1984).

\textbf{2.2.2 Fumigation-extraction (FE) method}

The chloroform fumigation-extraction method proposed by Brookes \textit{et al.} (1985b) is based on the observation that K$_2$SO$_4$-extractable NH$_4$-N increases when soils are fumigated with chloroform (Jenkinson and Powlson, 1976b), and a much larger increase occurs in total (i.e. organic and inorganic) N (Brookes \textit{et al.}, 1985a). This method requires exposure of the soils to chloroform vapour for 24h followed by immediate extraction with 0.5M K$_2$SO$_4$ under standardized conditions and the measurement of the increment in total N extracted. Sparling and West (1988b) measured NH$_4$-N in K$_2$SO$_4$-extracts using dichromate oxidation rather than Kjeldahl digestion, and found that the former was only slightly less (ca. 10%) efficient.

Brookes \textit{et al.} (1985b) calculated microbial biomass N in soil using the following equation:

$$\text{Biomass N} = \frac{1 \text{ day CHCl}_3-\text{N}}{0.54}$$
where 1 day CHCl₃-N is the difference between the amount of total N extracted with K₂SO₄ immediately after fumigation for 24h and the amount extracted from unfumigated soil at the beginning of fumigation, and 0.54 is an experimentally determined proportionality factor. The value of 0.54 is derived from the relationship between microbial N estimates obtained by the fumigation-incubation and fumigation-extraction methods where 1-day CHCl₃-N was 79% of the N mineralized during fumigation and incubation. They used a kₜ of 0.68 to calculate biomass N from the mineral-N flush.

The FE method has several advantages over the conventional fumigation-incubation method, as noted by Brookes et al. (1985b). These are: (a) the N can be extracted immediately after soil fumigation and hence no incubation is required, and (b) mineralization of the killed biomass N is carried out by chemical treatment rather than by the recolonizing microbial population. This method saves time and avoids the need for any correction for denitrification or immobilization (Brookes et al., 1985b). In addition, this method does not require complete fumigant removal. The FE method may be useful where the FI method breaks down, such as in acid soils, or in freshly sampled soils, or in soils that have recently received fresh organic substrates. The FE method may give accurate estimation in wet compacted soils, where the FI method appears to be unreliable (Ross, 1987). Despite these apparent advantages, the FE method still has some problems. It gives low estimates of microbial N because some of the organic N in K₂SO₄ extracts of unfumigated soil is derived from microbial sources (Azam et al., 1989a) and possibly single extraction may not be sufficient to remove all the microbial N from the fumigated soil. These aspects are investigated in detail in Chapter 4.

In recent years, several modifications to the direct extraction method have been proposed based on different approaches. For example, Amato and Ladd (1988) described a method for microbial N estimation in soils which involves the
measurement of ninhydrin-reactive N (mainly alpha-amino acid N and NH₄-N) released from soils which are fumigated with CHCl₃. In this method, the microbial N is calculated from the relationship: Microbial N = 3.1 x ninhydrin-reactive N, released from fumigated soils only, accepting a value of 6.7 : 1 for the C:N ratio of the soil microbial biomass (Shen et al., 1984). The factor of 3.1 is derived from the relationship between microbial C and ninhydrin-reactive N in fumigated soils where the relationship is given as: Microbial C = 21 x amount of released ninhydrin-reactive N.

Azam et al. (1989a) proposed a modification of the chloroform fumigation-extraction method which they termed the chloroform-extraction method (or simply CEM). In this method, K₂SO₄-containing chloroform was used to extract ¹⁵N-labelled microbial N produced in soil in situ using glucose and ¹⁵N-ammonium sulphate. The modified method proved superior to FE method for extracting microbial N and is comparatively simpler, more rapid and more convenient.

Widmer et al. (1989) modified the FE method for soils containing large amounts of inorganic N. In this method, the control soil is given a 15 min preliminary extraction, immediately followed by a 30 min extraction. At this time the 24 h chloroform fumigation is begun. The fumigated soil then receives an identical sequential extraction at the end of the 24 h period following chloroform removal. This method gives a small and non-significant decrease in measured biomass N.

2.2.3 Comparison of the methods

No one method appears to be adequate and reliable in all circumstances (Carter, 1986). The accuracy and reliability of any procedure depends on the calibration against other methods. The comparability of estimates of soil microbial N obtained by the FE and FI methods varies. For example, a number of studies (e.g.
Davidson *et al.*, 1989; Nannipieri *et al.*, 1990a) showed that the microbial N obtained by the FE method was more than double that obtained by the FI method. The reason appears to be that aerobic incubation of soils can result in N immobilization (Hart *et al.*, 1986) and thus anomalously low FI-N flush (Williams and Sparling, 1984; Sparling and Williams, 1986), particularly in acid organic soils. Nannipieri *et al.* (1990a) also attributed lower microbial N values estimated by the FI method to the immobilization of mineral N during incubation. Others (Azam *et al.*, 1989a; Davidson *et al.*, 1989;) have also found that estimates obtained by the FE and FI methods to be poorly correlated. Several other workers (e.g. Brookes *et al.*, 1985b; Sparling and West, 1988a,b) found 20 to 40% higher microbial N by the FI method than the FE method, while Brookes *et al.* (1985b) found similar estimates of microbial N after 5-day fumigation followed by direct extraction and the FI-N flush.

The correlation between the estimates of soil microbial N obtained by the FI and FE methods are good in some cases, but not in others. For instance, Gallardo and Schlesinger (1990) found a significant correlation (r = 0.77) between these two methods in soils from 0 - 20 cm depth of pH ranged from 3.70 to 3.94. They explained two reasons for this reasonable correlation: (i) use of a larger inoculum (3% of total mass rather than 0.4% which was used in the original method by Jenkinson and Powlson, 1976b) and (ii) they did not incubate unfumigated soil because they assumed that all the net increase in available N after fumigation following incubation was derived from mineralization of biomass, which was proposed by Voroney and Paul (1984). These workers, however, found no correlation in samples collected from the deeper horizon (up to 60 cm) of the soil profiles. They attributed this non-significant correlation to the lower reliability of both methods with low levels of microbial biomass. Some deep samples were wet and compacted which might have resulted in a lower estimate of microbial biomass, as previously noted by Ross (1987). Davidson *et al.* (1989) found a close correlation between the fumigation-extraction and fumigation-incubation methods in acid forest
soils when a variable $k_N$ was used to convert the Fl-N flush to microbial biomass N. Microbial biomass N obtained by the fumigation-extraction method also showed significant correlation with those obtained by chloroform fumigation extraction method (Azam et al., 1989b), direct microscopic counting technique (Martikainen and Palojarvi, 1990) and ninhydrin-reactive N (Ocio and Brookes, 1990a). There is also a significant correlation between the estimates of soil microbial N (FE method) and biomass C measured by a modified FI method (Brookes et al., 1985d). Overall, it is apparent that the relationship between the methods depends upon the size of the inoculum, soil moisture content and use of a single $k_N$ factor to convert the Fl-N flush to microbial biomass N.

2.3 Methods for determining microbial biomass C in soil

Soil microbial C can be estimated, both directly and indirectly, by a wide variety of methods. The most common methods are: (1) fumigation-incubation (or FI) (Jenkinson and Powlson, 1976b), (2) fumigation-extraction (or FE) (Vance et al., 1987c; Tate et al., 1988), (3) substrate-induced respiration (or SIR) (Anderson and Domsch, 1978b; West and Sparling, 1986) and (4) soil ATP determination (Paul and Johnson, 1977; Jenkinson and Oades, 1979). In addition, the following other methods are available, but they are not widely used: microbial biovolume derived by direct microscopic observation (Jenkinson et al., 1979), microcalorimetric methods and analyses of soil for specific biomass constituents. Detailed description of all these methods and discussion of their advantages and limitations can be found in the literature (see Paul and Voroney, 1980; Jenkinson and Ladd, 1981; Sparling, 1985; Vance and Brookes, 1987; Jenkinson, 1988; Nannipieri et al., 1990b). Discussion here is confined to recent advances, and evaluation of the advantages and unresolved issues.
2.3.1 Fumigation-incubation (FI) method

This method which involves fumigation with chloroform followed by incubation for a given period, has been used widely for microbial biomass C estimations in various soil systems. It was first proposed by Jenkinson (1966) and later modified by Jenkinson and Powlson (1976b). The original FI method is based on the assumption that the additional CO₂-evolved due to the fumigation and incubation comes from the microbial cells killed by the fumigant and their decomposition by the subsequent recolonizing microbial population (Jenkinson and Powlson, 1976b). Fumigants other than chloroform which have been used include methyl bromide (Powlson and Jenkinson, 1976), methyl bromide plus chloropicrin (Spalding, 1978), carbon disulphide (Kudeyarov and Jenkinson, 1976; Vance et al., 1987a) and propylene oxide (Kassim et al., 1981). None of these fumigants had shown greater efficiency than chloroform but most of them create problems because of the difficulty in removing them from soil after fumigation (Jenkinson, 1988).

In the FI method, microbial biomass C is usually calculated from the following relationship:

\[
\text{Biomass C} = \frac{F_C}{k_C}
\]

where \( F_C \) is CO₂-C released from a fumigated soil, less that evolved by unfumigated soil (under the same environmental conditions) and \( k_C \) is a constant, the fraction of the killed biomass C mineralized to CO₂ during incubation under the same conditions (Jenkinson and Powlson, 1976b). The validity of this relationship depends on two factors: the choice of unfumigated control and use of a suitable value for \( k_C \).

The unfumigated ‘control’ represents the respiration of non-microbial organic C during incubation. There are arguments about the choice of the control. Four methods of calculating soil microbial biomass C are available in the literature, each of which is based on a different or no control (Table 2.2).
Table 2.2 Calculation for soil microbial biomass C after chloroform fumigation-incubation using different controls.

<table>
<thead>
<tr>
<th>Approach</th>
<th>Method of calculation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \frac{[C_F(0-10) - C_{UF}(0-10)]}{k_c} )</td>
<td>Jenkinson and Powlson (1976b)</td>
</tr>
<tr>
<td>2</td>
<td>( \frac{[C_F(0-10) - C_{UF}(10-20)]}{k_c} )</td>
<td>Jenkinson and Powlson (1976b)</td>
</tr>
<tr>
<td>3</td>
<td>( \frac{[C_F(0-10) - C_{UF}(10-20)]}{k_c} )</td>
<td>Chaussod and Nicolardot (1982)</td>
</tr>
<tr>
<td>4</td>
<td>( \frac{[C_F(0-10)]}{k_c} )</td>
<td>Paul and Voroney (1980, 1984); Voroney and Paul (1984)</td>
</tr>
</tbody>
</table>

Note: \( C_F \) and \( U_F \) represent the CO\textsubscript{2}-C evolved during incubation from chloroform fumigated and unfumigated soils, respectively. \( k_C \) is the fraction of the C in the killed biomass that is released as CO\textsubscript{2} during incubation. Inside brackets, figures represent the period (days) of incubation.

of which is based on a different or no control (Table 2.2).

The first method (1) assumes that the rate of mineralization of soil organic matter is equal in both fumigated and unfumigated samples (Jenkinson, 1966). This may be applicable to soils which have received a pre-incubation for 7 - 10 days before fumigation to allow the respiration rates to stabilize. However, this method is unsuitable for soils which have received large quantities of fresh organic substrate or those in which decomposition is impaired in fumigated soil (Jenkinson and Powlson, 1976b). In such situation, this calculation method results in an underestimation (Shen et al., 1987).

The second method (2) was proposed for specific conditions in which part of the biomass had been killed just before fumigation (e.g. by sieving or freezing) and for freshly sampled soils (i.e. without pre-incubation) (Jenkinson and Powlson, 1976b). Lynch and Panting (1980a) recommended this method for sieved soils. Shen et al. (1987) concluded that this approach was inappropriate for soils which have received fresh organic substrate.
have received fresh organic substrate.

Because the timing of the incubation period significantly influences the production of CO₂ from unfumigated soil and hence the estimation of microbial C (Ross and Tate, 1984), Chaussod and Nicolardot (1982) proposed a method (3 in Table 2.2) where microbial biomass C is calculated using fumigated control over 10 - 20 days of incubation. Merckx et al. (1985) argued in favour of using CO₂ evolution during 10 - 20 days after fumigation as the control. Because they believed that the control CO₂ came from the same sample as the CO₂ evolved after fumigation. However this method has some limitations. Ross (1990b) reported that CO₂ production by fumigated soils during 0 - 10 days might be overestimated, because of an initial flush resulting from sampling and handling procedures which enhanced decomposition of organisms killed by these procedures and CO₂ production during 10 - 20 days could include a contribution from CHCl₃-killed organisms, that were still being decomposed after the 0 - 10 day period.

In the fourth method, no control is used because the rate of organic matter utilization in the unfumigated soil is very much less than in the fumigated soil (Paul and Voroney, 1980, 1984; Voroney and Paul, 1984). Many studies (e.g. Voroney and Paul, 1984; Schnurer et al., 1985; Shen et al., 1987) have shown that this method overestimates the amount of soil microbial C compared to other methods of calculation discussed above, particularly in soils where fresh organic substrates are added or in air-dried soils. Shen et al. (1987) strongly opposed this method of calculation.

Jenkinson (1988) in his review of methods emphasized the need for a control because a proportion of CO₂ evolved from the fumigated soil comes from sources other than killed microorganisms. Although the best procedure depends on the nature of the soil and its history, Jenkinson's (1988) conclusion was that soils should
be pre-incubated (usually for 7 - 10 days) over soda lime to reduce the effects of sampling, and then to fumigate. He also suggested that $F_C$ should be calculated from the evolved CO$_2$ from fumigated minus unfumigated soils over 0 - 10 days of incubation.

A second limitation to estimate biomass C by the FI method is the selection of an appropriate $k_C$ value. The $k_C$-factor is generally estimated by adding a known quantity of microbial C to a soil, fumigating and estimating the proportion of added microbial C mineralized under given conditions. The $k_C$-value depends on a number of microbial and environmental factors, such as age of microbial tissue (Ross et al., 1987), microbial species composition (Nicolardot et al., 1984), soil moisture content (Ross, 1987; Wardle and Parkinson, 1990a), soil texture (Nicolardot et al., 1984), soil pH (Vance et al., 1987a) and duration of prior incubation (Wardle and Parkinson, 1990a). Bacterial and fungal ratios may also affect the $k_C$-factor because dead tissue of the two groups of organisms appear to mineralize at different rates (Anderson and Domsch, 1978a).

Jenkinson (1966) initially proposed a value of 0.30 for $k_C$, which was later modified to 0.50, based on a greater number and wider variety of microorganisms (Jenkinson and Powlson, 1976b). In a later study, Jenkinson et al. (1979) indicated that the value for $k_C$ (0.50) might be slightly too high and that a value of 0.45 would probably be a better estimate. Anderson and Domsch (1978a) tested 15 species of fungi and 12 species of bacteria in four soils and proposed a $k_C$-value of 0.411 at 22 °C, assuming that the bacterial and fungal populations remain in a constant proportions. This value was accepted by Voroney and Paul (1984) and by Nicolardot et al. (1984), despite the fact that it was dependent on the type of soil used. Adams and Laughlin (1981) reported a value of 0.55 for $k_C$, based on 10 different organisms including both bacteria and fungi. Recently, Vance et al. (1987a) tested 4 species of fungi and 4 species of bacteria from 10 English forest soils with a wide range of pH
values (from 3.2 to 7.2) and found the mean value for $k_C$ in strongly acid soils was 0.30, while in soils above pH 4.5 it was 0.46. The $k_C$-factor can clearly differ with soil type and its management history, but Jenkinson (1988) concluded that in the absence of soil specific determinations, the best value to use was 0.45 at 25 °C.

There are further limitations to use of the FI method. This method gives anomalously low estimates of microbial biomass C in soils which have recently received large inputs of fresh organic materials (Jenkinson and Powlson, 1976b; Jenkinson et al., 1979; Sparling et al., 1981; Martens, 1985), or in acid soils of pH less than 4.5 (Jenkinson and Powlson, 1976b; Adams and Laughlin, 1981; Jenkinson and Ladd, 1981; Williams and Sparling, 1984; Vance et al., 1987a). The FI method may give low estimates in strongly acid soils at least for three reasons. These are: (i) a $k_C$-value of 0.45 is likely to be too high (Vance et al., 1987a), (ii) the reinoculation procedure used after fumigation is inadequate to allow full mineralization of killed biomass (Chapman, 1987a; Vance et al., 1987a), and (iii) the use of a unfumigated control is too large for acid soils compared to near-neutral soils (Powlson and Jenkinson, 1976; Sparling and Williams, 1986; Vance et al., 1987a). In acid soils, the microbial recolonizers in fumigated samples are unable to mineralize non-microbial soil organic matter to the same extent as does the indigenous microbial population in unfumigated samples, thus low CO$_2$-C is evolved from the fumigated soil during incubation than from the unfumigated soil. Grisi (1983) mentions other possible reasons for the failure of the FI method in acid soils. These were: (i) fumigation does not kill the biomass, or the biomass is resistant to fumigation because the biomass is largely present in the form of dormant spores, and (ii) fumigation kills the biomass, but these are not mineralized either because acidity prevents decomposition, or the fumigant is not removed completely from the soil, or phenolic compounds inhibit mineralization in acidic soils. However, he did not provide evidence for these mechanisms, and did not mention which reason was the most likely.
The FI method is also inconvenient in other situations, such as (a) in air-dried soils where native soil organic matter is solubilized and rendered decomposable, and where a portion of the biomass is killed (Powlson and Jenkinson, 1976), (b) in wet soils that are smeared and compacted during sieving (Ross et al., 1984, 1985; Ross, 1987), and (c) in waterlogged soil where CO₂ and CH₄ are produced under conditions that restrict diffusion of gases (Jenkinson, 1988).

2.3.2 Fumigation-extraction (FE) method

Because of the inherent drawbacks of the FI method discussed above, fumigation-extraction methods have recently been developed for measuring microbial biomass C in soils (Blagodatskiy et al., 1987; Merckx and Martin, 1987; Vance et al., 1987c; Tate et al., 1988) and these techniques appear attractive from several viewpoints. In most of the methods, microbial cells are killed by fumigation with chloroform and the C rendered extractable by K₂SO₄ (i.e. the extractable C-flush) is measured. Biomass C is calculated as the difference between the amount of oxidizable organic C extracted from fumigated and unfumigated soil.

The FE method requires a proportionality factor (termed k_EC, Sparling and West, 1988a) to convert from the extractable-C flush to biomass C. Vance et al. (1987c) worked with a wide range of English soils (pH ranged from 3.9 to 8.0) and showed that biomass C could be calculated from the expression:

\[ \text{Biomass } C = \frac{\text{FE-C flush}}{K_{EC}} \]

where FE-C flush (i.e. microbial derived C) is the difference between K₂SO₄-extractable organic-C from fumigated and unfumigated soils. The k_EC factor allows for the incomplete release and extraction of microbial C and is obtained by calibrating against other methods used to measure the microbial C, such as the fumigation-incubation technique (Vance et al., 1987c; Sparling and West, 1988b;
Tate et al., 1988; Ross, 1990c) which also uses a k-factor estimate or the SIR method (Sparling and West, 1988a,b). Calibration with soil microorganisms labelled *in situ* with ¹⁴C-glucose (Merckx and Martin, 1987; Sparling and West, 1988a) and with organisms grown *in vitro* (Tate et al., 1988) has also been used. The k_EC factors can vary markedly with the different calibration procedures, being 0.37 for the SIR method and 0.42 by ¹⁴C-labelling (Sparling et al., 1990). The k_EC factor also varies with soil type and the season of sample collection due to the variation of soil water content (Ross, 1990c; Sparling et al., 1990).

Vance et al. (1987c) reported a mean k_EC factor of 0.38 for a range of acid forest soils. Sparling and West (1988a) and Tate et al. (1988) suggested a provisional k_EC factor of 0.33 for a range of New Zealand pasture and arable soils of varying C content. In another study, Sparling and West (1988b) compared the FE, FI and the SIR methods and found a rather higher k_EC value of 0.39 for another group of soils. Lawrence and Germida (1988) use a value of 0.29, but give no details of its derivation.

The estimation of soil microbial C by the FE method has several advantages over the FI method. The FE method saves time due to the absence of an incubation step and may provide a rapid and direct estimation of soil microbial biomass C. It appears suitable for use in neutral and acid soils, freshly sampled soils, in soils where water content is low or in soils that have recently been amended with large inputs of fresh organic matter (Vance et al., 1987c; Sparling and West, 1988a,b; West et al., 1988a,b). This method may also prove useful in submerged soils (K. Inubushi; quoted by Jenkinson, 1988). Sparling and West (1988a) concluded that not only microbial C in soils, but also its rate of turnover could be estimated by the FE method.

Despite the apparent advantages of the FE method over the FI method, it also
has limitations. The results from the FE method depend upon laboratory extraction conditions, such as extraction time and shaking speed (Tate et al., 1988). Ross (1988) found marked increase in extractable-C flush due to vigorous shaking. Tate et al. (1988) attributed the change to the incomplete dispersion of the soil under standardized extraction conditions. The FE method is not applicable to soils with a high clay content because the soluble organic matter released by fumigation will partition between soil and extractant and this may not be identical in all soils (Jenkinson, 1988). This method is also unreliable in wet compacted soils of high organic matter content because the high water content of the soil protected cells against lysis due to low solubility of chloroform (Couteaux et al., 1990). Moreover, it is likely that K₂SO₄ may attack biomass C and some of the organic C in the K₂SO₄ extracts of unfumigated soil may derive from microbial sources. The reliability of the FE method is also dependent on the applicability of the kₑC factor used and on the basis of its calculation.

2.3.3 Substrate-Induced Respiration (SIR) Method

The substrate-induced respiration (SIR) method was developed by Anderson and Domsch (1978b). It is a simple and rapid technique for estimating the size of the active microbial biomass C in soil. Moreover, this method can be applied with selective inhibitors to separate the microbial biomass into bacterial and fungal components (Anderson and Domsch, 1978b). The SIR method is based on the initial respiratory response (measured as CO₂ evolution or O₂ uptake) of the microbial populations to amendment of the soil with an excess of substrate such as glucose. However, Jenkinson (1988) points out that not all the soil microbial biomass responds to a-glucose addition. Smith et al. (1985) found that addition of glucose plus nutrient broth resulted in evolution of more CO₂ than the addition of glucose alone and showed that the additional CO₂ was not derived from the glucose.
The maximum initial respiratory response is determined within 1 or 2 hours after the glucose amendment (Anderson and Domsch, 1978b). The pattern of response of the soil microbial population to glucose differs between soils (Anderson and Domsch, 1978b). Jenkinson (1988) mentioned three situations where the pattern of response varied considerably. According to him, some glucose amended soils respire at a constant rate for few hours before the rate rises as a result of cell division, in others the rate drops initially and then rises and in a third group of soils, the rate increases steadily from the beginning. Jenkinson and Ladd (1981) pointed out that the concentration of glucose must be carefully determined for each soil, because a too high concentration would lower the respiration while a too low concentration would not provide the maximum response.

A number of conversion factors to calculate microbial C from respiration rate have been published, based on the correlation between SIR data and microbial C determinations based on several other methods. The most recent formula for converting respiration rate to microbial C is given by Sparling et al. (1990), which is:

\[ \mu g \text{ microbial C} = 50 (\mu l \text{ CO}_2 \text{ g}^{-1} \text{ soil h}^{-1}) \]

Despite its simplicity and speed, there are several technical problems associated with the original SIR method. The method was restricted to soil within a narrow moisture range; thus it was difficult to apply to field samples. The problem was overcome by West and Sparling (1986) who added glucose to the soil as a solution rather than in a solid form. The modified method also has some problems with soils which have pH >6.0 because of dissolution of CO₂ in the soil water (West et al., 1988a). Secondly, the non-continuous flushing of the "Wosthoff Ultragas 3" CO₂-analyzer used by Anderson and Domsch (1978b) results in a large experimental error, when the pH value of the soil sample is greater than 6.0 (Martens, 1987). Recently, Cheng and Coleman (1989) have modified the original method by using 120% of field-water holding capacity as the standard water conditions during the
incubation, adding glucose in a liquid form and measuring CO₂ evolution under a continuous air-flow system. This modified method removes the problem of high experimental errors for soils with a pH >6.5 and reduces the danger of possible inhibition of SIR due to very high or very low CO₂ concentration in the head space of the incubator.

2.3.4 Soil ATP determination

The content of adenosine 5'-triphosphate (ATP) in soil has been used as an index of soil microbial biomass (Paul and Johnson, 1977; Jenkinson and Oades, 1979; Oades and Jenkinson, 1979; Nielson and Eiland, 1980; Inubushi et al., 1989a) because soil ATP content is related to microbial activity (Eiland, 1985). The basis for the measurement of ATP is that it is a relatively constant component of diverse microbial cells and that ATP is neither associated with dead cells nor adsorbed on soil components (Nannipieri et al., 1989b). In recent years, much emphasis has been given to this method, particularly in improving techniques for estimating soil ATP content (Ahmed et al., 1982; Tate and Jenkinson, 1982a; Verstraete et al., 1983; Verstraeten et al., 1983; Webster et al., 1984; West et al., 1986b).

A wide variety of acidic, neutral, alkaline and organic reagents have been used for extraction of ATP from soil (see Eiland 1979, 1983; Jenkinson and Oades, 1979) and their efficiency was discussed by Jenkinson (1988). Among these, trichloroacetic acid (TCA) together with phosphate and paraquat has been shown to be an efficient extractant of ATP from soil (Jenkinson and Oades, 1979). TCA is more efficient than the mild extractant, the mixture of NaHCO₃-CHCl₃-phosphate-adenosine (BGPA) (Martens, 1987; Ciardi and Nannipieri, 1990), but less efficient than the phosphoric acid (PA) (Ciardi and Nannipieri, 1990). Several reports have been published on the use of various TCA-based reagents for the extraction of ATP from soil (e.g. Verstrate et al., 1983; Angerosa et al., 1985; Inubushi et al., 1989b).
Most of them indicate that paraquat can be omitted from the extractant with little or no disadvantage. Inubushi et al. (1989b) concluded that ATP could be successfully measured in soil without the use of paraquat, although the presence of paraquat did not significantly improve the measurement precision. Further, different factors govern ATP levels in soil, such as the use of crude or purified enzymes and the differences in the procedures for measuring light emission (Eiland, 1983).

A wide range of conversion factors has been used to convert soil ATP data to soil microbial C. For example, Domsch et al. (1979) obtained 1 μg of ATP = 500 μg of microbial C, while Oades and Jenkinson (1979) found 1 μg of ATP = 120 μg of microbial C. This wide range suggests that a single conversion ratio may not apply. Indeed Ahmed et al. (1982) questioned the use of a single factor for conversion of ATP content to microbial C for soils of different texture and believed it was necessary to separate soils at least into loams and clays. They also found evidence that microbial C to ATP ratios changed during incubations and with other environmental variables.

Soil ATP is often assumed to be an unreliable index of soil biomass, because of the large ATP variations observed when soils are amended with glucose, nitrogen or phosphate (Nannipieri et al., 1978; Sparling et al., 1981; Elliot et al., 1983; Fairbanks et al., 1984). For instance, Sparling et al. (1981) found an appreciable increase in ATP content due to glucose addition. Soil treatments, such as air-drying, freeze-drying, water-logging or prolonged storage (Ahmed et al., 1982; Tate and Jenkinson, 1982b; Brookes et al., 1983; Sparling et al., 1986; West et al., 1986b) and temperature (Tate and Jenkinson, 1982a) alter the concentration of ATP in soil. Ahmed (1981) found a significant increase in ATP content after a short wetting phase prior to freeze-drying of air-dried soils. Jenkinson (1988) emphasized on the use of pre-incubation under strictly controlled conditions before soil ATP measurement, because pre-incubated soils only contained microbial ATP. Sparling et al. (1985)
reported that the living plant roots which contained a considerable amount of ATP died during pre-incubation. Thus soil ATP determination as a quantitative measure of soil biomass depends on extracting it efficiently from soil and relating the amount of soil ATP to the amount of biomass in that soil (Jenkinson and Oades, 1979). Nannipieri et al. (1990b) conclude that ATP measurements reflect the status of microbiological activity and not the size of soil microbial biomass when they are conducted immediately after sampling or after storage under conditions that reduce microbial metabolism.

2.3.5 Direct microscopic techniques

Direct microscopic methods for measuring soil microbial C are based on the measurement of numbers and sizes of microorganisms present in the soil. These methods, however, depend on the cell density, moisture content and carbon content of the microbial tissue. Three techniques are available, such as (a) soil smear technique (Conn, 1918), (b) agar slide technique, stained with phenolic aniline blue (Jones and Mollison, 1948) and (c) membrane filter technique (Hanssen et al., 1974). The latter method is more rapid than the agar slide and more precise than the soil smear method (Jenkinson and Ladd, 1981).

Many problems are associated with interpreting results since it is difficult to distinguish between live and dead organisms and impossible to be certain that all microorganisms are counted or measured (Parkinson and Paul, 1982). Vance and Brookes (1987) discussed these problems, including the method used to disperse soil organisms, the type of stain used, and the factors used to convert microscopic counts to biomass C. A major advantage of these methods is that they enable the location of organisms in relation to each other, organic matter and soil crumbs (Campbell, 1983). These methods can also be used in freshly sampled soils, where several other methods break down (Vance and Brookes, 1987).
2.3.6 Microcalorimetric methods

The use of microcalorimetry for measuring microbial activity in soil was suggested by Mortensen et al. (1973), and Ljungholm et al. (1979a,b) recorded changes in catabolic activity in various soils. Sparling (1981a) proposed a microcalorimetric method for estimating soil biomass. This method is non-destructive and offers potential for investigations of the activity and catabolism of the soil biomass (Sparling, 1981b).

2.3.7 Analyses of soil for specific biomass constituents

Estimation of particular component of soil microorganisms as a measure (index) of biomass appears to be unsatisfactory and is therefore briefly discussed here. Detailed review of these aspects is given by Paul and Voroney (1980), Jenkinson and Ladd (1981) and Sparling (1985).

Muramic acid, a cell component of bacteria and blue green algae, has been used for estimating bacterial biomass in soil (Millar and Casida, 1970; Moriarty, 1977). Millar and Casida (1970) determined the muramic acid content of 33 soils of the United States and reported that the cell walls of some gram-positive bacteria contained 19.4 mg muramic acid g⁻¹ biomass C. Jenkinson and Ladd (1981) concluded that this figure was unrealistically large, because of the large proportion of muramic acid extracted from soils which is derived from dead cells.

Chitin is a structural component of fungal cell wall which has been used as a measure of mycelial biomass (Swift, 1973a,b). Frankland et al. (1978) used chitin as a measure of fungal biomass, but Jenkinson and Ladd (1981) reported that the chitin content of fungi varies with age and species, as well as nutrient content of the substrate. In addition, chitin is also present in soil as non-fungal components, which
is also a source of error (Sparling, 1985). These factors all make it very difficult to relate chitin to soil biomass C.

Although variability in the level of diaminopimelic acid (DAP) limits its application as an index for soil microbial biomass (Synge, 1953), yet Steubing, (1970) proposed it as an estimate of bacterial biomass in soil.

Nucleic acids are present in all organisms and thus analysis for this component in soil could be a measure of the biomass (Sparling, 1985). Torsvik (1980) found that the DNA content of soils ranged from 90 to 187 mg kg⁻¹ soil. Jenkinson and Ladd (1981) pointed out its unreliability for use with soil samples, because some microbial DNA could survive in soil even after decomposition of cell structure (Anderson, 1979).

Overall, the usefulness of these constituents is limited by the fact that they occur in varying concentrations in different portions of organisms, and because the microbial products are stabilized in soil organic matter (Paul and Voroney, 1980).

2.3.8 Comparisons of the methods

Jenkinson and Powlson (1976b) observed close agreement between the FI method and the direct biovolume method for seven out of eight soils studied, the exception was a highly acid soil (pH <3.9). A number of other studies (e.g. Martikainen and Palojarvi, 1990) also showed highly significant correlation between these two methods for different types of acid forest soils. In contrast, some workers (Grisi, 1983; Hesebe et al., 1985) have found no relationship. Schnurer et al. (1985) found higher microbial C by the direct counting method than the FI method. This is probably because direct counting includes both biomass and necromass, thus resulting in higher estimates. Grisi (1983).found that the direct observation method
was also not significantly correlated with soil ATP content, but was better correlated with biomass C estimated by SIR.

Many workers (e.g. Anderson and Domsch, 1978b; Ross, 1980; Grisi, 1983; Martens, 1987) have found a close relationship \((r = 0.84 - 0.98)\) between SIR method and soil microbial C estimated by the FI method. Others (Sparling, 1981a; Williams and Sparling, 1984; Dumontet and Mathur, 1989) have observed a poor relationship between these estimates for acid organic soils. Wardle and Parkinson (1990b) found a poor correlation \((r^2 = 0.30)\) between SIR and FI estimates in a range of soils which had received different treatments such as rewetting at different moisture content and pre-incubation for various periods. However, West et al. (1986c) calibrated the modified SIR method against the FI method and found almost an identical relationship, but Sparling et al. (1986) did not observe any significant correlation in a wide range of New Zealand soils, many of them highly organic. The original SIR method showed positive and linear relationship \((r = 0.95)\) with the ATP content of soil (Grisi, 1983) or heat output, measured by microcalorimetric method (Sparling, 1981a). Anderson and Domsch (1978b) suggested that discrepancies between these two methods could be because young microorganisms may produce more CO\(_2\)-C per unit biomass following glucose amendment than older microbes. Another possible reason is that chloroform may not kill bacteria and fungi completely (Ingham and Horton, 1987). Moreover, where microbial activity is low (e.g. rewetting or after pre-incubation), the respiratory response of the soil microbial components to glucose may be delayed (Visser et al., 1984; Visser and Parkinson, 1989).

Several studies have shown strong positive correlations \((r = 0.85 - 0.98)\) between microbial C estimates by the FI method and soil ATP content in arable, grassland and forest soils (Oades and Jenkinson, 1979; Grisi, 1983; Verstraeten et al., 1983; Vance et al., 1987b). Ausmus (1973) observed significantly higher biomass content estimated from ATP data than from direct counting. Biomass C estimates
based on FE agreed with those based on either FI method (Sparling and West, 1988b; Tate et al., 1988; Vance et al., 1987c), or modified SIR method (Sparling and West, 1988a,b; West et al., 1988a; Sparling et al., 1990), or soil ATP content (Joergensen et al., 1989; Ocio and Brookes, 1990a).

Microbial C estimated by the FI method showed close correlations with ninhydrin-reactive N (Amato and Ladd, 1988; Ocio and Brookes, 1990a), mineral-N flush (Carter and McLeod, 1987; Christie and Beattie, 1989) or microbial N estimated by the FE method (Srivastava et al., 1989). Microbial N estimated by FE was also correlated well with the soil ATP content (Ocio and Brookes, 1990a), or with microbial C estimated by FE (West et al., 1988b). Soil ATP concentration showed a close correlation with ninhydrin-reactive N released during chloroform fumigation (Ocio and Brookes, 1990a).

The conclusion from all these studies is that the relationship between the estimates of different indices of microbial biomass depends upon the method used, experimental conditions and soil pH.

2.4 Experimental factors affecting the measurement of soil microbial biomass C and N

The accurate measurement of soil microbial biomass C and N estimates by different methods is influenced by many experimental factors. Most of these factors have been identified for soil microbial biomass C. Therefore, a considerable number of modifications of original methods have been reported in the literature. Brief discussion of various factors are given below.

The length of time after sampling (i.e. storage) is an important factor which affects soil microbial biomass. Soil microbial biomass measurements are normally
carried out within 24 hours of sampling (Jenkinson and Powlson, 1976b). In some cases, soils have been stored for some weeks usually at 2 °C or 4 °C (Bolton et al., 1985) or frozen indefinitely (Adams and Laughlin, 1981). Ross et al. (1980a) examined the effects of different lengths and temperatures of storage on soil biomass C and mineral-N flush. Their study indicated that keeping soil at 4 °C was satisfactory for retaining values of mineral-N flush and adequate for biomass C over a short periods (usually 28 days) of storage, while storage at -20 °C was the most suitable for minimizing changes. Lynch and Panting (1980b) observed no significant differences in microbial C between fresh and stored (24h at 25 °C) samples. Jenkinson and Powlson (1976a) showed some loss of microbial C in frozen and thawed soils and found that the loss was increased with duration of storage at -15 °C. Storage also increased soil ATP concentration (Tate and Jenkinson, 1982b). Thus prolonged storage of soil prior to biomass C measurement is best avoided (Ross et al., 1980a).

In the FI method, soil is usually sieved before fumigation. According to Ross and Tate (1984), use of at least eight different mesh sizes ranging from 2 to 10 mm have been reported in the literature, and unsieved soil has also been used (Lynch and Panting, 1980a,b). Jenkinson and Powlson (1980) reported three reasons for sieving soils prior to estimation of biomass C. These are: to allow easy penetration and removal of the fumigant, to allow thorough reinoculation of the fumigated soil, and to enable a relatively small sub-sample of much larger quantity of well-mixed soil to be used. They found little difference between unsieved and coarsely sieved samples. In contrast, Lynch and Panting (1980a) found that biomass C in coarsely-sieved soil (7 mm mesh) was less than about one-quarter of that present in unsieved soil cores. Ross and Tate (1984) observed an inconsistent effect of soil mesh size on biomass C estimation. Despite the different findings, Jenkinson and Powlson (1980) reported that soil biomass could be best measured in coarsely-sieved soil. Recently, Ocio and Brookes (1990b) compared biomass C obtained by
FE method in sieved (<2 mm-mesh) and unsieved soil and found no significant
difference between the estimates. However, these workers reported that the use of
unsieved soils might give unreliable results on soils which have moisture content of
<40 % WHC, because of the drying effects. Overall, it is clear that sieving is an
important variable to be considered in determining microbial biomass.

Pre-incubation (normally for 7-10 days; Jenkinson, 1988) before measurement
of biomass C and N by the FI method is often performed to minimize the effects of
disturbance during sample preparation (Jenkinson and Powlson, 1976b) and to
reduce interference from root-derived C, N and ATP (Sparling et al., 1985). The
microbial C present in the pre-incubated soil is not the same as that found in freshly
sampled soil, although the difference is usually small (Jenkinson and Powlson,
1976b; Ross et al., 1980a; Ross and Tate, 1984), except for soils sampled air-dry or
that have recently received fresh organic substrate (Jenkinson, 1988). Ross and
Tate (1984) and West et al. (1986a) found depressed soil microbial C in pre-
incubated samples, whilst Tate and Jenkinson (1982b) found an apparent increase in
microbial C in grassland soil after pre-incubation for 7 days at 25 °C which they
attributed to abiotic evolution of CO2 from the fresh soil. Recently, Wardle and
Parkinson (1990b) reported that duration of pre-incubation also affected the microbial
biomass C values. They found that the values were higher at day 3 or 10 compared
to those at 6 hours or 30 days (when a constant kC values used to convert FI-C flush
to biomass C). When different kC values were used , the 30-day values were
significantly higher. Although soil ATP concentration increases significantly during
the first day of incubation, it changes little during the following week (Tate and
Jenkinson, 1982b). On the other hand, there is little change in microbial biomass N
(West et al., 1986a). In conclusion, it is better to use fresh samples if data on the
status of a soil in the field are required. Pre-incubation also reduces the interference
of root-derived C, N and ATP in estimations of microbial constituents (Sparling et al.,
1985).
In the original FI method of Jenkinson and Powlson (1976b), 250 g of moist soil was used and incubated in a 3.75 litres glass jar. However, a number of modifications to the soil sample size and the incubation jar have been made. For example, sample size varies from 10 g (Sparling and Williams, 1986; West et al. 1986c; Myrold, 1987) to 100 g (Lynch and Panting, 1980a; Azam et al., 1986). Christie and Beattie (1987) investigated whether different weights of soil or size of incubation jar affected microbial C estimation. They found that sample weight was an important factor, while jar size was not important. However, they reported that soil samples dried out during handling which resulted in a smaller flush of soil respiration.

Soil microbial biomass C and N based on the FI method are affected by soil moisture content. Generally soil samples are adjusted to a water content between 50 to 60% of water holding capacity (WHC) prior to biomass measurements (Jenkinson and Powlson, 1976b; Ross and Tate, 1984; Azam et al., 1986; West et al., 1986a; Haynes, 1987). Marumoto et al. (1982b) adjusted soil samples to only 34% WHC, while others maintained a high water content, for up to 70 to 75% WHC (e.g. Adams and Laughlin, 1981; Bottner, 1985). Anderson and Domsch (1978b) did not adjust the water content.

Air-drying reduces soil biomass C (Powlson and Jenkinson, 1976; Sparling et al., 1985; West et al., 1986a; Shen et al., 1987), and on rewetting of an air-dried soil a substantial portion of the freshly killed biomass is readily decomposed by surviving microorganisms (Jenkinson and Powlson, 1976a; Marumoto et al., 1977a; Bottner, 1985; Chaussod et al., 1986). However, Wardle and Parkinson (1990b) showed highest microbial C at 25 to 45% moisture content (oven-dry weight) and lowest at 15%. In addition, direct extraction efficiency also depends on soil moisture content. Recently, Davidson et al. (1989) found higher microbial biomass N in wet soils. Cheng and Coleman (1989) also reported that the rates of SIR decreased when the soil water contents were either too low or too high. When the soil water content is
below the optimal level, lower water availability or non-uniform glucose distribution may occur, whilst in the reverse situation, the lower oxygen supply may inhibit aerobic activities of microorganisms (Orchard and Cook, 1983). Jenkinson (1988) noted that wet soils should be dried down to the desired moisture content and for very dry soils, they should be moistened.

There is argument about the choice of inoculum size to be used with the FI method, particularly in strongly acid soils. In the original method, Jenkinson and Powlson (1976b) used an inoculum of 0.4% in fumigated soils and found that incubation without inoculation reduced the $O_2$ consumption by about 7%. Subsequently, some workers (e.g. Nannipieri et al., 1983; Shen et al., 1984) have used this quantity of inoculum, but the range goes up to 20% (Ayanaba et al., 1976; Kassim et al., 1981; Anderson and Domsch, 1978a; Chapman, 1987a). Some workers have used a soil suspension as an inoculum (e.g. Lynch and Panting, 1980a; Grisi, 1983; Mulongoy, 1986). Usually the unfumigated sample is not inoculated, but Schnurer et al. (1985) used a 1% inoculum into both fumigated and unfumigated soils. In contrast, a number of studies indicate that inoculation often is not needed, because an adequate microbial population normally escapes fumigation (Paul and Voroney, 1980; Chausso and Nicolardot, 1982; Nicolardot et al., 1984; Voroney and Paul, 1984; Kieft et al., 1987).

Recently, Chapman (1987a) showed that a small inoculum of 0.4% (w/w) was better than no inoculation, but recommended a 10% inoculum for near-neutral soils and a 20% rate for strongly acid (pH below 4.5) soils. Vance et al. (1987a) also found similar results for highly acidic soils, and reported that the 0.4% inoculum was insufficient for those soils.

Overall, microbial biomass estimates vary with a number of experimental factors. A number of modifications are thus reported in the literature.
2.5 Amount of soil microbial biomass N and C in different ecosystems

Selected literature values for soil microbial N concentrations are summarized in Table 2.3. Most of the studies have considered only the 0 to 10 cm soil depth. The amount of microbial N varies with vegetation type, although there is no regular pattern. Within each broad vegetation type, microbial N varies appreciably with location. For example, in deciduous forests (hawthorn, ash, oak, hazel) in England, microbial N was 94 mg kg\(^{-1}\) soil (Vance \textit{et al.}, 1987c), while in India (under mixed dry deciduous forest dominated by \textit{Boswellia Serrata} Roxb. cx Colebr., \textit{Butea monosperma} Lamk., \textit{Lagerstroemia parviflora} Robx. and \textit{Wrightia tomentosa} R. and S.), the value was 75 mg N kg\(^{-1}\) soil (Srivastava \textit{et al.}, 1989). Microbial N concentrations also vary with the method of estimation. Sparling and West (1988b) obtained higher microbial N by the FI method than the FE method (average 129 versus 83 mg N kg\(^{-1}\) soil) in a range of soils. Azam \textit{et al.} (1989b) also found higher microbial N by the FI method than the FE method for a wide range of agricultural soils. Soil depth is another factor which influences the soil microbial N concentrations, with values usually being higher in the upper layers and decreasing with depth. As an example, Haines and Uren (1990) observed microbial N concentrations of 44, 18 and 7 mg N kg\(^{-1}\) soil for the 0 - 2.5, 2.5 - 7.5, and 7.5 - 25 cm, respectively.

The proportion of total soil N present as microbial N is relatively higher in agricultural soils than in other vegetation systems. The reason is probably because of the extensive use of fertilizer which increases soil N contents, whilst cultivation may decrease organic matter and biomass. With a few exceptions, soil microbial N represents 1 to 5% of total soil N. In their review, Smith and Paul (1990) also reported that microbial N contents accounted for 1 to 5% of total soil N.

From these selected literature values, it is apparent that the variation in soil
<table>
<thead>
<tr>
<th>Biomass N (mg kg(^{-1}) soil)</th>
<th>as % of total soil N</th>
<th>Method used(^a)</th>
<th>Values for (k_N) used</th>
<th>No. of soils</th>
<th>Depth (cm)</th>
<th>Vegetation type / Land use</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Range</td>
<td>Mean Range</td>
<td>Fl(^b) Variable(^c)</td>
<td>Fl</td>
<td>0.54</td>
<td>3</td>
<td>0 - 20</td>
<td>Pine</td>
</tr>
<tr>
<td>101 29 - 218</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>94 33 - 128</td>
<td>3.6</td>
<td>1.8 - 4.7</td>
<td>FE</td>
<td>0.54</td>
<td>3</td>
<td>0 - 23</td>
<td>Deciduous woodland</td>
</tr>
<tr>
<td>64 35 - 170</td>
<td>1.5</td>
<td>0.6 - 2.0</td>
<td>Fl</td>
<td>0.68</td>
<td>6</td>
<td>0 - 15</td>
<td>Pine, Oak</td>
</tr>
<tr>
<td>75 2.6</td>
<td>-</td>
<td>-</td>
<td>FE</td>
<td>0.54</td>
<td>1</td>
<td>0 - 10</td>
<td>Mixed dry deciduous</td>
</tr>
<tr>
<td>40 2.1</td>
<td>-</td>
<td>-</td>
<td>FE</td>
<td>0.54</td>
<td>1</td>
<td>0 - 10</td>
<td>Deforested</td>
</tr>
<tr>
<td>21(^d) 4 - 38</td>
<td>1.2</td>
<td>0.4 - 2.0</td>
<td>Fl</td>
<td>-</td>
<td>8</td>
<td>0 - 20</td>
<td>Agricultural</td>
</tr>
<tr>
<td>84 27 - 161</td>
<td>10.5</td>
<td>6.5 - 13.6</td>
<td>Fl</td>
<td>0.42</td>
<td>18</td>
<td>-</td>
<td>Agricultural</td>
</tr>
<tr>
<td>47 23 - 81</td>
<td>6.4</td>
<td>2.6 - 14.8</td>
<td>Fl</td>
<td>0.54</td>
<td>18</td>
<td>-</td>
<td>Agricultural</td>
</tr>
<tr>
<td>44 37 - 48</td>
<td>4.4</td>
<td>3.8 - 4.5</td>
<td>Fl</td>
<td>0.68</td>
<td>3</td>
<td>0 - 2.5</td>
<td>Wheat</td>
</tr>
<tr>
<td>18 17 - 19</td>
<td>2.3</td>
<td>2.2 - 2.4</td>
<td>Fl</td>
<td>0.68</td>
<td>3</td>
<td>2.5 - 7.5</td>
<td>Wheat</td>
</tr>
<tr>
<td>7 7 - 8</td>
<td>1.5</td>
<td>1.4 - 1.7</td>
<td>Fl</td>
<td>0.68</td>
<td>3</td>
<td>7.5 - 25</td>
<td>Wheat</td>
</tr>
<tr>
<td>43 37 - 48</td>
<td>3.9</td>
<td>3.3 - 4.3</td>
<td>Fl</td>
<td>0.57</td>
<td>4</td>
<td>0 - 10</td>
<td>Grain sorghum</td>
</tr>
<tr>
<td>101 59 - 144</td>
<td>5.6</td>
<td>3.9 - 6.8</td>
<td>Fl</td>
<td>0.68</td>
<td>5</td>
<td>0 - 20</td>
<td>Cereals</td>
</tr>
<tr>
<td>117(^d) 108 - 126</td>
<td>3.1</td>
<td>3.0 - 3.2</td>
<td>Fl</td>
<td>-</td>
<td>2</td>
<td>0 - 7.5</td>
<td>Grazed pasture</td>
</tr>
<tr>
<td>85(^d) 27 - 158</td>
<td>1.7</td>
<td>0.4 - 3.0</td>
<td>Fl</td>
<td>-</td>
<td>21</td>
<td>0 - 7.5</td>
<td>Improved pasture</td>
</tr>
<tr>
<td>129(^d) 53 - 209</td>
<td>2.5</td>
<td>1.1 - 4.1</td>
<td>Fl</td>
<td>-</td>
<td>9</td>
<td>0 - 7.5</td>
<td>Pasture</td>
</tr>
<tr>
<td>83(^d) 30 - 133</td>
<td>1.7</td>
<td>0.6 - 2.2</td>
<td>FE</td>
<td>-</td>
<td>9</td>
<td>0 - 7.5</td>
<td>Pasture</td>
</tr>
<tr>
<td>78 30 - 130</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
<td>0.68</td>
<td>8</td>
<td>0 - 10</td>
<td>Perennial ryegrass</td>
</tr>
<tr>
<td>100(^d) 59 - 167</td>
<td>3.5</td>
<td>2.0 - 5.1</td>
<td>Fl</td>
<td>-</td>
<td>9</td>
<td>0 - 8</td>
<td>Tossock grassland</td>
</tr>
<tr>
<td>124 -</td>
<td>4.8</td>
<td>-</td>
<td>FE</td>
<td>0.54</td>
<td>1</td>
<td>0 - 23</td>
<td>Grassland</td>
</tr>
<tr>
<td>35(^d) -</td>
<td>1.8</td>
<td>-</td>
<td>Fl</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>Paddy</td>
</tr>
<tr>
<td>36 22 - 52</td>
<td>3.0</td>
<td>2.0 - 5.1</td>
<td>Fl</td>
<td>0.50</td>
<td>6</td>
<td>0 - 10</td>
<td>Virgin (after grassland)</td>
</tr>
<tr>
<td>20(^d) -</td>
<td>1.1</td>
<td>-</td>
<td>Fl</td>
<td>-</td>
<td>1</td>
<td>0 - 15</td>
<td>Fallow (after maize)</td>
</tr>
</tbody>
</table>

\(^a\) Fl and FE represent the fumigation-incubation and fumigation-extraction methods, respectively; \(^b\) Fumigation following anaerobic incubation; \(^c\) Variable values depending upon C-flush / N-flush, according to Voroney (1983); \(^d\) Fl-N flush values, not converted to biomass N
microbial N concentration depends on a number of factors, such as vegetation type, method of measurement, soil depth, location due to topographic variation, and the amount of total soil N.

Table 2.4 summarises the effect of location, vegetation type or land use and soil depth on microbial biomass C. There is a considerable variation in the biomass C estimates due to the methods used for its estimation. For example, Hendricks and Pascoe (1988) measured microbial C in soils from oak grass savannah using four methods. They obtained the highest estimates of microbial biomass C by the SIR method, lower using FI and microwave treatments, and least by the microscopic technique. There was a 4-fold difference between the methods. Vance et al. (1987b) found less variation in estimates of biomass C obtained by FI, ATP and microscopic methods in acid forest soils.

Vegetation type or land use is another factor in producing a large variation in soil microbial C concentration. Generally, microbial C values increase in the order: grassland < forest < arable. In addition, biomass C contents also vary with soil depth. Haines and Uren (1990) estimated that biomass C was about double in the upper 0 - 2.5 cm and about half in the 7.5 - 25 cm depth compared to 2.5 - 7.5 cm depth.

Microbial biomass C content accounts for 1 to 5% of the total soil C (Jenkinson and Ladd, 1981; Smith and Paul, 1990), and this range appears to hold for the wide range of locations, vegetation types, land use and different methods of estimation summarised in Table 2.4. In a few cases, the amount of microbial C as the percentage of total soil C does not fall within this range. Variation in soil microbial biomass C content is, like soil microbial biomass N, attributable to variation in soil type, location, use of different measurement techniques and soil depth.
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Table 2.5 lists selected literature values for soil microbial C:N ratio in various ecotypes. Generally, the ratios are lower in arable soils than in forest soils. Smith and Paul (1990) attributed the lower C:N ratios in the soil microbial biomass in arable soils to the mineralization of soil organic matter due to the extensive use of N fertilizer. Another reason for higher microbial C:N ratios in forest soils is probably because of the higher proportion of fungal biomass which has a higher C:N ratio than that of bacteria. According to Jenkinson (1988), the C:N ratio of the soil microflora is relatively constant in soils which do not receive large amounts of plant material having a wide C:N ratio. Forest litter typically has a C:N ratio exceeding 50:1.

Overall, those soils which have the highest organic matter content also tend to have the highest microbial biomass C and N. A high clay content is a characteristic of a soil with a high capacity to protect soil organic matter (van Veen et al., 1984). Recently, Ladd et al. (1990) reported that the turnover of C and N through decomposer microorganisms was influenced by soil texture. The data for soil microbial N and C given in Tables 2.3 to 2.5 show that a considerable amount of both

<table>
<thead>
<tr>
<th>C:N ratio Mean</th>
<th>Range</th>
<th>No. of soils</th>
<th>Depth (cm)</th>
<th>Vegetation type / Land use</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.9</td>
<td>5.3 - 6.7</td>
<td>7</td>
<td>0 - 20</td>
<td>Pine</td>
<td>Myrol (1987)</td>
</tr>
<tr>
<td>6.5</td>
<td>4.5 - 9.4</td>
<td>3</td>
<td>0 - 23</td>
<td>Deciduous woodland</td>
<td>Vance et al. (1987c)</td>
</tr>
<tr>
<td>11.6</td>
<td>6.3 - 25.8</td>
<td>6</td>
<td>0 - 15</td>
<td>Pine, Oak</td>
<td>Diaz-Ravina et al. (1989)</td>
</tr>
<tr>
<td>11.6</td>
<td>-</td>
<td>- 1</td>
<td>0 - 10</td>
<td>Mixed dry deciduous</td>
<td>Srivastava et al. (1989)</td>
</tr>
<tr>
<td>5.9</td>
<td>5.0 - 7.2</td>
<td>9</td>
<td>0 - 25</td>
<td>Wheat</td>
<td>Haines and Uren (1990)</td>
</tr>
<tr>
<td>5.5</td>
<td>4.3 - 6.8</td>
<td>14</td>
<td>0 - 23</td>
<td>Wheat</td>
<td>Shen et al. (1989)</td>
</tr>
<tr>
<td>5.5</td>
<td>5.0 - 6.3</td>
<td>5</td>
<td>0 - 20</td>
<td>Cereal</td>
<td>Bonde et al. (1988)</td>
</tr>
<tr>
<td>5.4</td>
<td>5.1 - 5.9</td>
<td>4</td>
<td>0 - 25</td>
<td>Spring barley</td>
<td>Powlson et al. (1987)</td>
</tr>
<tr>
<td>7.4</td>
<td>6.6 - 8.6</td>
<td>4</td>
<td>0 - 10</td>
<td>Grain sorghum</td>
<td>Saffigna et al. (1989)</td>
</tr>
<tr>
<td>5.3</td>
<td>-</td>
<td>- 1</td>
<td>0 - 23</td>
<td>Grassland</td>
<td>Vance et al. (1987c)</td>
</tr>
<tr>
<td>7.3</td>
<td>6.7 - 7.9</td>
<td>5</td>
<td>0 - 20</td>
<td>Grass-legume</td>
<td>Nannipieri et al. (1990a)</td>
</tr>
<tr>
<td>10.6</td>
<td>8.7 - 13.2</td>
<td>6</td>
<td>0 - 10</td>
<td>Virgin (after grassland)</td>
<td>Dalal and Mayer (1987)</td>
</tr>
</tbody>
</table>
N and C are present in the active fraction of soil organic matter which may substantially contribute to the pool of mobile plant nutrients in soils.

2.6 Seasonal changes and the effects of treatments on soil microbial biomass C and N in different ecosystems

Seasonal changes and the effects of various forest management practices such as fertilization, liming, sewage sludge amendment and different regimes of low-intensity prescribed fire on the concentration of soil microbial biomass C and N is a part of this thesis. A brief introduction of these aspects are reviewed here and additional information is given in Chapter 6.

2.6.1 Seasonal changes in soil microbial biomass C and N

Environmental conditions affect the quality and quantity of soil organic matter by controlling both inputs of C and biomass turnover (Voroney et al., 1981; McGill et al., 1986). In addition to soil nutrient status, the size and the activity of soil microbial biomass varies temporally (Ross et al., 1981; Sarathchandra et al., 1984; Ritz and Robinson, 1988) because of fluctuations in soil moisture content (Granatstein et al., 1987; Ross, 1988) and temperature (Sarathchandra et al., 1988; Insam et al., 1989). The effects of these environmental changes can determine whether the biomass will take up nutrients for growth or release nutrients through death and decay. The activities of the microorganisms in response to environmental changes can therefore have important implications for nutrient availability and plant growth. In forests, seasonal litterfall and root turnover control input of C to soils, and affect both microbial biomass content and activity (Davidson et al., 1989).

There can be considerable variation in soil microbial C and N pools due to season. Several workers (e.g. Ross et al., 1981; Bolton et al., 1985; West et al.,
1986c) found depressed soil microbial C contents in pasture and cultivated soils during spring and higher levels in summer and autumn. Lynch and Panting (1980a) followed changes in microbial C over time under winter wheat and found that amounts were about three times greater in spring and summer than in the autumn and winter. In forest situations, Singh et al. (1989) found highest and lowest levels of microbial N in the summer and rainy season, respectively, and attributed this variation to the presence of a substantial amount of water at high tension during summer which is unavailable to plants but available to microorganisms.

In the field, soils are subjected to drying-wetting cycles and one of the most important effects of this is to accelerate mineralization of organic matter (Birch, 1960; Soulides and Allison, 1961; Marumoto et al., 1977b; Sparling and Ross, 1988). Many studies (e.g. Bolton et al., 1985; Kieft et al., 1987; West et al., 1988a,b) have found that drying-rewetting cycles significantly increased microbial activity and biomass concentrations in soil. Rewetting of dried soil makes the killed biomass potentially decomposable by the surviving micro-organisms, and is possibly responsible for the short-term respiratory increase associated with rewetting (Smith et al., 1985; Kieft et al., 1987). However, the importance of soil moisture in regulating the microbial community and its activity, and hence soil biomass is well documented (e.g. Bottner, 1985).

2.6.2 Effects of treatments on soil microbial biomass C and N

Fertilizer (N, P and K) is often applied to increase plant productivity in arable and intensively managed forest soils. Liming with phosphorus is also frequently required for successful crop production, particularly in acid forest soils which often have high phosphate-fixing capacity. Repeated sewage sludge applications can provide a cumulative positive effect on forest site quality and result in permanent increases in productivity (Brockway et al., 1986).
Soil microbial biomass responds more quickly to changes in management practices (e.g. fertilization) than does soil total organic C or N (Nannipieri, 1984; Granatstein et al., 1987) and, therefore, may be an indicator of early trends in changes to the soil organic matter pool (Schnurrer et al., 1985; Powlson et al., 1987).

Many factors influence the size and activity of the microbial biomass in soil. Fertilizer treatment (Lynch and Panting, 1982; Bolton et al., 1985; Nohrstedt et al., 1989), liming (Adams and Adams, 1983; Bekunda, 1987) and sewage sludge addition (Boyle and Paul, 1985), manure treatment (Eiland, 1980; Adams and Laughlin, 1981; Kanazawa et al., 1988), tillage (Doran 1980, 1987; Saffigna et al., 1989) and crop rotation (McGill et al., 1986; Roder et al., 1988) have all been shown to influence microbial biomass in soil.

The effects of fertilizer addition on soil microbial N and C are variable. For example, fertilizer N decreases microbial N and C in acid forest soils (Bekunda 1987; Nohrstedt et al., 1989) and in grassland soils (Christie and Beattie, 1989). But a number of studies have shown higher microbial C (Hesebe et al., 1985; Alef et al., 1988; Kanazawa et al., 1988) and N (Shen et al., 1989) after inorganic fertilizer addition to agricultural soils. McGill et al. (1986) found no significant differences in microbial biomass C and N due to N-fertilization. Form of fertilizer (Nohrstedt et al., 1989) and rate of its application (e.g. Ritz and Robinson, 1988; Shen et al., 1989) also affect the microbial C and N. Liming and sewage sludge amendment increase the concentration of soil microbial C and N (Sarathchandra and Upsdell, 1981; Adams and Adams, 1983; Boyle and Paul, 1985). On the other hand, Carter (1986) reported that the addition of lime alone either increased or did not affect the level of soil microbial biomass N.

Fire is used in forest as a management tool to reduce fuels and the subsequent risk of wildfire. In agricultural systems, cereal straws are burned to
remove the residues. The influences of burning on soil microbial numbers and activities depend on the severity of the fire, the types of organisms involved, and the post-fire environmental conditions and the frequency of fire and the total number of fires. Effects of fire can be considered as either short or longer-term (i.e. cumulative). Few studies so far have been conducted on the effects of forest fire or stubble burning on soil microbial C and N. Forest fire can decrease soil microbial N and C (Matson et al., 1987; Tateishi et al., 1989). Similarly, stubble burning also reduces the amount of biomass (Powlson et al., 1987; Haines and Uren, 1990). The decrease in microbial C and N after fire can be due to the short-term effects of soil heating (Raison, 1979) as well as to volatilization of C and N during combustion of fuels.

In summary, it is apparent from the examples given that the effects of soil management on microbial biomass C and N are variable and depend on many factors, including climate and soil type. Most studies have not investigated the processes leading to this change and are largely descriptive.

2.7 General Conclusions

In recent years, different methods for estimating microbial biomass N in soil have been developed. Among them, fumigation-incubation and fumigation-extraction methods are used widely, particularly in cultivated soils. Chloroform is usually used as the fumigant for both methods to lyse microbial cells, but it is a carcinogen and requires additional precaution.

As indicated in the literature review, the chloroform fumigation-incubation method gives poor results in acid soils and in soils which have recently received large inputs of fresh organic material. These two situations are common in forest ecosystems where large annual inputs of C come from litterfall and fine root turnover.
In acid soils, mineralization of N in the unfumigated samples often exceeds the amount of N mineralized in the fumigated samples. In freshly organic material amended soils, microbial immobilization of mineral N in the fumigated samples sometimes results in an underestimation of biomass N by the FI method due to the presence of microorganisms with a high C:N ratio. On the other hand, the fumigation-extraction procedure offers some promise for estimating soil microbial N. This method has not however been tested widely on acid forest soils, and the few studies that have been carried out (e.g. Brookes et al., 1985d; Davidson et al., 1989) have sometimes produced poor results (Brookes et al., 1985d). Thus the further development and testing of the existing fumigation-extraction method for forest soils was considered necessary and is described in Chapter 4.

In forest ecosystems, several management practices, such as fertilization, liming, sewage sludge addition and prescribed fire of varying frequency and intensity, influence the soil nutrient reserves and alter the rates of mineralization of organically bound nutrients. These practices are expected to affect the concentration of microbial biomass N and C. In order to explain patterns of these effects, it is essential to estimate microbial biomass at regular intervals over a few years for sufficient data. An understanding of the microbial biomass N and C and their link with nutrient cycling processes may lead to enlightened management of forest soil fertility.
CHAPTER THREE
CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

3.2 Description of Sites and Soils

3.3 Sample collection, preparation and storage

3.4 Soil Chemical Analyses

3.4.1 Comparison between different extractants to determine mineral-N in soil

3.4.2 Digestion methods to measure total-N in soil extract

3.5 Terminology

3.6 Determination of flushes from fumigation-extraction (FE-N flush) and fumigation-incubation (FI-N flush)

3.6.1 Fumigation-extraction (FE) method

3.6.2 Chloroform fumigation-incubation (FI) method

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(b) Effects of duration of chloroform fumigation prior to aerobic incubation

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(a) Effects of heating during oxidation on organic C estimates

(b) Comparison of hexanol and chloroform fumigation-extraction methods to estimate soil microbial C

3.7.2 Estimation of soil microbial biomass C by the Substrate-Induced Respiration (SIR) method

3.8 Data Presentation and Statistical Analysis
CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter deals with the general description of soils and the different analytical procedures which have been used in the thesis. It includes methods of sampling, preparing and storing soils. A number of preliminary tests carried out to validate some of the methods and any modifications to the methods are also described here.

3.2 Description of Sites and Soils

The experimental sites and soil sampling areas are located in the Cotter Area of the Australian Capital Territory (A.C.T.) and are shown in Fig. 3.1. All experiments have been performed with soils collected from under forests.

The geology, geochemistry and soils of the Australian Capital Territory have been described in detail by Owen and Wyborn (1979) and Talsma (1983). The study area is bounded to the north and west by the N.S.W. border, in the south by a line connecting Bendoara Dam through Tidbinbilla Natural Reserve to the Murrumbidgee River which forms the eastern boundary (Fig. 3.1). Three geomorphic units are recognized within this area (Owen and Wyborn, 1979). These are: (i) the Bimberi-Brindabella Upland in the west, which is an erosion-resistant mountainous region of Ordovician metasediments, Silurian granitoids and Devonian rhyolitic volcanics, (ii) the Mount Kelly Upland, bounded by the Cotter, Winslade and Murrumbidgee faults, which includes Ordovician and Adaminaby beds and the Silurian granitoid, and (iii) the Canberra Plain which comprises rolling country on Silurian volcanics. The area
Figure 3.1 Location map of the Cotter Area, Australian Capital Territory, A.C.T. (After Talsma, 1983).

Experimental and Soil Sampling Sites:

1. Biology of Forest Growth (Pierces Creek)
2. Uriarra Neds Block
3. Greens Catchment
4. Piccadilly Circus
encompasses a large range of altitude (Table 3.1) which influences both climate and native vegetation. Annual precipitation ranges from 820 to 1100 mm (Talsma, 1983), and winter frosts are common in high elevation areas. Highland areas are vegetated

Table 3.1 Description of experimental sites.

<table>
<thead>
<tr>
<th>Soil Classification</th>
<th>Pierces Creek</th>
<th>Uriarra</th>
<th>Greens Catchment</th>
<th>Piccadilly Circus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Soil Group</td>
<td>Yellow Podzolic (YP)</td>
<td>Red podzolic Duplex (D)</td>
<td>Red Podzolic (RP)</td>
<td>Red Earth (RE)</td>
</tr>
<tr>
<td>Principal Profile Form</td>
<td>Dy 3.61 Orthic Luvisol</td>
<td>Dr 2.41-Chromic Luvisol</td>
<td>Dr 2.21-Humic Ferralsol</td>
<td></td>
</tr>
<tr>
<td>FAO</td>
<td>Orthic Luvisol</td>
<td>-</td>
<td>Chromic Luvisol</td>
<td>Humic Ferralsol</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>594</td>
<td>750</td>
<td>794</td>
<td>1020</td>
</tr>
</tbody>
</table>

a According to Stace et al. (1968)
b According to Northcote (1979)

mainly by woodland *Eucalyptus pauciflora* (snowgum), whereas extensive lowland areas are covered by dry sclerophyll eucalypt forests. About 30% of the study area had been planted to *Pinus radiata*. Four soils were used for the studies with significant differences in profile morphology, physical and chemical properties (Table 3.2; Talsma, 1983). The soils are the yellow podzolic (YP, Site 1), red podzolic duplex (D, Site 2), red podzolic (RP, Site 3) and red earth (RE, Site 4). The soils are classified according to Stace et al. (1968) and Northcote (1979). The RE site supported sub-alpine eucalypt forest, whilst the other sites had been planted to *Pinus radiata*. 
Table 3.2 Selected physical and chemical properties of surface (0-10 cm, except for red podzolic which was 0-5 cm) soils.

<table>
<thead>
<tr>
<th></th>
<th>Yellow podzolic (YP)</th>
<th>Red Podzolic Duplex (D)</th>
<th>Red podzolic (RP)</th>
<th>Red Earth (RE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical Properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk density (kg m(^{-3}))</td>
<td>1.4</td>
<td>1.1</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Coarse sand (%)</td>
<td>54.2</td>
<td>20.3</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>Fine sand (%)</td>
<td>22.9</td>
<td>23.1</td>
<td>30.0</td>
<td></td>
</tr>
<tr>
<td>Silt (%)</td>
<td>14.0</td>
<td>35.5</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>Clay (%)</td>
<td>8.9</td>
<td>21.1</td>
<td>28.0</td>
<td></td>
</tr>
<tr>
<td><strong>Chemical Properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH (water)</td>
<td>5.4</td>
<td>5.7</td>
<td>5.2</td>
<td>4.8</td>
</tr>
<tr>
<td>pH (KCl)</td>
<td>4.6</td>
<td>4.8</td>
<td>4.3</td>
<td>3.8</td>
</tr>
<tr>
<td>% C</td>
<td>1.85</td>
<td>2.37</td>
<td>5.68</td>
<td>10.43</td>
</tr>
<tr>
<td>% N</td>
<td>0.060</td>
<td>0.054</td>
<td>0.135</td>
<td>0.330</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>30.2</td>
<td>43.9</td>
<td>42.2</td>
<td>31.3</td>
</tr>
<tr>
<td>Exchangeable Cations (me 100 g(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>0.26</td>
<td>0.48</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>3.89</td>
<td>2.33</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>0.54</td>
<td>0.74</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>0.09</td>
<td>0.23</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>0.00</td>
<td>1.88</td>
<td>7.62</td>
<td></td>
</tr>
<tr>
<td>Cation Exchange Capacity</td>
<td>4.81</td>
<td>6.03</td>
<td>12.16</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Compiled from data of author, Talsma (1983) and M.J. Connel (Personal communication).

\(^b\) D and RP soil have similar physical properties and exchangeable cations.

Description of sites, forest history, soils and field treatments are given below:

**Site 1**

(a) **Location**

The site is situated within the Commonwealth Scientific and Industrial Research Organization's (Division of Forestry and Forest Products) Biology of Forest Growth (BFG) comprehensive field experiment area at Pierces Creek, approximately 20 km west of Canberra (Linder *et al.*, 1987; Raison *et al.*, 1990; Khanna *et al.*, 1991).
(b) Forest history

The site was cleared of the original eucalypt woodland (*E. macrorrhyncha*, *E. rossii*) in 1934 - 35, the residues were burned *in situ*, and radiata pine (*Pinus radiata*) were planted in the winter of 1935. This tree crop was harvested in 1972, the slash burned, and following ripping along the planting lines to about 40 cm depth, replanting was carried out in June, 1973. Each tree was fertilized soon after planting with a fertilizer pellet (each pellet weighed 70 g and contains N, P and K as 6.28, 4.35 and 3.32%, respectively). The planting stock was 2-year old seedlings. The initial tree stocking was 997 stems ha⁻¹, but mortality had reduced this to around 700 stems ha⁻¹ by 1983 when trees were about 10 m high with a diameter at breast height of about 15 cm.

(c) Soil

The soil is a yellow podzolic (YP) (Stace *et al.*, 1968) derived from adamellite (coarse-grained, calcium rich, granitic rock) which has either a mottled or unmottled B horizon. The A horizon is up to 40 cm in depth, has a lower bulk density and higher hydraulic conductivity than the B horizon, and contains about 85 to 90% of the fine root system. The B horizon has very poor permeability and has a bulk density between 1.7 and 1.8 g cm⁻³. The soil is low in organic matter and hence, low in total nutrient reserves. Organic matter decreases from 2.4% (by weight) in the surface at 0 to 2.5 cm to 0.5% at 10 to 15 cm depth. Some physical and chemical properties of this soil is given in Table 3.2.

(d) Field treatments

The layout of field treatments at the BFG site is shown in Fig. 3.2. Treatment plots each of 0.25 ha were selected for similar stocking density and basal area, and
Figure 3.2 Location and layout of the Biology of Forest Growth (BFG) Research Project at Pierces Creek. Soils from plots 1, 4, 5, 6, 7 and 10 were used.
all trees were pruned to a height of 2 m before applying treatments. The objectives and detailed description of the treatments are given by Linder et al. (1987). Briefly, the treatments (see Fig. 3.2) are:

C - Control (Plot 6),
I - Irrigated (to maintain soil at about field capacity) (Plot 5),
F - Fertilized (with 400 kg N ha\(^{-1}\) as (NH\(_4\))\(_2\)SO\(_4\), 200 kg P ha\(^{-1}\) as superphosphate and 100 kg K ha\(^{-1}\) as potassium sulphate in 1983) (Plot 7),
IL - Irrigated and fertilizer applied in liquid form (with annual additions of about 300 kg N ha\(^{-1}\) as (NH\(_4\))\(_2\)SO\(_4\), and other balanced nutrients applied weekly in the irrigation water during the growing period) (Plots 1 and 2),
IF - Irrigated and fertilized (combination of I and F treatments) (Plots 3 and 4), and
S - Sewage sludge (with 13.3 t ha\(^{-1}\) of municipal sludge containing 176 kg N ha\(^{-1}\) and 237 kg P ha\(^{-1}\) applied in October 1983) (Plot 10).

The soils were sampled from these six treatments, with the exception that soils from plots 2 and 3 (IL and IF) were not collected because of the replication of plots. These soils are, however, designated as YPC, YPIL, YPIF, YPI, YPF and YPS. The YPC soil was sampled on six occasions from June 1986 to November 1989. The YPIL soil was collected twice in June and September 1986, while the other soils were sampled only once (September 1986). The selected chemical properties of these soils are given in Table 3.3. The YPF soil had relatively higher organic C and total N, while the pH is much greater for the YPS plot than in the other plots.
Table 3.3 Some chemical properties of differently treated yellow podzolic (YP) soil (0 - 5 cm) (Site 1). Samples taken in March 1987.

<table>
<thead>
<tr>
<th>Soil Treatment</th>
<th>pH (KCI)</th>
<th>% C</th>
<th>% N</th>
<th>C:N Ratio</th>
<th>% P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>4.34</td>
<td>2.09</td>
<td>0.10</td>
<td>20.9</td>
<td>0.017</td>
</tr>
<tr>
<td>IL</td>
<td>3.63</td>
<td>1.53</td>
<td>0.08</td>
<td>19.1</td>
<td>0.013</td>
</tr>
<tr>
<td>IF</td>
<td>4.18</td>
<td>1.69</td>
<td>0.07</td>
<td>24.1</td>
<td>0.022</td>
</tr>
<tr>
<td>I</td>
<td>4.39</td>
<td>1.43</td>
<td>0.08</td>
<td>17.9</td>
<td>0.016</td>
</tr>
<tr>
<td>F</td>
<td>4.57</td>
<td>2.84</td>
<td>0.10</td>
<td>28.4</td>
<td>0.028</td>
</tr>
<tr>
<td>Sludge</td>
<td>7.67</td>
<td>2.48</td>
<td>0.10</td>
<td>24.8</td>
<td>0.017</td>
</tr>
</tbody>
</table>

*a For soil symbols, see text (section 3.2).

Site 2

(a) Location

The site is known as Neds Block and is located in Uriarra forest (Fig. 3.1).

(b) Forest history

Radiata pine (*Pinus radiata*) was planted in 1960 and a nutritional trial described below was established in 1977.

(c) Soil

The soil is a typical duplex (Northcote, 1979), derived from Silurian Paddys River Volcanics. The A horizon has a lower bulk density than the B horizon and organic matter declines from 2.7% in the surface at 0-10 cm to 0.8% at 10-20 cm depth (Talsma, 1983). Some chemical properties of this soil are presented in Table 3.2.
(d) Field treatments

The layout of the field treatments at this site is shown in Fig. 3.3. Plots are square (0.05 ha) surrounded by a 5 m wide treated buffer and are arranged in a fully randomized layout. Following treatments were established in factorial combination using three replicates:

- R - Raked (annually each autumn to remove all litter),
- F - Fertilized (with 300 kg N ha$^{-1}$ as urea and 200 kg P ha$^{-1}$ as superphosphate in 1977), and
- T - Thinning (done by selection from a mean basal area of 35 m$^2$ ha$^{-1}$ to 20.7 m$^3$ ha$^{-1}$).

Soils from six plots (three from raked, plots 2, 9, 20, and three from fertilized, plots 6, 16, 24; Fig. 3.3) were sampled in June 1986. Hereafter, the soils were designated as DR1, DR2, DR3, DF1, DF2 and DF3, based on the soil name followed by treatment and replicate number.

Site 3

(a) Location

The site is located at Greens Catchment (Fig. 3.1).

(b) Forest history

The site was planted to radiata pine (*Pinus radiata*) in 1955.

(c) Soil

The soil is a red podzolic (Stace *et al.*, 1968), derived from Silurian Paddys River Volcanics with metasediment overlay.
Figure 3.3. Layout of Uriarra Neds Block. Soils from plots 2, 9 and 20 (raked), and from 6, 16 and 24 (fertilized) were used.
(d) Field treatments

There were no field treatments. Some physical and chemical characteristics are given in Table 3.2. This soil was sampled six times from June 1986 to November 1989.

Site 4

(a) Location

The site is located at Piccadilly Circus in the Brindabella Range about 50 km west of Canberra in the Australian Capital Territory (Fig. 3.1). A more detailed site description is given by Woods and Raison (1983).

(b) Forest history

The area is covered with natural sub-alpine eucalypt forest where communities dominated by:

(i) *Eucalyptus delegatensis* (alpine ash) - wet sclerophyll forest
(ii) *E. dives* (peppermint)-*E. dalrympleana* (mountaingum) - mixed species of dry sclerophyll forest, and
(iii) *E. pauciflora* (snowgum) - dry sclerophyll forest.

Alpine ash occupies moist, cool and southerly facing slopes, peppermint occurs on exposed ridges having northerly aspects and snowgum occupies higher elevation easternly aspects. Soils from each forest type were collected in May and December 1988.

(c) Soil

The soil supporting the forests is a strongly acid red earth (RE) (Stace et al.,
1968; Khanna et al., 1986), derived from highly weathered Ordovician sediments. The soil is highly organic, well aggregated and highly permeable, so that surface and subsurface runoff of water rarely occurs (Talsma and Hallam, 1980). Selected physical and chemical properties of the RE soil are given in Table 3.2.

(d) Field treatments

Studies (Chapters 6 and 7) have been concentrated in the snowgum forest which received the following treatments (H. Keith, Ph.D. Thesis, in preparation) applied to triplicate 20 x 20 m plots in December 1986:

- **C** - Control,
- **L+P** - 10 ton ha\(^{-1}\) lime + 500 kg P ha\(^{-1}\) as superphosphate,
- **N** - 300 kg N ha\(^{-1}\) as ammonium sulphate,
- **P** - 500 kg P ha\(^{-1}\) as superphosphate,
- **N+P** - combination of N and P treatments, and
- **Sugar (S)** - as sugar (sucrose), applied at the rate of 5 ton ha\(^{-1}\).

The control soil was sampled nine times between September 1986 and November 1989, and soils from L+P, N and N+P treatments were collected five times between October 1987 and March 1989, while soils from P and sugar treatments were sampled four times between June 1988 and March 1989.

The snowgum forest has also been deliberately burnt with low-intensity prescribed fires with the main aim of reducing fuel loads and hence wildfire risk. The soils from triplicate plots having the following fire treatments were sampled four times between June 1988 and March 1989:

- **UB** - Long unburnt (since 1973),
- **RB** - Regularly burnt (1962, 1973, 1980, 1987), and
A summary of the physical and chemical characteristics of soils from different sites is given in Table 3.2. There is a considerable range in both physical and chemical properties. For physical properties, the yellow podzolic (YP) soil has the highest coarse sand fraction and the lowest clay content, thus having a higher bulk density. The red earth (RE) soil contains less coarse sand and more clay. The low bulk density of RE soil is partly due to its high organic C content. The red podzolic (RP) soil is intermediate.

The RE soil has the highest organic C and total N content, and is more acidic than the other soils. The low pH of the RE soil is associated with high exchangeable Al content. The cation exchange capacity (CEC) in the RE soil is double that in the RP and about three-times greater than in the YP soil. The C:N ratios were high (>30) in all soils.

3.3 Sample collection, preparation and storage

Soil samples were collected from the different depths using either spade or soil cores (20 cm long and 54 mm diameter) after removal of litter layer. Soils were taken at random from each plot and sectioned by depth increments, where necessary, bulked and sieved (<5 mm). At least three replicates, except for few cases where duplicate or more than four replicates were used.

All sieved fresh soil samples were stored at 0 °C in closed plastic bags. Fresh soil was used to measure microbial N and C and fumigations were performed within 24 hours of sampling.
3.4 Soil Chemical Analyses

The soil pH was measured on a 1:5 (soil:water or soil:KCl) slurry after 1 hour of shaking using a glass electrode-calomel electrode assembly. Organic C was determined using the Walkley and Black wet digestion method, and total N after Kjeldahl digestion. Exchangeable cations and CEC were measured using 1M NH4Cl solution. All methods are described in detail by Heffernan (1985). Soil moisture content was determined by drying field moist soils at 105°C to constant weight.

3.4.1 Comparison between different extractants to determine mineral-N in soil

Mineral-N is usually extracted from soil with either 2M KCl (e.g. Ayanaba et al., 1976; Haynes, 1987; Myrold, 1987) or 1M KCl (e.g. Marumoto et al., 1982a, b; Sparling and Williams, 1986) or 0.5M K2SO4 (e.g. Shen et al., 1984; Voroney and Paul, 1984). The later extractant is usually used in the fumigation-extraction (FE) method for estimating microbial biomass N (Brookes et al., 1985b; West et al., 1988a, b). The main advantage of using 0.5M K2SO4 as an extractant in the fumigation-extraction method is the ease with which soil extracts can be digested to convert soluble organic N into NH4-N. It is assumed that Cl (from KCl) may form NH4Cl which has lower temperature of vaporization than (NH4)2SO4 and thus increase the risks of N loss during the digestion process. 2M KCl is double the salt content of 0.5M K2SO4, and high salt content may also create problems. However, Sparling and Williams (1986) reported similar levels of recovery of tryptophan and alanine in Kjeldahl digestions of KCl and K2SO4 extracts.

In this study, the efficiency of three extractants (viz. 0.5M K2SO4, 2M KCl and 1M KCl) in removing mineral N from nine soils (YPC, YPIL, RP, DR1, DR2, DR3, DF1, DF2 and DF3 sampled in June 1986) was tested.
Ten grams of moist soil was shaken for 1 hour (1:5, soil:extractant) in an end-over-end shaker and then filtered (Whatman No. 1). Total mineral (both NH$_4$ + NO$_3$) N was measured following reduction of NO$_3$ to NH$_4$ using Ti(SO$_4$)$_2$ by automated colorimetric procedure (Heffernan, 1985), as described by Raison et al., (1987). NO$_3$-N was determined as the difference between total mineral-N and NH$_4$-N. The results are expressed on oven-dry soil basis (105 °C, 24 h) as the mean of three replicates.

Results and Conclusion

The amounts of different forms of mineral-N extracted are presented in Table 3.4. The quantity of mineral-N in different extracts did not differ greatly within the

<table>
<thead>
<tr>
<th>Soila</th>
<th>0.5M K$_2$SO$_4$</th>
<th>2M KCl</th>
<th>1M KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min-N</td>
<td>NH$_4$-N</td>
<td>NO$_3$-N</td>
</tr>
<tr>
<td>YPC</td>
<td>33.9</td>
<td>30.4</td>
<td>3.5</td>
</tr>
<tr>
<td>YPIL</td>
<td>11.9</td>
<td>3.2</td>
<td>8.7</td>
</tr>
<tr>
<td>RP</td>
<td>13.0</td>
<td>12.5</td>
<td>0.5</td>
</tr>
<tr>
<td>DR1</td>
<td>4.5</td>
<td>3.4</td>
<td>1.1</td>
</tr>
<tr>
<td>DR2</td>
<td>3.2</td>
<td>1.9</td>
<td>1.3</td>
</tr>
<tr>
<td>DR3</td>
<td>4.4</td>
<td>3.3</td>
<td>1.1</td>
</tr>
<tr>
<td>DF1</td>
<td>3.5</td>
<td>2.4</td>
<td>1.1</td>
</tr>
<tr>
<td>DF2</td>
<td>3.9</td>
<td>3.2</td>
<td>0.7</td>
</tr>
<tr>
<td>DF3</td>
<td>4.0</td>
<td>2.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Mean</td>
<td>9.1</td>
<td>7.0</td>
<td>2.2</td>
</tr>
</tbody>
</table>

a For soil symbols, see text (section 3.2). Results are mean of three replicated values.
ND - Not determined

same soil, but to some extent. The average value was slightly higher in 0.5M K$_2$SO$_4$ extracts. Overall, the mineral-N in 0.5M K$_2$SO$_4$, 2M KCl and 1M KCl were 9.1, 8.5 and 8.0 mg N kg$^{-1}$ soil, respectively.
The 0.5M K₂SO₄ solution extracts as much mineral N from soils as KCl. It was used in all subsequent studies and has the advantage that organic N can also be determined in this extract (Brookes et al., 1985b).

3.4.2 Digestion methods to measure total-N in soil extract

Measurement of total (both organic and inorganic) N in the soil extract is usually determined after Kjeldahl digestion which converts organic N into NH₄-N (Brookes et al., 1985b). Sparling and West (1988b) compared Kjeldahl digestion with dichromate-oxidation, a method often used to measure oxidizable C (Sahrawat, 1982), and reported that the N values obtained by the latter method were slightly lower (about 10%).

A comparison was made between the standard Kjeldahl digestion, and a much simpler and more rapid acid-digestion which uses H₂SO₄ and H₂O₂ (Heffernan, 1985). Recently, Christianson and Holt (1989) reported that average recovery (compared to Kjeldahl digestion) of total soluble N from six soils was 98.2% when digested with H₂SO₄ and H₂O₂.

The soils used in this experiment were the same as those used for the previous study (section 3.4.1). The amounts of total N in both fumigated (1-day and 5-day chloroform fumigations; fumigation treatment is described in section 3.6.1) and unfumigated soils were determined after Kjeldahl digestion and digestion in H₂SO₄-H₂O₂ (Heffernan, 1985). In the Kjeldahl digestion, conc. H₂SO₄ (2.5 ml) and copper catalyst solution (180g CuSO₄. 10 H₂O /litre) were added to the K₂SO₄ soil extract (30 ml) in a heavy walled pyrex test tube and the mixture refluxed at 340 °C until clear (usually for 2 hrs). In the H₂SO₄-H₂O₂ digestion, the 30 ml extract was treated with 2.5 ml conc. H₂SO₄ and heated at 120 °C for overnight to reduce the volume to
about 3 ml. After cooling, 2 ml $\text{H}_2\text{O}_2$ was added to each tube and the sample digested by heating for about 40 min at 360 °C to produce a clear solution. After cooling, the digests were diluted to 50 ml with distilled water. In both cases, total-N was analyzed by the automated indophenol-blue procedure (Heffernan, 1985).

Results and Conclusion

Figure 3.4 shows the comparison of the two digestion procedures. Although small variations in the amounts of total-N as measured by the two methods were observed in both fumigated and unfumigated soils, the differences were not significant. This was the case for extracts from both unfumigated and chloroform fumigated soils covering the range of 8 - 95 mg N kg$^{-1}$ soil.

There was a good agreement between the two digestion methods. For this reason the simpler of the two, the $\text{H}_2\text{SO}_4$-$\text{H}_2\text{O}_2$ digestion method is recommended and was used in all further studies.

3.5 Terminology

The following terms and symbols have been used in this thesis:

(a) Fumigation-extraction or FE-N flush: The amount of total N extracted by 0.5M $\text{K}_2\text{SO}_4$ from fumigated soil, less that extracted from unfumigated soil. For the 1-day hexanol-fumigation, 1-day CHCl$_3$-fumigation and 5-day CHCl$_3$-fumigation, the FE-N flushes are termed 1-day-Hexanol-N, 1-day CHCl$_3$-N and 5-day CHCl$_3$-N, respectively.

(b) Microbial N Index: (Total N in the fumigated sample - mineral N in the unfumigated soil) x factor to account for incomplete removal of microbial biomass-derived N in a single $\text{K}_2\text{SO}_4$ extraction. The detailed rationale for this
$K_2SO_4$-extractable total N (mg N kg$^{-1}$ soil) after $H_2SO_4$-$H_2O_2$ digestion

Figure 3.4 Comparison of the acid-digestion ($H_2SO_4$-$H_2O_2$) and Kjeldahl digestion methods for estimating total N in 0.5M $K_2SO_4$ soil extracts. For chloroform-fumigated soils, $y = 3.69 + 0.91x$; $r^2 = 0.94$, $P<0.001$ and for unfumigated samples, $y = 0.99 + 0.94x$; $r^2 = 0.95$, $P<0.001$. 
approach for calculating biomass N is given in Chapter 4 (section 4.6).
(c) Fumigation-incubation or Fl-N flush: The difference between the amount of
N mineralized in chloroform fumigated and unfumigated soil after subsequent
re-inoculation and aerobic incubations.
(d) Fumigation-extraction or FE-C flush: The amount of oxidizable organic C
extracted by 0.5M K$_2$SO$_4$ from fumigated soil, less that extracted from
unfumigated soil.

The FE-N flush and Fl-N flush were not converted to microbial biomass N
because of validity of the $k_N$ factors, as proposed by Shen et al. (1984) and Brookes
et al. (1985b), have not been established for soils used in this study. The FE-C
flushes were not converted to microbial biomass C for the same reason.

3.6 Determination of flushes from fumigation-extraction (FE-N flush) and
fumigation-incubation (Fl-N flush)

3.6.1 Fumigation-extraction (FE) method

The chloroform-fumigation technique used was similar to that described by
Jenkinson and Powlson (1976b). Briefly, two portions of field moist soil, each
containing 20 g, unless otherwise mentioned, were placed in 150 ml glass bottles
and fumigated with ethanol-free chloroform (a pyrex glass beaker containing 50 ml of
purified chloroform and a few anti-bumping granules) in large desiccators (30.5 cm
i.d.) lined with moist paper and also containing another glass beaker with 25 g of
soda lime. The desiccators were evacuated until the chloroform boiled vigorously
and then the tap closed. The desiccators were left in the dark at room temperature
for either 1 or 5 days. After fumigation, the beaker of chloroform and the paper were
removed, and chloroform vapour was removed from the soil by repeated evacuation
(6 times) of the desiccator. Fumigated samples were then extracted with 0.5M
K$_2$SO$_4$ (1:5, soil:extractant). Unfumigated soils were extracted in a similar way at the time fumigation commenced.

Hexanol fumigation was done with liquid hexanol (4 ml was added to 10 g moist soils) for 1 day (McLaughlin et al., 1986). After fumigation, liquid hexanol was removed by evaporation for 24 h in a fume cupboard. Fumigated soils were then extracted with 0.5M K$_2$SO$_4$, as described earlier.

Mineral (NH$_4$ + NO$_3$)-N and total (organic + mineral)-N in the soil extracts were determined as described in section 3.4.

3.6.2 Chloroform fumigation-incubation (FI) method

The FI method used was as described by Jenkinson and Powlson (1976b) and Shen et al. (1984). Soils (10 g field moist) were fumigated with purified chloroform for 24 h in a glass bottle and after removal of chloroform vapour the samples were re-inoculated with 1 g of fresh soil. Unfumigated soils were not inoculated. After fumigation, soils were covered with polythene film (with a few holes for aeration) and incubated for 10 days at 25 °C, unless otherwise stated. Small amounts of water lost during fumigation were not replaced. Following incubation soils were extracted with 0.5M K$_2$SO$_4$ and mineral-N in the soil extracts was determined as described in subsection 3.4.1.

The FI-N flush is an index of soil microbial biomass N and is calculated as the N mineralized by the fumigated soil less the amount mineralized in the unfumigated soil during either a 10-day (Ayanaba et al., 1976; Jenkinson and Powlson, 1976b; Shen et al., 1984) or a 20-day aerobic incubation (Sarathchandra et al., 1984). It has also been estimated by subtracting the NH$_4$-N released by the unfumigated soil from that released by the fumigated soil during either a 7-day (Myrold, 1987) or a 14-day
(Inubushi et al., 1984; Azam et al., 1988, 1989b) anaerobic incubation. Several problems have been identified with the calculation of Fl-N values. These problems are discussed in detail in Chapter 2 (section 2.2.1).

(a) Methods of calculating Fl-N flush

In the following experiment, the influence of using a control on the estimate of Fl-N flush was examined using three acid forest soils (e.g. YP, RP and RE), with the YP soil having received different fertilizer and irrigation treatments. Soils (0-5 cm) were sampled in September 1986. Both chloroform-fumigated (1 day) and unfumigated samples were incubated under aerobic conditions at 25 °C for 10 days. The Fl-N flush was calculated in two ways, using a selected control or no control:

\[
\text{FI-N1} = \text{Amount of mineral N (NH}_4^-\text{-N and NO}_3^-\text{-N) produced during incubation by the chloroform-fumigated soil minus that produced by the unfumigated soil during the same period of time, under identical conditions (e.g. methods of Ayanaba et al., 1976 and Shen et al., 1984), and}
\]

\[
\text{FI-N2} = \text{Amount of mineral N (as NH}_4^-\text{-N) accumulated during incubation of the fumigated soil minus NH}_4^- \text{in the soil before fumigation (Voroney and Paul, 1984).}
\]

Results and Discussion

The Fl-N flush calculated by the two methods varied significantly only for two soils (i.e. YP1F and RE), whilst other soils showed no significant differences (Fig. 3.5). Overall, these results agree with those reported by Voroney and Paul (1984), who found insignificant differences between microbial biomass N estimates obtained with or without subtracting the unfumigated control. Ross (1990b) reported that the Fl-N flush values were not influenced appreciably by the control used. The Fl-N
Figure 3.5 Comparison of Fl-N flush (mg N kg\(^{-1}\) soil) calculated either with or without using an unfumigated control. For each soil, values not marked with the same letter are significantly different (P<0.05).
flush values calculated by these two procedures were highly correlated ($r^2 = 0.99$, $P<0.001$).

**Conclusion**

The Fl-N flush values calculated using two different controls gave very similar estimates, except for two soils. Good agreement between the methods suggests that either approach could be used. The Fl-N1 method (i.e. subtraction of the N mineralized in the control) is used in this thesis and is referred to simply as Fl-N flush.

(b) Effects of duration of chloroform fumigation prior to aerobic incubation

The effect of duration of chloroform fumigation before reinoculation and subsequent aerobic incubation for 10 and 20 days on the estimates of Fl-N flush was tested on soils collected in September 1986. The chloroform fumigation and incubation procedures were as described in section 3.6.2. Mineral N in the $K_2SO_4-$ extracts was determined as described in section 3.4.

**Results**

Results presented in Fig. 3.6. show that the amounts of Fl-N flush obtained after 1-day chloroform fumigation were, in general, relatively higher than those obtained after a 5-day fumigation. During the 10-day incubation, the average Fl-N flush values were 11.5 and 8.5 mg N kg$^{-1}$ soil for the 1-day and 5-day chloroform fumigation, respectively. Similarly, during the 20-day incubation, the respective mean values were 10.8 and 6.7 mg N kg$^{-1}$ soil. Thus on average, 1-day fumigation followed by incubations increased the Fl-N flush by 33%, when data for 10 and 20
Fl-N flush (mg N kg\(^{-1}\) soil)
after 5-day fumigation

Fl-N flush (mg N kg\(^{-1}\) soil)
after 1-day fumigation

Figure 3.6 Comparison of Fl-N flush values (mg kg\(^{-1}\) soil) obtained after 1-day and 5-day chloroform fumigation prior to aerobic incubation for 10 days (open) or 20 days (closed) of a range of acid forest soils.
days incubation were combined. The lower Fl-N flushes after 5-day fumigation compared to those obtained after 1-day fumigation may have resulted in less survival of cells in soil micropores in the former treatment and therefore less effective attack on tissues of killed adjacent cells.

There was reasonable agreement between the 1-day and 5-day chloroform fumigation flush obtained with incubation for 10 days ($r^2 = 0.77$) and 20 days ($r^2 = 0.77$) (Table 3.5). When the data were pooled the correlation was 0.73. The slopes of the regression was not much affected by the incubation period.

Table 3.5 Regressions of Fl-N flush (mg N kg\(^{-1}\) soil) values obtained after 1-day and 5-day chloroform fumigation during 10 or 20 days of aerobic incubation.

<table>
<thead>
<tr>
<th>y</th>
<th>x</th>
<th>Days of incubation</th>
<th>No. of samples</th>
<th>Regression equation</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-day</td>
<td>1-day</td>
<td>10</td>
<td>28</td>
<td>$y = 2.7 + 0.50 x$</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>28</td>
<td>$y = -0.4 + 0.59 x$</td>
<td>0.77</td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td></td>
<td>56</td>
<td>$y = 1.3 + 0.53 x$</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Note: All $r^2$ are significant at 0.1% level.

Conclusion

In conclusion, a 1-day chloroform-fumigation prior to estimating Fl-N flush was considered best and was used in all further studies.
3.7 Soil microbial biomass C measurements

3.7.1 Estimation of microbial C using the hexanol fumigation-extraction procedure

Introduction

Soil microbial biomass C can be measured by a number of methods, each of which is based on different principles. Many factors are known to affect the reliability of these methods (Jenkinson and Powlson, 1976b; Jenkinson, 1988). These factors are discussed in detail in Chapter 2 (section 2.3).

A direct extraction method for measuring microbial biomass N in acid forest soils is described in Chapter 4 and compared with the chloroform fumigation-extraction method of Brookes et al. (1985b) and the FI method of Shen et al. (1984) (see Chapter 5). The method outlined in Chapter 4 involves the measurement of microbial N by fumigating soils with liquid hexanol followed by extraction of solubilized N with K$_2$SO$_4$. The possibility of measuring the release of both C and N in the extract of hexanol-fumigated soils was evaluated. It was hypothesized that residual hexanol might result in an over-estimation of microbial C estimates because hexanol contains long chain C atoms. A number of experiments were performed to examine the effects of hexanol contamination on the estimation of soil microbial biomass C using the direct extraction method. Estimations were also compared with those obtained using the chloroform fumigation-extraction method.
(a) Effect of heating during oxidation on organic C estimates

Materials and Methods

The red earth (RE) soil was used and samples were taken from 0-2.5, 2.5-5 and 5-10 cm depths. Hexanol fumigation and extraction procedures were described in section 3.6.1. A known amount of $K_2SO_4$-extract from both unfumigated and fumigated soils was placed in a glass beaker and 2 ml of 0.25M $K_2Cr_2O_7$ was added. One part was left unheated for 1 hour and another part was heated on a sandbath for 30 min followed by addition of 10 ml of conc. $H_2SO_4$ to achieve oxidation. After cooling (1 hour), distilled water (100 ml) was added to each beaker and the solutions allowed to cool further. Oxidizable organic C in the extracts was calculated following a potentiometric titration (Raveh and Avnimelech, 1973) to determine the unused dichromate utilizing back titration with ferrous ammonium sulphate (Walkley and Black, 1934).

Another experiment was performed to test the recovery of added glucose-C from unfumigated and fumigated soil extracts. Glucose solution (0.5 mg C ml$^{-1}$) was added to three soils (YP, RP and RE) and the samples were heated before and/or during oxidation. All other steps were the same as described above.

A further experiment examined the effect of hexanol contamination on the estimation of oxidizable organic C. This involved the addition of known amounts of hexanol to $K_2SO_4$ extracts. The extracts were then heated for the following periods to test removal (volatilization) of hexanol. The heating periods were 0, 5, 10, 15 and 20 min on a sandbath (ca. 105 °C). Oxidizable C was determined as described earlier.
All results are expressed as mg C 100 g\(^{-1}\) soil, and are the mean of three replicates for each treatment.

**Results and Discussion**

Heating on the sandbath for 30 min during oxidation significantly increased the amount of organic C removed in extracts of unfumigated soils from the 0-2.5 cm depth, but not at lower depths (Fig. 3.7). Extractable organic C contents in non-heated extracts of unfumigated soils were 39.6, 41.3 and 33.7 mg C 100 g\(^{-1}\) soil (mean 38.2) for the 0-2.5, 2.5-5 and 5-10 cm depths, respectively. For the heated extracts, the corresponding values were 50.3, 51.7 and 44.4 mg C 100 g\(^{-1}\) soil (mean 48.8). Thus the increase was about 28% when averaging over all depths. Heating apparently increased the oxidation of some of the organic compounds in the soil extracts.

Table 3.6 shows the recovery of glucose - C from unfumigated soil extracts

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatments(^b)</th>
<th>Recovery of glucose-C (%)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td>YP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RE</td>
<td>-</td>
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<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\) 0-5 cm depth.

\(^b\) Heating was done at 70\(^\circ\) C for 1 hour on both occasions. Plus and minus signs represent with or without heat.

\(^c\) Mean of three replicates.
**Extractable organic C**
*(mg 100 g⁻¹ soil)*

![Bar chart showing the effect of heating on the k₂so₄-extractable organic carbon of unfumigated red earth soil.](image)

**Figure 3.7** Effect of heating for 30 min on a sandbath during oxidation on the K₂SO₄-extractable organic carbon of unfumigated red earth soil. Bars represent the SE of the mean.
with or without heating before and during oxidation. Heating resulted in recovery of almost all added C. Samples which received no heating at any stage recovered slightly less C.

Heating on a sandbath for 30 min during oxidation significantly increased measured organic C of extracts of fumigated soils (Fig. 3.8). Extractable organic C measured in the non-heated extracts were 630, 646 and 630 mg C 100 g⁻¹ soil (mean 635) for the 0-2.5, 2.5-5 and 5-10 cm depths, respectively. By contrast, the corresponding values for the heated fumigated samples were 1343, 1387 and 1451 mg C 100 g⁻¹ soil (mean 1396). The average increase was 120%. The main reason for this increase is probably because of the presence of some hexanol in the extracts which is oxidized and recorded as organic C.

Heating prior to oxidation drastically reduced the amounts of hexanol C in K₂SO₄ solutions, with the amount decreasing on average by 98% during the first 5 minutes (Table 3.7). After 20 minutes, virtually no carbon was detected in the K₂SO₄ solution which indicated that hexanol was removed (evaporated) during this period. The results demonstrate the need to remove hexanol from K₂SO₄ extracts of fumigated soils and this can be done by heating for 20 minutes prior to oxidation step to measure organic carbon.

Table 3.7 Changes in hexanol C in K₂SO₄ solutions during heating.

<table>
<thead>
<tr>
<th>Duration of heating (minutes)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg C 100 g⁻¹ soil)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>128.6 (4.3)b</td>
<td>128.6</td>
<td>2.6</td>
<td>0.7</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>(0.4)</td>
<td>(0.0)</td>
<td>(0.1)</td>
<td>(0.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Heating was done on a sandbath (ca. 105 °C).
b SE of the mean.
Figure 3.8 Effect of heating for 30 min on a sandbath during oxidation on the $K_2SO_4$-extractable organic carbon of hexanol fumigated red earth soil. Bars represent the SE of the mean.
Conclusions

The hexanol fumigation-extraction method could be useful to estimate soil microbial C, but residual hexanol-C must be removed before oxidation. Hexanol can be removed by heating at 70 °C for 30 min. Unfumigated soil does not require heating. The estimate of microbial C measured by the hexanol fumigation-extraction method was compared with that obtained by the chloroform fumigation-extraction method in the following experiments.

(b) Comparison of hexanol and chloroform fumigation-extraction methods to estimate soil microbial C

The YP, RP and RE soils were used to compare the FE-C flush (i.e. fumigated minus unfumigated) after hexanol and chloroform fumigation and extraction.

Materials and Methods

The effect of eight repeated extractions was measured in unfumigated soil. The effects of either a single 24 hour fumigation or two 24 hour fumigations were examined, with extractions with K$_2$SO$_4$ after each, i.e. a) either fumigation, extraction, extraction, or b) fumigation, extraction, fumigation, extraction treatments. Organic C in the extracts was then determined as described previously. Hexanol fumigated soils were heated prior to oxidation at 70 °C for 30 min. The chloroform fumigation and extraction was done using the method of Vance et al. (1987c). The FE-C flush was calculated as the difference between the amount of oxidizable C extracted from fumigated and unfumigated soil.
Results and Discussion

The organic C contents of eight sequential $K_2SO_4$ extracts of unfumigated soils is shown in Fig. 3.9. The first extraction removed most soluble organic C from all soil. Significant amounts of C were removed in second and subsequent extractions. For the RE soil the values gradually decreased, whilst in the other two soils fluctuations were less. The reduction between the first and subsequently extracted amounts of soluble C suggests that the $K_2SO_4$ extract attacks the microbial biomass (see Chapter 4, section 4.2 for a detailed treatment). This will result in the unfumigated value being an overestimate as a control in calculating the flush due to fumigation.

The second $K_2SO_4$ extraction with or without a further fumigation produced almost 50% of the organic C in the initial extracts of fumigated soils (Fig. 3.10). The amount was greater than from the unfumigated soil for second extraction (Table 3.8). The second fumigation did not result in any increase in extractable organic C. The results suggest that a single fumigation is sufficient, but that at least two extractions are needed to remove all the C released by fumigation. Similar results have been found for N (Chapter 4, section 4.5), and a correction factor is needed to adjust the results if only a single extraction is used.

Table 3.8 Quantities (mg C 100 g⁻¹ soil) of organic C extracted by 0.5M $K_2SO_4$ from unfumigated and fumigated soils during second extraction.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Organic C during second extraction</th>
<th>Unfumigated</th>
<th>Fumigated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hexanol</td>
<td>Chloroform</td>
</tr>
<tr>
<td>YP</td>
<td>19.0 (1.4)a</td>
<td>34.7 (2.3)</td>
<td>31.1 (2.4)</td>
</tr>
<tr>
<td>RP</td>
<td>22.2 (2.0)</td>
<td>45.9 (5.1)</td>
<td>50.8 (2.5)</td>
</tr>
<tr>
<td>RE</td>
<td>72.9 (2.0)</td>
<td>118.3 (8.1)</td>
<td>107.1 (4.9)</td>
</tr>
</tbody>
</table>

a SE of the mean value in parentheses.
Figure 3.9  Amounts of organic carbon extracted from unfumigated soils during sequential extraction with 0.5M $K_2SO_4$. 

**Extractable organic C**  
(mg 100g$^{-1}$ soil)
Figure 3.10 Quantities of organic C extracted by 0.5M K$_2$SO$_4$ from hexanol (a) and chloroform (b) fumigated soils during a first extraction, and a second extraction without (-F) and with (+F) second fumigation. Bars represent the SE of the mean.
The microbial C contents were calculated as the difference between the amount of C extracted from fumigated and unfumigated soil after correcting for biomass C in the unfumigated soil and incomplete extraction. The same correction factor was used for microbial C as was used for microbial N, assuming that the effects are same for N and C. The microbial C were similar for hexanol and chloroform fumigation (Fig. 3.11); when averaged across all three soils, the microbial C was 10% greater for hexanol fumigation. The microbial C contents from hexanol and chloroform fumigations correlated significantly \((r^2 = 0.99; \text{Fig. 3.12})\). These results suggest that either fumigant could be used.

**Conclusions**

Both the hexanol and chloroform fumigation-extraction methods result in an underestimate of the flush because some microbial C is extracted from unfumigated soil. The good agreement between the hexanol and chloroform fumigation-extraction methods suggest that hexanol fumigation could be used to estimate both microbial N and C.

The following sections describe studies to determine suitable conditions under which to determine microbial biomass C using the Substrate-Induced Respiration (SIR) technique.

### 3.7.2 Estimation of soil microbial biomass C by the Substrate-Induced Respiration (SIR) method

**Introduction**

The Substrate-Induced Respiration (SIR) procedure of Anderson and Domsch (1978b), as modified by West and Sparling (1986), was also used to estimate soil
Figure 3.11 Quantities of microbial C (mg C 100 g⁻¹ soil) estimated by the hexanol and chloroform fumigation-extraction methods. Bars represent the SE of the mean.
Figure 3.12 Relationship between microbial C (mg C 100 g\(^{-1}\) soil) estimates obtained from hexanol and chloroform fumigation-extraction methods.
microbial biomass C. In this method the size of the active soil microbial biomass is calculated from the magnitude of the initial respiratory response after addition of a non-limiting amount of glucose to the soil. The SIR method was chosen because it appears to give reliable estimates of biomass C in a range of soils of varying pH conditions (Anderson and Domsch, 1978b; West and Sparling, 1986; Cheng and Coleman, 1989; Dumontet and Mathur, 1989). Experiments were performed to assess a suitable concentration of added glucose, and to determine the duration of incubation needed to achieve a maximal respiration in the acid forest soils. Because the pattern of response of the soil microbial population to added glucose differs between soils (Anderson and Domsch, 1978b). The concentration of added glucose must be determined for each soil (Jenkinson and Ladd, 1981). The required frequency of headspace sampling also depends on the response of the soil to glucose addition (Anderson and Domsch, 1978b).

Materials and Methods

To determine suitable glucose concentration and duration of incubation sampling, 0-5 cm samples from the contrasting yellow podzolic (YP) and red earth (RE) soils were used. Triplicate portions of each soil (1 g oven-dry weight) were placed in McCartney bottles (28.5 ml) and amended with 2 ml of glucose solution so that the final concentration in the soil solution (initial soil water plus added glucose solution) was either 2.5, 5, 10 and 15 mg glucose ml⁻¹ (5, 10, 20 and 30 mg glucose g⁻¹ soil). The bottles containing soil and glucose solution were left open to the atmosphere (25 °C) for 30 min, swirled violently for 5 seconds on a vortex mixer before sealing with a suba seal, and then incubated at 25 °C. The headspace was sampled before and after 1 hour of incubation and the amount of CO₂ (expressed as µl g⁻¹ oven-dry soil h⁻¹) in the gas samples was estimated by sampling 1 ml of headspace gas with a micro-syringe and analysing it with a portable CO₂-analyzer (Model LI-6200, LI-COR Inc., Nebraska, U.S.A.; Leuning and Sands, 1989).
Two experiments examined the effects of incubation time: in the first, soils were treated with 5 mg glucose g\(^{-1}\) soil and the headspace sampled hourly for 5 hours. Because this study showed low respiration in the second hour, a second study in which 2 g freshly collected RE soil was amended with either 5 or 10 mg glucose g\(^{-1}\) soil and respiration measured for 0 - 2 and 2 - 3 hour was conducted.

Results and Discussion

Figure 3.13 shows the effect of glucose concentration on the rate of respiration for the YP and RE soils. Increasing glucose addition from 5 to 20 mg g\(^{-1}\) had little effect on respiration rate. There was a trend towards a slight increase from 20 to 30 mg glucose g\(^{-1}\) soil. The pattern of respiratory response for the two soils was almost identical. West and Sparling (1986) found optimal respiration response (as \(\mu l CO_2 g^{-1} soil h^{-1}\)) of soil (25% w/w water content) with glucose amendments in the range of 2.5 to 10 mg glucose g\(^{-1}\) oven-dry soil. They also showed that <2.5 mg or >10 mg glucose g\(^{-1}\) soil resulted in significantly reduced responses. Thus they recommended a glucose concentration of 7.5 mg g\(^{-1}\) soil, except for soils having a very high organic matter content. Cheng and Coleman (1989) measured glucose-amended respiration of a soil (0 to 5 cm, 35% w/w soil water content during incubation) over the range of glucose addition of 1 to 12 mg g\(^{-1}\) oven-dry soil. They found that respiration rate gradually increased for up to 4 mg g\(^{-1}\) soil and then remained unchanged. They recommended 8 mg glucose g\(^{-1}\) soil as an acceptable amount.

Figure 3.14 depicts the respiration rates during incubation for up to 5 hours following the addition of glucose solution at the rate of 5 mg g\(^{-1}\) soil. The maximum response was observed during the first hour and respiration rates dropped during the second hour. From 3 to 5 hours, rates were relatively constant in both soils.
Figure 3.13 Effects of different rates of glucose addition on soil respiration rates ($\mu$L CO$_2$ g$^{-1}$ oven-dry soil hr$^{-1}$). Measurements made after 1 hr of glucose addition. Bars represent the SE of the mean.
Figure 3.14 Temporal changes in respiration rate (μl CO₂ g⁻¹ soil hr⁻¹) of YP and RE soils after addition of 5 mg glucose g⁻¹ soil. Bars represent the SE of the mean.
Dumontet and Mathur (1989) also found decreased respiration rate between 1 and 2 h after glucose addition in seven out of eight soils. However, these authors did not give any explanation for their results. In contrast, West and Sparling (1986) found no great variation in hourly CO₂ respiration rates with the duration assay (0.5 to 5.5 h). In general, the pattern of microbial response after glucose addition is variable (see Jenkinson, 1988). It is important to avoid the effects of significant cell growth on respiration rates i.e. to keep the incubation period short. The two soils examined had a similar respiratory response.

An another study on freshly collected RE soil showed that there was no significant effect of glucose concentration or incubation period up to 3-5 hours (Table 3.9).

Table 3.9 Comparison of respiration rates (μl CO₂ g⁻¹ soil h⁻¹) with different concentrations of glucose and incubation time using a large amount (2 g) of fresh RE soil (0 - 5 cm).

<table>
<thead>
<tr>
<th>Glucose concentration (mg g⁻¹ soil)</th>
<th>Duration of incubation (hour)</th>
<th>0 - 2</th>
<th>2 - 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>30.4 (2.1)ᵃ</td>
<td>31.8 (0.7)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>28.5 (0.7)</td>
<td>28.0 (2.5)</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ SE of the mean value.

Conclusions

Overall, a glucose concentration of either 5 or 10 mg g⁻¹ generates an optimal respiration response in the two soils studied. Measurement of respiration rates can be done over 2 to 3 h of incubation. West and Sparling (1986) recommended a minimum of 2.5 h after the addition of glucose solution. The SIR method with above characteristics was used as an index of microbial biomass C changes induced by different forest fertilization and low intensity prescribed fire on the highly acid RE soil (see Chapter 6).
3.8 Data Presentation and Statistical Analysis

The means of treatment or analytical replicates are presented in Tables and Figures along with their standard error (SE), a measure of variability among the individual measurements. The SE was calculated by dividing standard deviations by the square root of number (n) of observations constituting the mean.

The significant difference between the two population means was analyzed by Student's t-test and values used in the text refer to P<0.05, unless otherwise specified. Analysis of variance (ANOVA) was used to analyze most of the data presented in Chapter 6 using the GENSTAT Computer Program (GENSTAT Manual, 1980). Significant differences were compared using the Fisher least significant difference (LSD) test (P<0.05) (Steel and Torrie, 1980). The relationships between measured parameters were investigated by using regression analysis (Steel and Torrie, 1980).
CHAPTER FOUR

DEVELOPMENT OF A DIRECT EXTRACTION METHOD
FOR MEASURING SOIL MICROBIAL BIOMASS NITROGEN
USING HEXANOL AS A FUMIGANT

4.1 Fumigation-extraction (or FE) procedure - comparison of hexanol and chloroform as fumigants

4.1.1 Comparison of N extracted in K$_2$SO$_4$ from soils fumigated for 1 day with hexanol and chloroform

4.1.2 Duration of exposure of soil to hexanol and chloroform
   (a) Effects of duration of exposure of soil to hexanol on the flush of FE-N
   (b) Effects of 1- and 5-day chloroform fumigation on the flush of FE-N and comparison with 1-day hexanol fumigation

4.2 Effects of multiple extractions on the quantity of N extracted from fumigated and unfumigated soils

4.3 Effects of multiple fumigations on the quantity of N extracted from soils

4.4 Assessment of microbial N

4.5 Assessment of non-microbial and microbial N in K$_2$SO$_4$-extracts of unfumigated and fumigated soils

4.6 Calculation of soil microbial N

4.7 Relationships between the estimates of microbial N based on three fumigation-extraction methods
CHAPTER FOUR

DEVELOPMENT OF A DIRECT EXTRACTION METHOD FOR MEASURING SOIL MICROBIAL BIOMASS NITROGEN USING HEXANOL AS A FUMIGANT

4.1 Fumigation-extraction (or FE) procedure - comparison of hexanol and chloroform as fumigants

Introduction

Chemical biocides are generally used as fumigants in soil microbial biomass studies, although other treatments (e.g. gamma irradiation, autoclaving, heating and drying, and microwave irradiation) have also been used (e.g. Powlson and Jenkinson, 1976; Spalding, 1978; Ferris, 1984; Speir et al., 1986; Hendricks and Pascoe, 1988; Zagal, 1989).

Chloroform has been the most extensively used chemical to estimate various elements present in the soil microbial biomass, e.g. for C (Jenkinson and Powlson, 1976b; Vance et al., 1987c; Tate et al., 1988), N (Shen et al., 1984; Voroney and Paul, 1984; Brookes et al., 1985b), P (Brookes et al., 1982; Hedley and Stewart, 1982), S (Saggar et al., 1981; Strick and Nakas, 1984; Chapman, 1987b) and other nutrients (Sparling, 1985; Diaz-Ravina et al., 1989). Chloroform is generally believed to be a very effective fumigant (Jenkinson and Powlson, 1976a,b; Chaussod et al., 1986). Other fumigants, such as carbon disulphide (Kudeyarov and Jenkinson, 1976; Vance et al., 1987a), methyl bromide (Powlson and Jenkinson, 1976), methyl bromide plus chloropicrin (Spalding, 1978), propylene oxide (Kassim et al., 1981), and combination of pure ethanol and propanol (Hedley and Stewart, 1982) have also been used to estimate soil microbial biomass C by the fumigation-incubation method. But all of them are either more difficult to remove from soil after fumigation, or
experimentally less convenient and less effective than chloroform (Jenkinson and Powlson, 1976a; Jenkinson, 1988). These fumigants have not been used in microbial biomass N studies.

Although chloroform has been extensively used as a fumigant for determining microbial biomass N, its use in acid forest soils may pose problems. Furthermore, chloroform is hazardous and extreme care must be exercised in handling it.

McLaughlin et al. (1986) found that hexanol and chloroform were similarly effective in extracting P present in soil micro-organisms. It is thus likely that hexanol could be as effective as chloroform for estimating microbial biomass N in soil. Hexanol is less hazardous and cheaper than chloroform.

To test the usefulness of hexanol as a fumigant, a number of experiments which are now described were performed on a range of acid forest soils. The main objective was to evaluate and compare the effectiveness of hexanol and chloroform for the estimation of microbial biomass N in acid forest soils.

4.1.1 Comparison of $N$ extracted in $K_2SO_4$ from soils fumigated for 1 day with hexanol and chloroform

Experimental details

Seventeen acid forest soils of varying properties were used. Soil samples were collected in June 1986 (see Fig. 4.1a) and in September 1986 (Fig. 4.1b). Detailed descriptions of these soils and sampling procedure were given in Chapter 3 (section 3.2). The field moist soils were sieved through <2 mm-mesh and stored at 4 °C for 2 to 3 days before use. The soil moisture contents varied from 14.9 to 27.9% for June, 1986, and from 10.0 to 45.1% for September, 1986 samples.
Fumigation was done for 1-day with liquid hexanol and chloroform vapour, as
described in Chapter 3 (section 3.5.1). Both fumigated and unfumigated soils were
extracted with 0.5M K₂SO₄ (1:5, soil:extractant). Mineral N (NH₄-N and NO₃-N) and
total N (inorganic and organic) in the soil extracts were determined as described in
Chapter 3 (section 3.3).

Results and Discussion

The amount of total N extracted in K₂SO₄ for each soil is shown in Fig. 4.1. Total N (mg N kg⁻¹ soil) in the unfumigated samples ranged from 10.0 - 59.5 (mean 22.2), while the values varied from 23.1 to 118.3 (mean 50.0) and from 28.9 to 131.6 (mean 52.1) for the hexanol and chloroform fumigated soils, respectively. There were few significant differences between the effects of hexanol and chloroform fumigation. Higher amounts of N were sometimes extracted after hexanol fumigation of soils collected in June (Fig. 4.1a), whilst the reverse situation was found for the September samples (Fig. 4.1b). The reason for this variability is not known. Overall, hexanol and chloroform gave very similar total N values.

The additional total N after fumigation (i.e. FE-N flush = fumigated -
unfumigated) varied several-fold among the soils. After hexanol fumigation the FE-N
flush (mg N kg⁻¹ soil) ranged from 12.0 to 82.4 (mean 27.7), whilst after chloroform
fumigation it varied between 13.9 and 95.2 (mean 29.8). The two estimates showed
a significant and linear correlation (r² = 0.82, P<0.001; Fig. 4.2), but chloroform
resulted in lower values (slope = 0.82). The highly acid red earth had a high average
effect on the regression line. When this soil was excluded from the analysis, r² was
only 0.42 (P<0.001, n = 46). The size of the FE-N flush was not related to the
amount of total N extracted from unfumigated soils (Fig. 4.3).
Figure 4.1 Quantities of total N extracted by 0.5M K$_2$SO$_4$ from different acid forest soils before and after one day fumigation with either hexanol or chloroform. Bars represent the SE of the mean and values not marked with the same letter are significantly different within the soil. (P<0.05, t-test).
Hexanol flush

\[ y = 1.9 + 0.82 x \]
\[ r^2 = 0.82, \ P < 0.001, \ n = 50 \]

Chloroform flush

Figure 4.2 Relationship between FE-N flush (mg N kg\(^{-1}\) soil) based on one day hexanol and chloroform fumigation and direct extraction.
Figure 4.3 Relationships between initial total N in K₂SO₄ extracts and the FE-N flush values obtained after one day hexanol and chloroform fumigation followed by direct extraction.
Conclusions

Hexanol and chloroform released similar amounts of total N after 1 day fumigation. Thus hexanol may be a useful alternative fumigant to chloroform for microbial biomass N estimation. However, the adequacy of 1 day fumigation in acid forest soils required investigation. The effect of duration of hexanol and chloroform fumigations on the FE-N flush in various acid forest soils is reported in the following section.

4.1.2 Duration of exposure of soil to hexanol and chloroform

(a) Effects of duration of exposure of soil to hexanol on the flush of FE-N

The following experiment examined the effect of duration of hexanol fumigation, ranging from 4 to 120 hours.

Experimental details

The yellow podzolic (YP), red podzolic (RP) and red earth (RE) soils were used for this study. They were collected in June 1989. Nine sub-samples were taken at random from each site to a depth of 5 cm and composited prior to sieving (<5 mm).

Three replications of 10 g portions of field moist soil were exposed to liquid hexanol (4 ml for each replication) sealed in plastic bottles and kept for either 4, 8, 16, 24, 48 or 120 hours. After fumigation, soils were extracted by shaking for 1 hour with 50 ml 0.5M K₂SO₄ followed by filtering (Whatman No.1). Unfumigated control soils were extracted similarly at the time fumigation commenced.
The extraction and analytical procedures for determining total N in extracts were described in detail in Chapter 3 (section 3.3).

Results and Discussion

Figure 4.4 shows the effect of time of hexanol fumigation on the amount of N extracted from the three soils.

Increasing time of fumigation increased mineral N rapidly during the initial 8 h and then more gradually up to 120 h in all three soils. The amount of total N extracted from the YP and RP soils peaked after 24 h of fumigation, and did not increase significantly (P<0.05) with longer fumigation. In contrast, the amount of total N in the RE soil did increase significantly from 24 h to 120 h. The further increase was 17.1 mg N kg⁻¹ soil, which was only 18% of the 24 h value. The increase in total N from the 4 h value was not large in any soil. The pattern of mineral and total N extracted was reasonably similar in all three soils, although the quantities varied markedly between the soils.

Quantitatively hexanol fumigation had a greater effect on total N than on mineral N. The proportion of the total N in K₂SO₄-extracts of fumigated samples in the organic N fraction ranged from 63 to 88% (Table 4.1). There was a tendency for the proportion to decrease with increasing fumigation time, suggesting that enzymes may have continued to degrade soluble organic N compounds during fumigation. With P, a large proportion of total P released by chloroform was inorganic, around 90% (Brookes et al., 1982; Bekunda, 1987) and by hexanol it was 57% (McLaughlin et al., 1986). The pattern of extractable-soil N released by hexanol was very similar to that released by chloroform (Brookes et al., 1985a), who reported that about 34% of total N extracted after 15 days was present as mineral N.
Figure 4.4 Effects of duration of exposure of soils to hexanol on (a) mineral N and (b) total N extracted with 0.5M K$_2$SO$_4$. Bars represent the SE of the mean.
Table 4.1 Organic N as a percentage of total N in K$_2$SO$_4$ - extracts during different periods of hexanol fumigation.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Duration of hexanol fumigation (h)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>24</td>
<td>48</td>
<td>120</td>
</tr>
<tr>
<td>YP</td>
<td>78.6</td>
<td>77.8</td>
<td>75.2</td>
<td>75.7</td>
<td>69.7</td>
<td>62.9</td>
</tr>
<tr>
<td></td>
<td>(1.06)$^a$</td>
<td>(0.36)</td>
<td>(1.40)</td>
<td>(2.49)</td>
<td>(1.81)</td>
<td>(1.75)</td>
</tr>
<tr>
<td>RP</td>
<td>82.4</td>
<td>79.7</td>
<td>79.1</td>
<td>76.0</td>
<td>76.2</td>
<td>68.8</td>
</tr>
<tr>
<td></td>
<td>(0.31)</td>
<td>(0.74)</td>
<td>(0.64)</td>
<td>(2.03)</td>
<td>(0.58)</td>
<td>(0.34)</td>
</tr>
<tr>
<td>RE</td>
<td>87.9</td>
<td>86.3</td>
<td>86.1</td>
<td>83.1</td>
<td>82.1</td>
<td>76.9</td>
</tr>
<tr>
<td></td>
<td>(0.79)</td>
<td>(0.97)</td>
<td>(0.74)</td>
<td>(0.88)</td>
<td>(3.06)</td>
<td>(1.14)</td>
</tr>
</tbody>
</table>

$^a$ SE of the mean value in parentheses.

Conclusions

Exposure of soil to hexanol for 16 to 24 hours is likely to be sufficient to ensure complete fumigation in most cases. A longer fumigation in the RE soil gave slightly higher (18% more than the 24 h value) values of extractable total N. The increase in mineral N during fumigation was significant and hence the quantity of total (inorganic + organic) N released must be the basis for subsequent estimates of microbial biomass N.

b) Effects of 1 and 5-day chloroform fumigation on the flush of FE-N and comparison with 1-day hexanol fumigation

Introduction

Brookes et al. (1985a) showed that both the amount of NH$_4$-N and total N released by chloroform increased with the duration of fumigation up to 5 days, but
did not increase during a further 10-day period. Inubushi et al. (1985) found that the quantity of released N increased with chloroform-fumigation time up to 11 h, but was then almost constant up to 24 h. Williams and Sparling (1984) reported that most of the soluble- and mineral-N released by chloroform-fumigation were formed after only 2h of treatment and then was constant up to 18 h. Recently, Davidson et al. (1989) reported that the plateau levels of extractable N after chloroform fumigation were achieved after 1 day with a forest soil, but required 5 to 7 days in grassland soils. These observations indicated the importance of examining the effects of chloroform fumigation time on FE-N flush in acid forest soils. In addition, these results were compared with the 1-day hexanol which was shown to be effective in previous experiments.

Experimental details

The effects of fumigation with chloroform vapour for 1 and 5 days on the N extracted from 17 soils used earlier (section 4.1) was determined. Comparison between 1- day hexanol and 5-day chloroform fumigations were made on these soils and nine additional soils which were collected in April and October 1987 (Figs. 4.7c and d). Description of these soils are given in Chapter 3 (section 3.2).

Results and Discussion

Effects of duration of chloroform fumigation on extractable-total N

Eight of the 17 soils showed significant differences (P<0.05) in the total N extracted after 1-day or 5-day chloroform fumigation (Fig. 4.5). However, the 5-day fumigation did not give consistently higher values; the extractable total N (mg N kg⁻¹ soil) ranged from 28.9 to 132.0 (mean 52.1) and from 23.4 to 122.4 (mean 51.4) for
Figure 4.5 Effects of duration of exposure of soils to chloroform on total N extracted with 0.5M K$_2$SO$_4$. Bars represent the SE of the mean and values not marked with the same letter are significantly different within the soil (P<0.05, t-test).
the 1-day and 5-day chloroform fumigated soils, respectively. For the YPC, YPIL and RP (common in both seasons), total N did not differ significantly between the fumigation treatments, except for the YPC September sample. For the June samples, total N in the 1-day extracts were relatively higher than in the 5-day extracts, while the opposite pattern was found for the September samples. The reasons for this result are not known, but these results differ from those reported by Brookes et al. (1985a), who showed that total N released by chloroform increased with fumigation time for periods up to 5 days. The high acidity of soils in this study contrast with those used by Brookes et al. (1985a).

The FE-N flushes (total N in fumigated soil - total N in unfumigated soil) after 1-day and 5-day chloroform fumigation showed a significant positive relationship ($r^2 = 0.83$, $P<0.001$; $n = 49$) (Fig. 4.6). Again, the highly acid red earth (RE) soil markedly influenced the regression line. When this soil was excluded from the analysis, the coefficient of determination was poor ($r^2 = 0.35$, $P<0.001$, $n = 45$), thus the slope of the regression (0.81) which suggests that overall 1-day values are greater than 5-day values may be misleading.

Comparison of 1-day hexanol with 5-day chloroform fumigation

The amount of total N extracted from 26 soils after 1-day hexanol or 5-day chloroform fumigation are shown in Fig. 4.7. Most of the soils showed similar amounts of total N (mg N kg$^{-1}$ soil) in the extracts, varying from 23.1 to 132.0 (mean 60.0) and from 23.4 to 137.9 (mean 63.8) after 1-day hexanol and 5-day chloroform fumigation, respectively. The RE soils had the highest values in all seasons. Slightly higher values were observed after the 5-day chloroform fumigation for the April and October 1987 samples. However, overall there was a very close correlation between the FE-N flush after 1-day hexanol and 5-day chloroform fumigations ($r^2 = 0.93$, $P<0.001$; Fig. 4.8). The slope of the regression indicates that on average the 1-day hexanol fumigation results in a slightly lower FE-N flush.
$y = 3.5 + 0.81 \times$
$r^2 = 0.83, P < 0.001, n = 49$

Figure 4.6 Relationship between the FE-N flush (mg N kg$^{-1}$ soil) based on one day and five day chloroform fumigation and direct extraction.
Total N in extracts
(mg N kg⁻¹ soil)

(a) June, 1986

(b) September, 1986

(c) April, 1987

(d) October, 1987

Figure 4.7 Comparison of total N in K₂SO₄ extracts of one day hexanol and five day chloroform fumigated soils. For explanation, see Fig. 4.1.
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Figure 4.8 Relationship between FE-N flush (mg N kg⁻¹ soil) based on one day hexanol and five day chloroform fumigation and direct extraction.

\[ y = 0.9 + 0.86x \]

\[ r^2 = 0.91, \ P < 0.001, \ n = 88 \]
General Conclusions

In conclusion, increasing the exposure of soil to chloroform vapour from 1 day to 5 days had no clear effect on total N extracted. The 1-day hexanol fumigation gave a similar FE-N flush to the 5-day chloroform fumigation. Thus a strong relationship was found between these two fumigation treatments. Longer (>24 h) fumigation with hexanol produced 10 to 20% increases in extractable total N, but a remaining question was whether a single fumigation was sufficient to release all microbial biomass N in all soils or whether repeated fumigations were required.

Other important unresolved points were:

a) is a single extraction with K₂SO₄ sufficient to remove all soluble biomass N?
b) does the time of extraction with K₂SO₄ affect the amount of N in the soil extract? and
c) is the microbial biomass N extracted by K₂SO₄ in unfumigated soils? If so, how much and how can this be accounted for in the calculation of biomass N?

The experiments described below were carried out to address these questions.

4.2 Effects of multiple extractions on the quantity of N extracted from fumigated and unfumigated soils

Introduction

As described previously, a single fumigation followed by immediate extraction has been used to estimate the nutrients held in the soil microbial biomass. The
underlying assumption in these studies is that a single fumigation kills all the soil biomass and that a single extraction removes all the dead cells and products into the solution. No study has reported on the possible effects of multiple extractions after fumigation on soil microbial biomass N estimates. The following study examined the changes in K$_2$SO$_4$-extractable total N content and FE-N flush after a single fumigation followed by repeated extractions.

**Experimental details**

The yellow podzolic (YP), red podzolic (RP) and red earth (RE) soils were used. Moist samples (0-5 cm) were collected in November, 1989 at randomly selected locations at each site and bulked prior to sieving (<5 mm). Samples were stored field moist in sealed plastic bags at 0 °C until analyzed, usually within a few days.

For the unfumigated soils, four replicates (8 g) were shaken for 1 h in 40 ml 0.5M K$_2$SO$_4$. After shaking, the soil mixtures were centrifuged for 5 min at 5000 rpm. The supernatants were then removed and filtered (Whatman No. 1). The soil residue was then weighed, further K$_2$SO$_4$ added (to 40 ml total) and the samples shaken for a further 1 h before again centrifuging to separate the supernatant. This extraction procedure was repeated eight times. Fumigation of field moist soil (8 g) was performed using either liquid hexanol or chloroform vapour for 24 h. Description of both fumigation procedures are given in Chapter 3. After fumigation, hexanol fumigated soils were left open for few hours in the fume cupboard, while chloroform vapours were removed by repeated evacuation. Soils were then extracted by shaking with 0.5M K$_2$SO$_4$ (40 ml) for 1 h. The soil mixtures were then centrifuged and re-extracted a total of eight times in a similar way to the unfumigated soils.

The mineral and total N in soil extracts were determined as described in sections 3.3.1 and 3.3.2. All results are expressed as the mean of four replicates on
an oven-dry soil basis.

**Results and Discussion**

Mineral N in the K$_2$SO$_4$ - extracts of unfumigated and total N in both unfumigated and fumigated samples extracted sequentially are shown in Fig. 4.9. During the sequential extraction, mineral N continued to be extracted from all the unfumigated soils (Fig. 4.9a). The amount tended to be decrease with increasing number of extractions. When expressed as a percentage of first extraction, the amounts of mineral N extracted during second and subsequent extractions were significantly different (Table 4.2, part a). Total N in the unfumigated soils behaved similarly (Fig. 4.9b). For the first extraction, total N contents of YP, RP and RE soils were 11.2, 12.6 and 29.1 mg N kg$^{-1}$ soil, respectively. During the second and subsequent extractions, the amount of total N was almost constant. When expressed as the percentage of first extraction, about 40 to 50% total N was extracted during the second or subsequent extractions (Table 4.2, part b).

In the fumigated soils, an important quantity of total N was extracted during the second and further extractions (Figs. 4.9c and d). For the first extraction, hexanol fumigated extracts of YP, RP and RE soils contained 30.3, 42.1 and 86.2 mg N kg$^{-1}$ soil, and chloroform fumigated extracts contained 28.4, 31.6 and 76.3 mg N kg$^{-1}$ soil, respectively. During the second extraction, total N in the fumigated samples were 26 to 44% of that extracted during the first extraction, depending on fumigants (Table 4.2, part c and d). By the eighth extraction the amount was only 9 to 13%.

Figure 4.10 shows the ratios of hexanol-to-chloroform for total N in sequential extracts. In most cases, the ratio was close to 1.0, but in some of the samples the amounts of total N in hexanol fumigated extracts were slightly higher.
Figure 4.9 Effects of sequential extraction on $K_2SO_4$-extractable mineral N (a), total N of unfumigated soil (b), total N of hexanol (c) and chloroform (d) fumigated soils. Bars represent the SE of the mean.
Figure 4.10 Ratios of total N in K$_2$SO$_4$ extracts of hexanol and chloroform fumigated samples during single fumigation and sequential extractions.
Table 4.2 Soil N in K$_2$SO$_4$ - extracts during sequential extractions, expressed as a percentage of first extraction value.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Second extraction</th>
<th>Fourth extraction</th>
<th>Eighth extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Mineral N in unfumigated extracts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP</td>
<td>30.7</td>
<td>46.2</td>
<td>23.5</td>
</tr>
<tr>
<td>RP</td>
<td>46.7</td>
<td>68.2</td>
<td>77.2</td>
</tr>
<tr>
<td>RE</td>
<td>44.3</td>
<td>51.2</td>
<td>43.9</td>
</tr>
</tbody>
</table>

b) Total N in unfumigated extracts

<table>
<thead>
<tr>
<th>Soil</th>
<th>Second extraction</th>
<th>Fourth extraction</th>
<th>Eighth extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>YP</td>
<td>48.6</td>
<td>45.9</td>
<td>44.3</td>
</tr>
<tr>
<td>RP</td>
<td>47.8</td>
<td>53.9</td>
<td>44.4</td>
</tr>
<tr>
<td>RE</td>
<td>40.4</td>
<td>47.2</td>
<td>26.2</td>
</tr>
</tbody>
</table>

c) Total N in hexanol extracts

<table>
<thead>
<tr>
<th>Soil</th>
<th>Second extraction</th>
<th>Fourth extraction</th>
<th>Eighth extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>YP</td>
<td>25.7</td>
<td>19.2</td>
<td>9.9</td>
</tr>
<tr>
<td>RP</td>
<td>30.7</td>
<td>19.0</td>
<td>11.9</td>
</tr>
<tr>
<td>RE</td>
<td>33.8</td>
<td>17.2</td>
<td>13.1</td>
</tr>
</tbody>
</table>

d) Total N in chloroform extracts

<table>
<thead>
<tr>
<th>Soil</th>
<th>Second extraction</th>
<th>Fourth extraction</th>
<th>Eighth extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>YP</td>
<td>27.2</td>
<td>11.5</td>
<td>8.5</td>
</tr>
<tr>
<td>RP</td>
<td>44.1</td>
<td>21.9</td>
<td>12.9</td>
</tr>
<tr>
<td>RE</td>
<td>42.1</td>
<td>20.2</td>
<td>13.4</td>
</tr>
</tbody>
</table>

The amount of mineral and total N in the unfumigated extracts and total N in fumigated samples reached a nearly constant value after the third or fourth extraction. The continuous extraction of total N in fumigated soils suggest that the sources of N is non-microbial soil organic matter. In unfumigated soils, there was a decrease from the first extraction to second and third extractions, indicating that a part of total N in the first extraction comes from microbial sources. Part of the biomass N in unfumigated soil appears to be attacked by K$_2$SO$_4$ and extracted into the solution. In a recent study, Azam et al. (1989a) showed that a significant amount of organic N appeared in K$_2$SO$_4$ extracts of unfumigated soil was derived from biomass. Subtraction of this N may yield lower values for microbial biomass N (Azam et al., 1989a, b). The results also indicated that the single extraction did not extract all the dead cells into the solution. Thus more than one extraction may be required to obtain higher microbial biomass N values.
The question of fumigation efficiency was explored using multiple fumigations with hexanol and chloroform, and the results are discussed in the following section.

4.3 Effects of multiple fumigations on the quantity of N extracted from soil

Experimental details

Soils used and the extraction technique for unfumigated samples were the same as used for the previous experiment (section 4.2). Fumigation with hexanol or chloroform was repeated five times with extraction of N in K$_2$SO$_4$ between each fumigation. For the second and subsequent fumigations, liquid hexanol and liquid chloroform were mixed into the K$_2$SO$_4$-saturated soils with a small glass rod. Liquid chloroform was added, because it was assumed that chloroform vapour would not provide efficient fumigation in the K$_2$SO$_4$-saturated soils. However, chloroform fumigated soils were not re-fumigated with chloroform vapour, as done by Widmer et al. (1989). All other steps were identical to those described previously (section 4.2).

Results and Discussion

The amounts of total N extracted from hexanol and chloroform fumigated samples during repeated fumigations and extraction are shown in Fig. 4.11. The total N was much higher during the first fumigation / extraction cycle for all three soils. The amount extracted approached a constant value after the second or third fumigation particularly for the YP and RP soils. By contrast, the total N extracted from the RE soil gradually declined up to the fourth fumigation and then remained constant. When total N in the fumigated extracts was expressed as a percentage of that amount of total N contained in the first extract, about 13 to 37% was present in
Figure 4.11 Changes in total N extracted by 0.5M K$_2$SO$_4$ from (a) hexanol and (b) chloroform fumigated soil after repeated fumigations and extractions. Bars represent the SE of the mean.
the second extract and this gradually decreased to 14 to 20% in the fifth extract (Table 4.3).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Second extraction</th>
<th>Third extraction</th>
<th>Fourth extraction</th>
<th>Fifth extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Hexanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP</td>
<td>28.4</td>
<td>19.8</td>
<td>13.2</td>
<td>15.9</td>
</tr>
<tr>
<td>RP</td>
<td>37.0</td>
<td>23.9</td>
<td>15.5</td>
<td>19.5</td>
</tr>
<tr>
<td>RE</td>
<td>32.3</td>
<td>25.9</td>
<td>14.9</td>
<td>17.1</td>
</tr>
<tr>
<td>b) Chloroform</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP</td>
<td>16.8</td>
<td>21.2</td>
<td>19.2</td>
<td>16.4</td>
</tr>
<tr>
<td>RP</td>
<td>13.5</td>
<td>11.7</td>
<td>17.2</td>
<td>13.6</td>
</tr>
<tr>
<td>RE</td>
<td>33.6</td>
<td>23.0</td>
<td>17.0</td>
<td>15.4</td>
</tr>
</tbody>
</table>

The ratios (hexanol-to-chloroform) for total N in the extracts were close to 1.0, except for three soils which showed high variability between replicates as shown by the large SE (Fig. 4.12).

A comparison of total N extracted from soils after single and repeated fumigation are depicted in Fig. 4.13. There were few differences between the single and multiple fumigations. Variations in the RP soil can not be explained. The overall conclusion is that a second fumigation is unlikely to be required for any of the soils studied.

**Conclusions**

Single and repeated fumigations had similar effects on extractable total N. The effects of fumigants differed slightly, depending on soil type and extraction or fumigation conditions. However, a single extraction does not appear to remove all
Figure 4.12 Ratios of total N in K$_2$SO$_4$ extracts of hexanol and chloroform fumigated samples during repeated fumigations and extractions.
Figure 4.13 Comparison of the effects of single and repeated fumigation on total N in K$_2$SO$_4$ extracts of three forest soils.
microbial biomass N and a second extraction is required. After a second extraction, the total N in the extract approached a constant value, indicating that the third and subsequent extractions removed non-microbial biomass N from the soil (Table 4.4).

Table 4.4 Comparison of total N (mg N kg$^{-1}$ soil) extracted by 0.5M K$_2$SO$_4$ from unfumigated and fumigated samples during third and further extractions.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment$^a$</th>
<th>Total N (mg N kg$^{-1}$ soil) during extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Third</td>
</tr>
<tr>
<td>------</td>
<td>----------------</td>
<td>--------</td>
</tr>
<tr>
<td>YP</td>
<td>UF Nil</td>
<td>4.8 (0.46)$^b$</td>
</tr>
<tr>
<td></td>
<td>HF Single</td>
<td>7.1 (0.40)</td>
</tr>
<tr>
<td></td>
<td>HF Repeated</td>
<td>4.9 (0.40)</td>
</tr>
<tr>
<td></td>
<td>CF Single</td>
<td>6.4 (0.18)</td>
</tr>
<tr>
<td></td>
<td>CF Repeated</td>
<td>6.0 (0.66)</td>
</tr>
<tr>
<td>RP</td>
<td>UF Nil</td>
<td>6.3 (0.48)</td>
</tr>
<tr>
<td></td>
<td>HF Single</td>
<td>8.7 (0.28)</td>
</tr>
<tr>
<td></td>
<td>HF Repeated</td>
<td>9.5 (0.20)</td>
</tr>
<tr>
<td></td>
<td>CF Single</td>
<td>9.5 (0.46)</td>
</tr>
<tr>
<td></td>
<td>CF Repeated</td>
<td>5.0 (1.07)</td>
</tr>
<tr>
<td>RE</td>
<td>UF Nil</td>
<td>15.4 (0.67)</td>
</tr>
<tr>
<td></td>
<td>HF Single</td>
<td>18.0 (0.08)</td>
</tr>
<tr>
<td></td>
<td>HF Repeated</td>
<td>23.5 (0.84)</td>
</tr>
<tr>
<td></td>
<td>CF Single</td>
<td>20.9 (0.27)</td>
</tr>
<tr>
<td></td>
<td>CF Repeated</td>
<td>19.1 (1.86)</td>
</tr>
</tbody>
</table>

$^a$ UF, HF and CF represent the unfumigation, hexanol fumigation and chloroform fumigation, respectively.

$^b$ SE of the mean value in parentheses.
4.4 Assessment of microbial N

Introduction

With the fumigation-extraction method, microbial biomass N is usually calculated as the difference between the amount of total N extracted by K$_2$SO$_4$ immediately after fumigation and the amount extracted from the unfumigated soil at the time fumigation commenced (Brookes et al., 1985b). Because K$_2$SO$_4$ extracts biomass N from unfumigated soil as shown in Fig. 4.9a, the calculation of microbial biomass N as defined above will be an underestimation (i.e. the 'control' or unfumigated value will be an overestimate of non-microbial N). In a recent study, Azam et al. (1989a) also showed that a significant amount (15 to 28%) of biomass N appeared in K$_2$SO$_4$ extracts of unfumigated soils. The following experiment was conducted to determine the effects of time of exposure to K$_2$SO$_4$ on the amount of microbial N extracted from unfumigated soils.

Experimental details

The YP, RP and RE soils were used. Two sets of field-moist soils (8 g each) were extracted with 40 ml of 0.5M K$_2$SO$_4$ for 0, 15, 30, 60, 120 and 240 min and then the mixtures were centrifuged for 5 min at 5000 rpm. The supernatants were removed and filtered (Whatman No. 1) and then the tubes reweighed. After the first extraction, K$_2$SO$_4$-saturated soils were either fumigated with hexanol (one set) or extracted with K$_2$SO$_4$ for further 60 min (second set). Simultaneously, a set of moist soils were fumigated with hexanol or chloroform for 24 h without any prior extraction with K$_2$SO$_4$. Unfumigated and fumigated soils were extracted with K$_2$SO$_4$. There were four replicates for each treatment combinations.
Results and Discussion

Figure 4.14 depicts the amounts of mineral N extracted from soils during first extraction up to 240 min, and from unfumigated and hexanol fumigated soils during a further 60 min second extraction. The duration of the first extraction had little effect on the amount of mineral N extracted (Fig. 4.14a), and during the second extraction of unfumigated soils (Fig. 4.14b), there was a small effect of the length of the first extraction period. Fumigation increased the amount of mineral N extracted irrespective of previous extraction treatments (Fig. 4.14c).

Increasing duration of exposure of soil to K₂SO₄ increased the amount of total N extracted (Fig. 4.15a). The increase was small in YP and RP soils, but more in the RE soil. The quantity of N removed during the second extraction of K₂SO₄-saturated unfumigated and fumigated soils showed a sharp drop due to the initial period during the first 15 min of extraction (Figs. 4.15b and c). Effects of longer initial extraction were minor. This indicates that K₂SO₄ attacks the biomass during the short period (15 min) of exposure and extracts some biomass N from the unfumigated soil. Widmer et al. (1989) also reported that the longer exposure of soil to K₂SO₄ increased the amount of total N extracted from unfumigated soil and they demonstrated this was derived from biomass N solubilized by K₂SO₄.

The effect of very short exposure (0-15 min) of soil to K₂SO₄ was further examined using the same soils and procedures, but this experiment was performed about three weeks later. The pattern of total N extracted from soil during the first extraction or from unfumigated and fumigated samples during a further 60 min extraction (Fig. 4.16) was very similar to that obtained in the previous study (Fig. 4.15). There was an increase in total N after a few minutes of extraction with K₂SO₄, particularly in the RE soil. During the second extraction, total N in the unfumigated
Figure 4.14 Quantities of mineral N extracted by 0.5M K$_2$SO$_4$ during first extraction up to 240 min (a), from unfumigated (b) and hexanol fumigated (c) samples during a further 60 min second extraction.
Figure 4.15 Quantities of total N extracted by 0.5M K₂SO₄ during first extraction up to 240 min (a), and unfumigated (b) and hexanol fumigated (c) samples during a further 60 min second extraction.
Figure 4.16 Quantities of total N extracted by 0.5M K$_2$SO$_4$ during first extraction up to 15 min (a), and unfumigated (b) and hexanol fumigated (c) samples during a further 60 min second extraction.
RE soil dropped sharply, indicating that the \( \text{K}_2\text{SO}_4 \) attacked biomass N very quickly. The effect of short period prior extraction on the amount of N extracted after fumigation were very marked for all three soils (Fig. 4.16c). The total N extracted after 15 min was much less in the second experiment, probably because of the effects of soil storage for three weeks.

**Conclusions**

The above experiments show that \( \text{K}_2\text{SO}_4 \) extracts a large amount of organic N (Tables 4.5) which varies with soils and extraction conditions (i.e. duration of extraction), and that part of the organic N is of microbial origin in unfumigated soils. Thus it is important to determine the amount of non-microbial N in unfumigated soils and the following experiments were performed to develop a way of determining this.

Table 4.5 Quantities (mg N kg\(^{-1}\) soil) of organic N extracted by 0.5M \( \text{K}_2\text{SO}_4 \) during first extraction up to 240 min (a), and from unfumigated (b) and hexanol fumigated (c) samples during a further 60 min second extraction.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Duration of extraction (min)</th>
<th>a) During first extraction</th>
<th>b) Unfumigated soils during a further 60 min extraction</th>
<th>c) Hexanol fumigated soils during a further 60 min extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>YP</td>
<td>10.2 (1.55)(^a)</td>
<td>9.0 (0.98)</td>
<td>8.5 (0.32)</td>
<td>8.0 (0.91)</td>
</tr>
<tr>
<td>RP</td>
<td>10.6 (0.67)</td>
<td>14.6 (0.83)</td>
<td>10.9 (1.47)</td>
<td>14.3 (0.95)</td>
</tr>
<tr>
<td>RE</td>
<td>28.9 (0.46)</td>
<td>31.3 (0.87)</td>
<td>31.8 (1.27)</td>
<td>36.7 (1.16)</td>
</tr>
</tbody>
</table>

\(^a\) SE of the mean value in parentheses.
4.5 Assessment of non-microbial and microbial N in $K_2SO_4$-extracts of unfumigated and fumigated soils

A previous study (section 4.3) showed that a single fumigation was as good as multiple fumigation. However, a single extraction was not sufficient to extract all microbial N released by fumigation. The following approach was used to separate the total N extracted in 0.5M $K_2SO_4$ from both unfumigated and fumigated soils into microbial and non-microbial components. If the soil is extracted sequentially after fumigation, the first few extractions will contain both microbial and non-microbial N, but subsequent extracts will contain a relatively constant amount of non-microbial N.

Figure 4.17 shows the cumulative amount of total N extracted sequentially with $K_2SO_4$ from three unfumigated soils. Extraction of N was approximately linear between 1 and 8 extractions. After 5 extractions the rate of N solubilization declined slightly, but a linear regression accounted for >99% of the variation for all soils (Table 4.6). Clearly the first extraction (shown as a dotted line in Fig. 4.17) removes both microbial and non-microbial N. The intercepts for the YP and RP soils were comparable (6.52 versus 6.40), but the slope was slightly lower for the YP soil. Both the intercept and the slope were much higher for the RE soil. If it is assumed that the extraction of non-microbial N is relatively constant as suggested by the equations given in Table 4.6, then the microbial component of the total N removed in the first extraction of unfumigated soil is equal to the intercept in these equations. An underlying assumption in this approach is that the extraction of non-microbial N remains relatively constant for all extractions performed. This then allows the microbial component of the total N removed in the first extraction of unfumigated soil to be calculated from splitting the curve. Previous experiments (see Figs. 4.15 and 16) also indicated that microbial N is mostly extracted from unfumigated soils during the initial extractions.
Figure 4.17 Cumulative total N extracted sequentially with 0.5M K$_2$SO$_4$ from three unfumigated forest soils.
Table 4.6 Regressions of cumulative total N extracted by 0.5M K$_2$SO$_4$ from unfumigated soils during sequential (eighth times) extractions.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Regression line ( y = a + b x^* )</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>YP</td>
<td>( y = 6.5 + 5.06 x )</td>
<td>0.998</td>
</tr>
<tr>
<td>RP</td>
<td>( y = 6.4 + 6.33 x )</td>
<td>0.998</td>
</tr>
<tr>
<td>RE</td>
<td>( y = 18.9 + 12.23 x )</td>
<td>0.991</td>
</tr>
</tbody>
</table>

* \( y = \) Total N (mg N kg$^{-1}$ soil) in K$_2$SO$_4$-extracts of unfumigated soils; \( x = \) Number of extractions; \( a = \) Intercepts, and \( b = \) Slope of the regression line.

Similarly, cumulative total N in the K$_2$SO$_4$-extracts of hexanol and chloroform fumigated soil samples are shown in Fig. 4.18. During the first three extractions, the total N extracted showed a curvilinear relationship, while from the third extraction the relationships were linear. This indicates that the first three extractions contained both microbial and non-microbial fraction of N, and that subsequent extracts contained only non-microbial N. It was assumed that the fumigation was complete, but that three extractions were required to remove all the microbial N. Thus linear regressions for the fumigated samples were calculated from third to eighth extraction and are presented in Table 4.7. Slopes and intercepts were very similar for the two fumigants, but varied among the soils. The slopes of these lines are very similar to those obtained using unfumigated soils (Table 4.6) indicating that K$_2$SO$_4$ removes similar amounts of non-microbial N from both unfumigated and fumigated soils. The intercepts of the equations in Table 4.7 represent the amount of extracted N derived from microbial sources (i.e. about 32, 41 and 96 mg N kg$^{-1}$ soil when averaged for the two fumigants for the YP, RP and RE soils, respectively).

It is possible to calculate the amount of microbial N contained in each sequential extract by subtracting the non-microbial N from the measured total N. These are shown in Table 4.8 and Fig. 4.19. During the first extraction, a large
Figure 4.18 Cumulative total N extracted sequentially with 0.5M K$_2$SO$_4$ from (a) hexanol and (b) chloroform fumigated soils.
Figure 4.19  Microbial N (mg N kg\(^{-1}\) soil) extracted with 0.5M K\(_2\)SO\(_4\) from (a) hexanol and (b) chloroform fumigated soils during single fumigation and sequential extraction.
Table 4.7  Regressions of cumulative total N extracted by 0.5M K₂SO₄ from fumigated soils during single fumigation and sequential (third to eighth) extractions.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Regression line</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>y = a + b x*</td>
<td></td>
</tr>
<tr>
<td>a) Hexanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP</td>
<td>y = 33.2 + 4.26 x</td>
<td>0.991</td>
</tr>
<tr>
<td>RP</td>
<td>y = 44.9 + 6.65 x</td>
<td>0.993</td>
</tr>
<tr>
<td>RE</td>
<td>y = 95.8 + 12.91 x</td>
<td>0.998</td>
</tr>
<tr>
<td>b) Chloroform</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP</td>
<td>y = 30.5 + 3.74 x</td>
<td>0.973</td>
</tr>
<tr>
<td>RP</td>
<td>y = 37.2 + 5.99 x</td>
<td>0.995</td>
</tr>
<tr>
<td>RE</td>
<td>y = 95.6 + 11.82 x</td>
<td>0.994</td>
</tr>
</tbody>
</table>

* see Table 4.6

amount of microbial N was extracted from all soils and this then decreased substantially during the second and subsequent extractions, and very little microbial N was detected in the fourth and subsequent extractions (Fig. 4.19). The pattern of microbial N release from the fumigated soils was very similar for the hexanol and chloroform fumigants.

Table 4.8  Estimated microbial N (mg N kg⁻¹ soil) from single extraction based on correction for non-microbial N in K₂SO₄-extracts.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Extractable total N</th>
<th>Non-microbial N (using regression)</th>
<th>Microbial N</th>
<th>Microbial N as % of extractable total N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg N kg⁻¹ soil</td>
<td>mg N kg⁻¹ soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Hexanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP</td>
<td>24.91</td>
<td>4.26</td>
<td>20.65</td>
<td>82.9</td>
</tr>
<tr>
<td>RP</td>
<td>39.77</td>
<td>6.65</td>
<td>33.12</td>
<td>83.3</td>
</tr>
<tr>
<td>RE</td>
<td>91.60</td>
<td>12.91</td>
<td>78.69</td>
<td>85.9</td>
</tr>
<tr>
<td>b) Chloroform</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP</td>
<td>28.51</td>
<td>3.74</td>
<td>24.77</td>
<td>86.9</td>
</tr>
<tr>
<td>RP</td>
<td>42.64</td>
<td>5.99</td>
<td>36.65</td>
<td>85.9</td>
</tr>
<tr>
<td>RE</td>
<td>84.34</td>
<td>11.82</td>
<td>75.52</td>
<td>86.0</td>
</tr>
</tbody>
</table>
The cumulative total N extracted sequentially after multiple fumigated soils are shown in Fig. 4.20. Applying similar procedures as just described to these soils yielded the estimates of microbial N (equivalent to the intercept of the equation) shown in Table 4.9. The amount of microbial N removed after each fumigation and extraction are shown in Fig. 4.21. The results from this analysis are essentially the same as after a single fumigation followed by multiple extractions.

Table 4.9 Regressions of cumulative total N extracted by K₂SO₄ from fumigated soils during repeated fumigations (third to fifth) and extractions.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Regression line y = a + b x*</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Hexanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP</td>
<td>y = 26.0 + 3.62 x</td>
<td>0.997</td>
</tr>
<tr>
<td>RP</td>
<td>y = 42.9 + 6.94 x</td>
<td>0.996</td>
</tr>
<tr>
<td>RE</td>
<td>y = 100.6 + 14.58 x</td>
<td>0.998</td>
</tr>
<tr>
<td>b) Chloroform</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP</td>
<td>y = 24.2 + 5.09 x</td>
<td>0.998</td>
</tr>
<tr>
<td>RP</td>
<td>y = 33.9 + 6.55 x</td>
<td>0.998</td>
</tr>
<tr>
<td>RE</td>
<td>y = 90.9 + 13.63 x</td>
<td>0.999</td>
</tr>
</tbody>
</table>

* see Table 4.6

4.6 Calculation of soil microbial N

Usually microbial biomass N in soil is calculated (when using the fumigation-extraction method) as the difference in total N extracted by K₂SO₄ from fumigated and unfumigated soils (Brookes et al., 1985b). This method, however, will lead to underestimation of biomass N, because some of the organic N in the K₂SO₄ extracts of unfumigated soils is derived from biomass sources (Azam et al., 1989a). An alternative approach for estimating microbial N is to estimate the amount of non-microbial N extracted from fumigated soil as described above. The non-microbial N is derived from both inorganic N initially present, and organic N solubilized by the
Figure 4.20 Cumulative total N extracted with 0.5M K$_2$SO$_4$ after repeated fumigation with (a) hexanol and (b) chloroform.
Figure 4.21 Microbial N (mg N kg$^{-1}$ soil) extracted with 0.5M K$_2$SO$_4$ after repeated fumigation with (a) hexanol and (b) chloroform.
extractant. The latter is a feature of the soil, and the former can be extracted from an unfumigated 'control'.

Microbial N in soil was calculated in the following ways:

1) using the difference method of Brookes et al. (1985b),
2) correcting for non-microbial N in the fumigated soil using the regression approach developed above, and
3) subtracting the mineral N in unfumigated soil from the total N obtained in a single K₂SO₄ extract of a single fumigation.

A summary of data obtained using different calculation methods for microbial N is shown in Table 4.10. The most theoretically acceptable values are those obtained by method 2 above and these were the highest, but not greatly different to those obtained by method 3. These can be corrected to account for effects of incomplete extraction (Table 4.11). The microbial N values determined using method 1 are considerable underestimates and need much correction (Table 4.11). Method 2 whilst theoretically the best is rather complicated and impractical for routine use. Thus the use of method 3 is practical and an experimentally determined correction factor for each soil can be applied so that:

Microbial N = (Total N in once fumigated and extracted soil minus mineral N in unfumigated soil) x factor to account for incomplete extraction of microbial N and contribution of non-microbial N.

Conclusions

Subtracting the total N in extracts of unfumigated soils from fumigated soils gives appreciably lower (i.e. 50 to 80%) estimates of microbial N than when only the
Table 4.10 Comparison of estimates of microbial N (mg N kg\(^{-1}\) soil) in different soils calculated using three different methods.

<table>
<thead>
<tr>
<th>Method of Calculation(^a)</th>
<th>Soil</th>
<th>YP</th>
<th>RP</th>
<th>RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Hexanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curve-split</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29.6</td>
<td>43.9</td>
<td>98.2</td>
<td></td>
</tr>
<tr>
<td>(F_T - UF_M)</td>
<td>24.3 (1.79)(^b) [82](^c)</td>
<td>39.1 (0.58) [89]</td>
<td>84.7 (2.25) [86]</td>
<td></td>
</tr>
<tr>
<td>(F_T - UF_T)</td>
<td>16.4 (0.88) [55]</td>
<td>28.3 (1.27) [64]</td>
<td>59.8 (2.59) [61]</td>
<td></td>
</tr>
<tr>
<td>b) Chloroform</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curve-split</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.3</td>
<td>35.6</td>
<td>93.2</td>
<td></td>
</tr>
<tr>
<td>(F_T - UF_M)</td>
<td>27.3 (0.76) [100]</td>
<td>35.6 (0.82) [100]</td>
<td>76.1 (1.66) [82]</td>
<td></td>
</tr>
<tr>
<td>(F_T - UF_T)</td>
<td>17.2 (0.92) [63]</td>
<td>24.5 (1.23) [69]</td>
<td>51.2 (1.17) [55]</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Microbial N by curve-splitting calculated as mean of intercepts in Tables 4.7 and 4.9. Microbial N by \(F_T - UF_M\) (i.e. total N in fumigated soil - mineral N in unfumigated soil) calculated from single fumigation and single extraction (mean of two studies, i.e. 8 and 5 extractions). Microbial N by \(F_T - UF_T\) (i.e. total N in fumigated soil - total N in unfumigated soil) calculated from single fumigation and single extraction (mean of two studies, i.e. 8 and 5 extractions).

\(^b\) SE of the mean values

\(^c\) Values are expressed as percentages of values obtained after curve-splitting.

Table 4.11 Correction factor for calculating soil microbial N.

<table>
<thead>
<tr>
<th>Calculation method(^a)</th>
<th>Soil</th>
<th>Hexanol</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(F_T - UF_M)</td>
<td></td>
<td>(F_T - UF_T)</td>
<td>(F_T - UF_M)</td>
</tr>
<tr>
<td>YP</td>
<td>1.22</td>
<td>1.81</td>
<td>1.00</td>
</tr>
<tr>
<td>RP</td>
<td>1.12</td>
<td>1.55</td>
<td>1.00</td>
</tr>
<tr>
<td>RE</td>
<td>1.16</td>
<td>1.64</td>
<td>1.23</td>
</tr>
</tbody>
</table>

\(^a\) see Table 4.10
mineral N in the unfumigated soils is subtracted. The latter approach still requires use of a correction factor of <20% (Table 4.11), but has a better theoretical basis than the method of Brookes. For each soil, the correction factors established and given in Table 4.11 were used in further studies to convert N flushes after fumigation to microbial N contents.

In the following section, microbial N was estimated using the above correction factors for a range of soils, sampling times and fumigation procedures. The relationships between the estimates were determined by linear regression analysis.

4.7 Relationships between the estimates of microbial N based on three fumigation-extraction methods

Estimates of microbial N in a range of acid forest soils were obtained using 1-day hexanol, 1-day and 5-day chloroform fumigation-extraction methods. Soil samples were collected on different occasions, e.g. June and September 1986, and April and October 1987. All soils were used in the earlier experiments (see section 4.1). Concentrations of microbial N in soil were calculated by the method (Method 3) as given in the previous section. The assumption was made that the correction factors applied to soils collected in different seasons and that it also applied to 5-day chloroform fumigation (although derived using the 1-day fumigation). However, the correction factors were determined on the basis of 1-day hexanol and chloroform fumigations which may not be applicable for 5-day chloroform fumigation.

Firstly, the coefficient of determination ($r^2$) between the fumigation-extraction methods was tested on the basis of time of soil sampling and the results are presented in Table 4.12. There were highly significant relationships between the 1-day hexanol and 5-day chloroform fumigation-extraction methods at all sampling
Table 4.12 Seasonal relationships ($r^2$)* between estimates of microbial N (mg N kg$^{-1}$ soil) measured by the three different fumigation-extraction procedures of a range of acid forest soils. The soil moisture contents varied from 14.9 to 27.9% for June 1986, 10.0 to 45.1% for September 1986, 10.0 to 29.8% for April 1987, and 10.4 to 41.4% for October 1987 samples.

<table>
<thead>
<tr>
<th>Fumigation-extraction</th>
<th>Month of sample collection during 1986-1987</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June (winter)</td>
</tr>
<tr>
<td></td>
<td>(n = 18)</td>
</tr>
<tr>
<td>1-d Hexanol</td>
<td>1-d Chloroform</td>
</tr>
<tr>
<td>1-d Hexanol</td>
<td>5-d Chloroform</td>
</tr>
<tr>
<td>5-d Chloroform</td>
<td>1-d Chloroform</td>
</tr>
</tbody>
</table>

* All $r^2$ are significant at 0.1% level; ND - not determined.

The relationships between the 1-day hexanol and 1-day chloroform, and between 1-day and 5-day chloroform methods were better for the winter 1986 samples than the spring 1986 samples.

When data for all soil samples and seasons were combined, the relationships between 1-day hexanol and 1-day chloroform, and between 1-day and 5-day chloroform fumigation-extraction methods were relatively poor (Figs. 4.22a and b). By contrast, the relationship between 1-day hexanol and 5-day chloroform methods was strong and linear ($r^2 = 0.86$) (Fig. 4.22c). The better agreement between 1-day hexanol and 5-day chloroform estimates may be partly because of the larger number of soils used and the wider range of the data. When the 1-day and 5-day chloroform fumigation data were pooled, these were well correlated with 1-day hexanol microbial N estimates (Fig. 4.23).

Conclusions

Good agreement between the hexanol and chloroform fumigation-extraction estimates of soil microbial N (Fig. 4.23) indicate that any of the methods could be
Figure 4.22 Relationships between soil microbial N (mg N kg$^{-1}$ soil) estimated using three different fumigation-extraction methods.

(a) $y = 5.0 + 0.98x$, $r^2 = 0.44$, n=46

(b) $y = 8.5 + 0.61x$, $r^2 = 0.46$, n=46

(c) $y = 12.3 + 0.87x$, $r^2 = 0.86$, n=86
Figure 4.23 Relationships between microbial N estimates based on hexanol and chloroform fumigation. The pooled regression equation is $y = 8.9 + 0.89 \times$ ($r^2 = 0.84, P<0.001; n=132.$)
used. The microbial N values may not be the real biomass N values. It is thus still unknown whether the use of a $k_N$-factor is required to convert microbial N to biomass N estimates. Therefore the estimate is considered as biomass N index, and simply used in all further cases as 'microbial N'.

Soil microbial biomass N was also estimated using the chloroform fumigation-incubation (or FI) method in a range of acid forest soils. This method, its evaluation, and comparison of FI-N flush (essentially microbial biomass N) estimates with those obtained using fumigation-extraction methods are described in the following chapter.
CHAPTER FIVE
CHAPTER FIVE

MEASUREMENT OF MINERAL-N FLUSH (FI-N FLUSH) BY THE FUMIGATION-INCUBATION METHOD, AND ITS COMPARISON WITH MICROBIAL N OBTAINED BY THE FUMIGATION-EXTRACTION PROCEDURES

5.1 Effects of duration of aerobic incubation on the mineralization of soil N in unfumigated and fumigated samples, and on the FI-N flush values

5.1.1 Experimental details
5.1.2 Results and Discussion
   (a) Effects of duration of incubation on N mineralization in unfumigated and chloroform-fumigated soils
   (b) Effects of duration of incubation on the FI-N flush values

5.2 Relationship between microbial N estimated by fumigation-extraction and fumigation-incubation procedures

5.2.1 Introduction
5.2.2 Materials and Methods
5.2.3 Results and Discussion
   (a) Comparison between fumigation-extraction and fumigation-incubation methods
   (b) Ratios of FI-N flush-to-microbial N

5.3 General conclusions from studies reported in this chapter
CHAPTER FIVE

MEASUREMENT OF MINERAL-N FLUSH (FI-N FLUSH) BY THE FUMIGATION-INCUBATION METHOD, AND ITS COMPARISON WITH MICROBIAL N OBTAINED BY THE FUMIGATION-EXTRACTION PROCEDURES

Introduction

The chloroform fumigation-incubation (FI) method has been used extensively for soil microbial biomass N determination. This method yields unreliable results for soils which have received a large inputs of fresh organic substrate, or soils rewetted prior to fumigation-incubation or recently sampled or strongly acid soils of pH <4.5 (Jenkinson and Powlson, 1976b; Vance and Brookes, 1987; Jenkinson, 1988). The FI method fails in such situations because of immobilization of mineral N due to the presence of microorganisms with a high C-to-N ratio, or incomplete mineralization of soil N in dry conditions or mineralization of N in the unfumigated samples sometimes exceeds the amount of N mineralized in the fumigated samples of acid soils (Jenkinson et al., 1979; Williams and Sparling, 1984).

Net mineralization of N in the chloroform-fumigated soil is high (Paul and Voroney, 1984; Diaz-Ravina et al., 1989); in some cases more than five times that of unfumigated soil (Jenkinson and Powlson, 1976a; Shen et al., 1984). This is because freshly killed biomass is more susceptible to decomposition than non-biomass organic matter (Amato and Ladd, 1980; Marumoto, 1984; Marumoto et al., 1982b). Anderson and Domsch (1978a) reported that both the chloroform-fumigated and unfumigated dead fungal materials mineralized to the same extent in soil during a 10-day incubation.
It was considered important to establish the optimum period of aerobic incubation for acid (pH <5.0 in KCl; see Table 3.2) forest soils. The FI method was applied to a range of acid forest soils differing in pH, organic matter content and fertilizer history. Mineralization of N in unfumigated and chloroform-fumigated soils was carried out during 10 day, 20 day or longer periods. Finally, the amount of FI-N flush obtained was compared with the microbial N (Chapter 4) estimated by the different fumigation-extraction methods.

5.1 Effects of duration of aerobic incubation on the mineralization of soil N in unfumigated and fumigated samples, and on the FI-N flush values

5.1.1 Experimental details

Soils (0 - 5 cm) used in the experiments were yellow podzolic (YP), red podzolic (RP) and red earth (RE) sampled in October 1987. The field experiments which applied on RE soil, and the procedure for sample collection have been described in Chapters 3 and 4.

Field moist soils (10 g) were fumigated with purified chloroform for 24 h, as described in Chapter 3. After removal of chloroform vapour by repeated evacuation, fumigated soils were inoculated with 1 g of fresh moist soil and mixed thoroughly. The glass bottles containing unfumigated and fumigated samples were then covered with thin polythene film (which had a few holes to allow aeration) and incubated aerobically for either 10, 20, 40 or 60 days at 25 °C. The small amount of water lost during fumigation and subsequent removal of chloroform was not replaced. After each incubation, soils were extracted by shaking with 50 ml 0.5M K₂SO₄ for 1 h followed by filtration (Whatman No. 1). Soil extracts were kept at 4 °C for a few days prior to analysis of inorganic N.
The Fl-N flush was calculated as the difference between the N mineralized in the fumigated and the unfumigated soils (see Chapter 3, section 3.6.2). The results represent the mean of four replicates, and are expressed on an oven-dry soil basis.

5.1.2 Results and Discussion

(a) Effects of duration of incubation on N mineralization in unfumigated and chloroform-fumigated soils

Figure 5.1 summarises the results for aerobic incubations up to 60 days. A substantial amount of N was mineralized in the unfumigated soils and fumigation markedly increased mineralization. In all soils, the extractable mineral N values in the unfumigated samples during incubation were relatively lower than those in the fumigated samples. A similar observation was also reported by Jenkinson and Powlson (1976a), Shen et al. (1984) and Diaz-Ravina et al. (1989). However, extending the incubation from 10 to 60 days did not increase mineralization in both unfumigated and fumigated soils, and sometimes decreased it. The reason is difficult to explain, but could be related to the reduction in NO\textsubscript{3}-N due to denitrification or microbial immobilization of mineral N (Table 5.1). Gaseous losses of N during incubation following fumigation is also very common (see Jenkinson and Powlson, 1976b; Nannipieri et al., 1990a) and many workers (e.g. Adams and Laughlin, 1981; Nannipieri, 1984, Voroney and Paul, 1984; Nicolardot et al., 1986) attributed decreased N mineralization in the fumigated soils to the microbial immobilization of mineral N. Jenkinson and Powlson (1976b) attributed the reduction in NO\textsubscript{3}-N content in the incubated soils after fumigation to the loss of NO\textsubscript{3}-N through denitrification during or after fumigation. Immobilization of mineral N in the fumigated sample may occur in soils with a high C-to-N ratios of the microorganisms because of the release of decomposable organic substances by fumigation which are otherwise beyond microbial attack (Jenkinson, 1976; Nannipieri et al., 1985;
Figure 5.1 Mineral N (mg N kg\(^{-1}\) soil) extracted by 0.5M \(\text{K}_2\text{SO}_4\) from unfumigated and fumigated samples of untreated YP and RP soils, and variously treated RE soil during 60 days of aerobic incubation. Bars represent the SE of the mean.
Table 5.1 Distribution of mineral N (mg N kg\(^{-1}\) soil) in the unfumigated and chloroform-fumigated samples of untreated YP and RP soils and variously treated RE soil during 60 days of aerobic incubation.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Before incubation</th>
<th>Unfumigated</th>
<th>After incubation</th>
<th>Fumigated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 days</td>
<td>20 days</td>
<td>40 days</td>
</tr>
<tr>
<td>YP</td>
<td>NH(_4)-N</td>
<td>2.3 (0.4) (^a)</td>
<td>4.2 (1.1)</td>
<td>2.7 (0.6)</td>
</tr>
<tr>
<td></td>
<td>NO(_3)-N</td>
<td>1.2 (0.2)</td>
<td>2.2 (0.3)</td>
<td>2.7 (0.2)</td>
</tr>
<tr>
<td>RP</td>
<td>NH(_4)-N</td>
<td>1.6 (0.3)</td>
<td>3.1 (0.4)</td>
<td>3.3 (0.5)</td>
</tr>
<tr>
<td></td>
<td>NO(_3)-N</td>
<td>1.5 (0.2)</td>
<td>2.2 (0.6)</td>
<td>3.4 (0.4)</td>
</tr>
<tr>
<td>RE(C)</td>
<td>NH(_4)-N</td>
<td>3.8 (0.5)</td>
<td>6.2 (0.6)</td>
<td>7.6 (0.8)</td>
</tr>
<tr>
<td></td>
<td>NO(_3)-N</td>
<td>1.8 (0.1)</td>
<td>5.6 (0.7)</td>
<td>5.4 (0.5)</td>
</tr>
<tr>
<td>RE(L+P)</td>
<td>NH(_4)-N</td>
<td>8.6 (1.8)</td>
<td>12.1 (2.4)</td>
<td>12.2 (1.7)</td>
</tr>
<tr>
<td></td>
<td>NO(_3)-N</td>
<td>1.7 (0.2)</td>
<td>8.3 (1.5)</td>
<td>5.5 (0.7)</td>
</tr>
<tr>
<td>RE(N)</td>
<td>NH(_4)-N</td>
<td>28.3 (4.6)</td>
<td>35.5 (4.3)</td>
<td>36.0 (4.0)</td>
</tr>
<tr>
<td></td>
<td>NO(_3)-N</td>
<td>0.5 (0.2)</td>
<td>16.4 (1.6)</td>
<td>10.4 (1.3)</td>
</tr>
<tr>
<td>RE(N+P)</td>
<td>NH(_4)-N</td>
<td>29.4 (2.9)</td>
<td>34.5 (2.6)</td>
<td>35.2 (2.1)</td>
</tr>
<tr>
<td></td>
<td>NO(_3)-N</td>
<td>0.1 (0.1)</td>
<td>13.9 (0.9)</td>
<td>9.7 (0.9)</td>
</tr>
</tbody>
</table>

\(^a\) SE of the mean in parentheses.
ND - Not detectable
Azam et al., 1986). Brookes et al. (1985a) showed that either denitrification or immobilization of N by the soil microorganisms during incubation could mask the fumigant induced release of N.

Generally, higher amounts of mineral N were extracted from unfumigated and fumigated soils after 10 or 20 days of incubation. Williams and Sparling (1984) reported that the amounts of mineral N extracted from unfumigated and fumigated samples of three peats and a mineral soil was higher during the 5 to 12 days. Overall, mineralization of N was highest in the RE soils, irrespective of treatment, lower in RP soil and lowest in YP soil.

Similar patterns of N mineralization were also observed for other forest soils sampled on different times. These data were not included here because of the repetition.

In conclusion, longer incubation did not increase N mineralization in both unfumigated and fumigated soils. Generally, higher amounts of N were extracted after 10 or 20 days of aerobic incubation.

(b) Effects of duration of incubation on the Fl-N flush values

Figure 5.2 depicts the amounts of Fl-N flush in the unfertilized YP and RP soils and variously treated RE soil. In the YP and RP soils, the significantly higher Fl-N flush was observed during 10 days of incubation. The Fl-N flushes were decreased by 45 and 42% for the YP and RP soils, respectively, after 20 days of incubation compared to those obtained after 10 days. The RE(C) soil had slightly higher Fl-N flush values after 10 days than after the other periods, and the difference was not significant with that obtained after 20 days. In the plot which received lime and phosphorus (i.e. L+P treatment), the amount of Fl-N flush was very similar
Figure 5.2 Values (mg N kg\(^{-1}\) soil) of Fl-N flush of untreated YP and RP soils, and variously treated RE soil.
during the incubation, although the value after 40 days did not significantly differ from
the other periods. By contrast, the values in the N and N+P soils were maximum
after 20 days, but the values were not significantly different than the 10-day values.
At the end of the incubation, the FI-N flush values were declined by 25, 4, and 36%
in control (C), L+P and N+P treated soils, respectively, while the FI-N flush value was
increased by 23% in the N-fertilized (N) plot. Despite the different soil properties and
pH, these three soils (i.e. YP, RP and RE) behaved similarly. Of the three soils, the
RE soil had the highest values, irrespective of treatment, and the YP the least. A
similar trend was also found for other forest soils sampled on different occasions.
Again these results were not included because of repetition.

In conclusion, the results show that duration of incubation did change the level
of FI-N flush. Generally, higher values were observed after 10 days of incubation
than after the other periods.

The following section examines the relationship between the estimates of soil
microbial N obtained by the fumigation-extraction methods using both hexanol and
chloroform, and FI-N flush, as measured by the chloroform fumigation-incubation (FI)
method in a range of acid forest soils.

5.2 Relationships between microbial N estimated by fumigation-extraction and
fumigation-incubation procedures

5.2.1 Introduction

Soil microbial N estimated after the fumigation-extraction (FE) procedure is
often calibrated against the widely used fumigation-incubation (FI) method. The
agreement between these two methods is variable, ranging from good for arable
soils (Brookes et al., 1985b) and some forest soils (Davidson et al., 1989) to very poor for other groups of forest (Brookes et al., 1985d; Gallardo and Schlesinger, 1990) or grassland soils (Davidson et al., 1989). Azam et al. (1989b) determined soil microbial N in several agricultural soils by using chloroform fumigation-extraction and a modification of this method where soils are extracted with 0.5M K₂SO₄ containing different amounts of chloroform [the method is proposed by Azam et al. (1989a) and is termed as chloroform-extraction method or CEM] and found a highly significant correlation between these two methods. Recently, Martikainen and Palojarvi (1990) calibrated fumigation-extraction procedure with microscopic counting technique for the determination of microbial N in a range of forest soils and found a close correlation between these two methods. Moreover, microbial N from fumigation-extraction was well correlated with biomass C and soil ATP content (Brookes et al., 1985d; Ocio and Brookes, 1990a).

The following section compares microbial N in acid forest soils estimated by different fumigation-extraction methods (Chapter 4) and the F1-N flush measured by the chloroform fumigation-incubation method (section 5.1).

5.2.2 Materials and Methods

Soils (0 - 5 cm) used in this study were sampled on four occasions: June and September 1986, and April and October 1987. The field treatments of the YP, duplex and RE soils have been described in Chapter 3. Sample collection and analytical procedures were the same as described earlier (Chapters 3 and 4).
5.2.3 Results and Discussion

(a) Comparison between fumigation-extraction and fumigation-incubation methods

The amounts of microbial N obtained after 1-day hexanol, 1-day and 5-day chloroform fumigations followed by immediate extraction were always greater than the amounts of Fl-N flush obtained by chloroform fumigation-incubation (Table 5.2).

Table 5.2 Values (mg N kg⁻¹ soil) of microbial N and Fl-N flush for a range of acid forest soils.

<table>
<thead>
<tr>
<th>Soils</th>
<th>Month of collection</th>
<th>Microbial N by fumigation-extractions</th>
<th>Fl-N flush</th>
<th>Biomass N⁡¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-day Hexanol 1-day CHCl₃ 5-day CHCl₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YP(C)</td>
<td>Jun, 1986</td>
<td>88.8 (2.1) 58.6 (4.2) 51.2 (0.6)</td>
<td>19.7 (1.6)</td>
<td>34.6 (2.7)</td>
</tr>
<tr>
<td>YP(IL)</td>
<td>Jun, 1986</td>
<td>37.7 (1.3) 22.0 (1.5) 29.1 (5.1)</td>
<td>4.5 (0.8)</td>
<td>7.9 (1.3)</td>
</tr>
<tr>
<td>RP</td>
<td>Jun, 1986</td>
<td>51.0 (0.1) 32.9 (0.6) 35.1 (1.7)</td>
<td>17.6 (1.6)</td>
<td>31.2 (2.7)</td>
</tr>
<tr>
<td>DR1</td>
<td>Jun, 1986</td>
<td>46.3 (3.4) 30.7 (3.3) 32.2 (0.5)</td>
<td>21.4 (0.2)</td>
<td>37.6 (0.4)</td>
</tr>
<tr>
<td>DR2</td>
<td>Jun, 1986</td>
<td>45.5 (0.8) 34.3 (0.5) 34.2 (0.7)</td>
<td>18.9 (0.5)</td>
<td>33.2 (0.8)</td>
</tr>
<tr>
<td>DR3</td>
<td>Jun, 1986</td>
<td>50.7 (3.6) 36.6 (0.4) 37.9 (1.9)</td>
<td>24.0 (0.9)</td>
<td>42.0 (1.5)</td>
</tr>
<tr>
<td>DF1</td>
<td>Jun, 1986</td>
<td>59.4 (1.0) 42.6 (0.1) 42.1 (1.1)</td>
<td>24.3 (0.8)</td>
<td>42.7 (1.4)</td>
</tr>
<tr>
<td>DF2</td>
<td>Jun, 1986</td>
<td>66.5 (0.5) 42.6 (1.2) 47.6 (0.1)</td>
<td>29.3 (1.8)</td>
<td>52.3 (3.1)</td>
</tr>
<tr>
<td>YP(C)</td>
<td>Sep, 1986</td>
<td>34.8 (5.6) 45.3 (5.6) 27.3 (3.6)</td>
<td>12.8 (2.7)</td>
<td>22.5 (4.7)</td>
</tr>
<tr>
<td>YP(IL)</td>
<td>Sep, 1986</td>
<td>24.2 (3.8) 32.0 (2.9) 18.0 (4.2)</td>
<td>5.8 (1.4)</td>
<td>10.2 (2.4)</td>
</tr>
<tr>
<td>YP(IF)</td>
<td>Sep, 1986</td>
<td>19.3 (2.2) 26.4 (2.8) 17.9 (1.5)</td>
<td>3.8 (1.4)</td>
<td>6.6 (2.4)</td>
</tr>
<tr>
<td>YP(I)</td>
<td>Sep, 1986</td>
<td>24.1 (0.8) 31.9 (0.6) 25.0 (0.4)</td>
<td>6.7 (1.0)</td>
<td>11.8 (1.8)</td>
</tr>
<tr>
<td>YP(F)</td>
<td>Sep, 1986</td>
<td>27.3 (2.9) 29.9 (1.6) 21.2 (1.6)</td>
<td>7.5 (1.2)</td>
<td>13.1 (2.1)</td>
</tr>
<tr>
<td>YP(S)</td>
<td>Sep, 1986</td>
<td>58.6 (6.9) 64.0 (7.8) 44.2 (4.9)</td>
<td>19.2 (5.5)</td>
<td>33.6 (9.6)</td>
</tr>
<tr>
<td>RP</td>
<td>Sep, 1986</td>
<td>60.7 (4.4) 44.6 (3.4) 42.0 (4.8)</td>
<td>24.5 (4.5)</td>
<td>42.9 (7.8)</td>
</tr>
<tr>
<td>YP(C)</td>
<td>Apr, 1987</td>
<td>32.3 (0.7) ND 28.9 (1.6)</td>
<td>8.8 (1.1)</td>
<td>15.4 (1.9)</td>
</tr>
<tr>
<td>RP</td>
<td>Apr, 1987</td>
<td>45.4 (1.4) ND 46.1 (2.5)</td>
<td>10.6 (0.7)</td>
<td>18.6 (1.2)</td>
</tr>
<tr>
<td>RE(C)</td>
<td>Apr, 1987</td>
<td>142.2 (3.1) ND 138.2 (9.3)</td>
<td>34.5 (2.9)</td>
<td>60.6 (5.1)</td>
</tr>
<tr>
<td>YP(C)</td>
<td>Oct, 1987</td>
<td>29.3 (2.2) ND 30.0 (1.8)</td>
<td>7.1 (1.0)</td>
<td>12.4 (1.8)</td>
</tr>
<tr>
<td>RP</td>
<td>Oct, 1987</td>
<td>58.1 (5.7) ND 53.9 (4.9)</td>
<td>26.5 (2.6)</td>
<td>46.4 (6.3)</td>
</tr>
<tr>
<td>RE(C)</td>
<td>Oct, 1987</td>
<td>116.7 (11.7) ND 120.7 (4.0)</td>
<td>58.9 (5.2)</td>
<td>103.4 (9.2)</td>
</tr>
<tr>
<td>RE(L+P)</td>
<td>Oct, 1987</td>
<td>92.7 (5.3) ND 105.7 (6.9)</td>
<td>51.1 (8.0)</td>
<td>89.6 (14.1)</td>
</tr>
<tr>
<td>RE(N)</td>
<td>Oct, 1987</td>
<td>104.7 (4.7) ND 109.2 (8.8)</td>
<td>42.0 (6.6)</td>
<td>73.7 (11.6)</td>
</tr>
<tr>
<td>RE(N+P)</td>
<td>Oct, 1987</td>
<td>104.6 (6.0) ND 105.4 (9.3)</td>
<td>33.4 (4.7)</td>
<td>58.6 (8.2)</td>
</tr>
</tbody>
</table>

⁡¹ Biomass N = Fl-N flush / 0.57 (see Jenkinson, 1988).

⁡² SE of the mean in parentheses.
The Fl-N flush (over 25 soil/time comparisons summarized in Table 5.2) was 35 to 90% (mean 65%) less than the microbial N obtained by the 1-day hexanol fumigation-extraction procedure. For the same soils, the Fl-N flush comprised 21 to 89% (mean 63) and 21 to 86% (mean 60%) of the microbial N obtained by 1-day and 5-day chloroform fumigation-extractions, respectively. Thus the results demonstrate that after chloroform fumigation less N was mineralized than was extracted. This is probably due to less accumulation of inorganic N.

In many instances, aerobic incubation of soils resulted in N immobilization (Hart et al., 1986), and exceptionally low Fl-N flush (Williams and Sparling, 1984; Sparling and Williams, 1986), particularly from acid organic soils. However, the results obtained here did agree well with those reported by a number of workers (e.g. Davidson et al., 1989; Gallardo and Schlesinger, 1990; Nannipieri et al., 1990a), but contrast with other studies (e.g. Brookes et al., 1985b; Sparling and West, 1988a,b). Davidson et al. (1989) reported that fumigation-extraction yielded over twice as much N as the fumigation-incubation method for forest soils at one sampling time, but not at another time. Nannipieri et al. (1990a) found biomass N values determined by the fumigation-extraction method (using a factor of 0.54 to convert microbial N to biomass N) were about 4 and 2.5 times higher than the values obtained by the fumigation-incubation method (using a factor of 0.57) in samples collected 5 to 9 days and 18 - 545 days, respectively, after the addition of urea to a clay-loam soil under a grass-legume association. These authors also attributed lower biomass N values determined by the Fl method to the immobilization of mineral N. By contrast, Sparling and West (1988a) found about 62% less microbial N (i.e. CHCl₃-N) than the Fl-N flush after incubation of pasture and arable soils. In an another study, Sparling and West (1988b) showed that the microbial N after Kjeldahl digestion and dichromate oxidation comprised 69 and 58% of the Fl-N flush for a range of soils. These authors, however, did not explain their results. Similarly, Brookes et al. (1985b) found a 1:1 ratio between the FE-N flush after 5-day fumigation and Fl-N
flush in several agricultural soils and concluded that the origin of extractable and decomposable biomass fractions were the same.

Overall, fumigation-extraction method is likely to prove more realistic approach than the fumigation-incubation method for determining microbial N in acid soils. This conclusion is based on the similar estimates of microbial N obtained by the direct extraction procedures. It has been already suggested that the FI method breaks down in acid soils or in soils which received large addition of fresh organic material. (e.g. Jenkinson and Powlson, 1976b; Jenkinson, 1988).

The Fl-N flush values were converted to biomass N by assuming a $k_N$ factor of 0.57 (see Jenkinson, 1988) and the results showed that the biomass N measured in this way were generally lower than the values of microbial N measured by the fumigation-extraction methods (Table 5.2).

Amount of microbial N (fumigation-extraction) and Fl-N flush (fumigation-incubation) is compared for individual sampling times by means of regression analyses in Table 5.3. There were no relationships between microbial N and Fl-N flush ($r^2 = 0.17$ to 0.19) for the June 1986 (winter) samples. Correlations between these estimates were still poor for the September 1986 and October 1987 (spring) samples ($r^2 = 0.44$ to 0.75 and 0.55 to 0.59, respectively), but were very close to one for the April 1987 (autumn) samples ($r^2 = 0.96$ and 0.97) (Table 5.3). The slopes of the regression lines between the fumigation-extraction and fumigation-incubation methods varied seasonally. Generally, slopes were greater than 1.0, except for the winter samples where slopes were less than 0.8. The reason why fumigation-extraction procedures yielded lower estimates than the FI method in winter samples is not known.
Table 5.3  Simple linear regressions and coefficient of determination ($r^2$) between fumigation-extraction and fumigation-incubation methods to estimate microbial N and Fl-N flush in a range of acid forest soils sampled at different seasons. Regression equations are of the form $y = a + bx$, where: $y =$ Microbial N after fumigation-extractions, $x =$ Fl-N flush after fumigation-incubation, $a =$ intercept, and $b =$ slope of regression line.

<table>
<thead>
<tr>
<th></th>
<th>Regression lines</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>June 1986, Winter (n = 18)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-day Hexanol</td>
<td>$y = 39.5 + 0.79x$</td>
<td>0.19</td>
</tr>
<tr>
<td>1-day CHCl$_3$</td>
<td>$y = 26.7 + 0.50x$</td>
<td>0.17</td>
</tr>
<tr>
<td>5-day CHCl$_3$</td>
<td>$y = 30.5 + 0.38x$</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>September 1986, Spring (n = 28)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-day Hexanol</td>
<td>$y = 16.4 + 1.55x$</td>
<td>0.75</td>
</tr>
<tr>
<td>1-day CHCl$_3$</td>
<td>$y = 26.9 + 1.07x$</td>
<td>0.44</td>
</tr>
<tr>
<td>5-day CHCl$_3$</td>
<td>$y = 15.4 + 1.09x$</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>April 1987, Autumn (n = 12)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-day Hexanol</td>
<td>$y = -0.1 + 4.09x$</td>
<td>0.97</td>
</tr>
<tr>
<td>5-day CHCl$_3$</td>
<td>$y = 0.4 + 3.97x$</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>October 1987, Spring (n = 28)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-day Hexanol</td>
<td>$y = 40.6 + 1.27x$</td>
<td>0.59</td>
</tr>
<tr>
<td>5-day CHCl$_3$</td>
<td>$y = 40.8 + 1.35x$</td>
<td>0.55</td>
</tr>
</tbody>
</table>

The different indices of microbial N (based on FE methods) and the Fl-N flush were combined and the relationships are shown in Fig. 5.3. There was reasonable agreement ($r^2 = 0.66$ and 0.64) between the microbial N obtained by 1-day hexanol and 5-day chloroform fumigation-extraction methods, and the Fl-N flush. In both cases, slopes of regression lines were greater than 1.7. In contrast, the relationship was poor ($r^2 = 0.23$) between the microbial N obtained by 1-day chloroform fumigation-extraction and the Fl-N flush (slope 0.6). This result contrasts with that of Brookes et al. (1985b) who found a 1:1 relationship between Fl-N flush and microbial N (following 5-day chloroform fumigation) in a range of agricultural and grassland soils. Whilst Gallardo and Schlesinger (1990) found a less significant
Figure 5.3 Relationships between microbial N obtained by fumigation-extraction procedures and the FL-N flush measured by the fumigation-incubation method in a range of acid forest soils.

- **(a) 1-day Hexanol**
  \[ y = 22.3 + 1.73x \]
  \[ r^2 = 0.66 \] (n=86)

- **(b) 1-day Chloroform**
  \[ y = 29.0 + 0.62x \]
  \[ r^2 = 0.23 \] (n=46)

- **(c) 5-day Chloroform**
  \[ y = 14.8 + 1.84x \]
  \[ r^2 = 0.64 \] (n=86)
correlation ($r = 0.77$) between the estimates of microbial biomass N after 5-day chloroform fumigation following immediate extraction and fumigation-incubation methods in the top 20 cm of highly acid forest soils. They found no relationship to the deeper samples (up to 60 cm) of the soil profiles and attributed to the inefficiency of these methods with low levels of microbial biomass. Another reason they mentioned that some deep soil samples were wet and compacted which might resulted an underestimation of biomass N by the FI method, as previously noted by Ross (1987). However, Brookes et al. (1985b) found a close relationship ($r^2 = 0.92$) between the microbial N obtained by the 1-day chloroform fumigation-extraction and FI-N flush by the fumigation-incubation method, but reported that the microbial N was only 79% of FI-N flush. Davidson et al. (1989) found significant relationships ($r^2 = 0.92$ and 0.96 for the November and April sampling times) between these two methods in acid forest soils. On the other hand, Sarathchandra et al. (1988) did not find any significant correlation between FI-N flush and microbial N in pasture soils sampled over 2-year periods. These workers have speculated that the FI-N flush are likely to be more affected than those of microbial N by the soil conditions at the time of sampling and during incubation, because their soils received a large inputs of urine over short periods from dairy cattle. They also mentioned immobilization of N during incubation was another contributing factor.

(b) Ratios of FI-N flush-to-microbial N

Measurement of microbial biomass N by the fumigation-incubation method involves the use of a $k_N$-factor, which is defined as the fraction of the killed microbial biomass N mineralized during a given incubation period. Generally, the $k_N$-factor is determined from the mineralization of laboratory grown organisms added to soils (Ross, 1987; Ross et al., 1987) or $^{15}$N-labelled microbial populations developed in situ (Voroney and Paul, 1984; Nicolardot et al., 1986). The principal problem in calculating soil microbial biomass N from the FI-N flush lies in establishing this $k_N$
factor (Jenkinson, 1988), because it varies considerably due to variable N contents of soil organisms and changes in their relative proportions in soils (McGill et al., 1986) and due to the seasonal changes in soil moisture content (Ross, 1987). Thus a wider range of $k_N$ values, ranging from 0.20 (Voroney and Paul, 1984) to 0.68 (Shen et al., 1984), have been used. Recently, the value of 0.57 for $k_N$ was proposed by Jenkinson (1988), based on calculations using a large group of soils from different countries. But in this calculation values of the C-to-N ratio of soil biomass of greater than 6.7 were excluded.

The ratios of Fl-N flush-to-microbial N obtained by 1-day hexanol fumigation-extraction were calculated for each soil and are given in Table 5.4. The ratios varied appreciably, depending on soil type and fertilizer treatments. For the differently fertilized YP soils, the ratios ranged from 0.10 to 0.53 (mean 0.27), being generally higher for the sewage treated (YPS) soil. For the differently fertilized RE soils, the

<table>
<thead>
<tr>
<th>Soil</th>
<th>No. of samples</th>
<th>Mean</th>
<th>(SE)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>YP(C)</td>
<td>14</td>
<td>0.28</td>
<td>(0.03)</td>
<td>0.17 - 0.46</td>
</tr>
<tr>
<td>YP(IL)</td>
<td>6</td>
<td>0.21</td>
<td>(0.05)</td>
<td>0.10 - 0.42</td>
</tr>
<tr>
<td>YP(IF)</td>
<td>3</td>
<td>0.23</td>
<td>(0.04)</td>
<td>0.16 - 0.29</td>
</tr>
<tr>
<td>YP(I)</td>
<td>4</td>
<td>0.28</td>
<td>(0.04)</td>
<td>0.17 - 0.33</td>
</tr>
<tr>
<td>YP(F)</td>
<td>4</td>
<td>0.29</td>
<td>(0.06)</td>
<td>0.12 - 0.39</td>
</tr>
<tr>
<td>YP(S)</td>
<td>4</td>
<td>0.32</td>
<td>(0.07)</td>
<td>0.24 - 0.53</td>
</tr>
<tr>
<td>D(R)</td>
<td>6</td>
<td>0.41</td>
<td>(0.03)</td>
<td>0.32 - 0.50</td>
</tr>
<tr>
<td>D(F)</td>
<td>6</td>
<td>0.44</td>
<td>(0.04)</td>
<td>0.32 - 0.60</td>
</tr>
<tr>
<td>RP</td>
<td>14</td>
<td>0.41</td>
<td>(0.03)</td>
<td>0.21 - 0.64</td>
</tr>
<tr>
<td>RE(C)</td>
<td>8</td>
<td>0.37</td>
<td>(0.05)</td>
<td>0.21 - 0.59</td>
</tr>
<tr>
<td>RE(L+P)</td>
<td>4</td>
<td>0.54</td>
<td>(0.07)</td>
<td>0.34 - 0.65</td>
</tr>
<tr>
<td>RE(N)</td>
<td>6</td>
<td>0.39</td>
<td>(0.04)</td>
<td>0.27 - 0.56</td>
</tr>
<tr>
<td>RE(N+P)</td>
<td>6</td>
<td>0.33</td>
<td>(0.05)</td>
<td>0.17 - 0.51</td>
</tr>
<tr>
<td>All data (mean)</td>
<td>85</td>
<td>0.35</td>
<td>(0.05)</td>
<td>0.10 - 0.65</td>
</tr>
</tbody>
</table>
ratios ranged between 0.17 and 0.65 (mean 0.41); the maximum value was found for the L+P treatment. The control (C), N and N+P fertilized RE soils showed very similar values. For the RP soil, the values ranged from 0.21 to 0.64 (mean 0.41) and for the duplex soils, the mean ratios were 0.41 (range 0.32 to 0.50) for the raked and 0.44 (range 0.32 to 0.60) for the fertilized plots. Thus the use of a factor of 0.57 (see Jenkinson,1988) seems to be too high for many of the soils studied, but the overall mean value is comparable to that of 0.37 for soils that have not received decomposable organic material (Nicolardot et al., 1989). Voroney and Paul (1984), who produced 14C- and 15N-labelled microbial biomass in soil in situ by incubating 14C-glucose and K15NO3 under aerobic conditions, reported kN values between 0.2 and 0.3, and attributed the variability to the C-to-N ratio of the microbes.

The ratios of Fl-N flush-to-microbial N obtained by chloroform fumigation-extractions were also calculated using seasonal data and the results are presented in Table 5.5. The mean values for the 1-day and 5-day chloroform fumigation-extractions were very similar to those obtained by hexanol fumigation-extraction.

Table 5.5 Seasonal changes in the ratios of Fl-N flush-to-microbial N obtained by the hexanol and chloroform fumigation-extraction procedures in a range of acid forest soils.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Season</th>
<th>1-day Hexanol</th>
<th>1-day CHCl₃</th>
<th>5-day CHCl₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 1986</td>
<td>Winter</td>
<td>0.38 (0.03)a</td>
<td>0.53 (0.05)</td>
<td>0.51 (0.04)</td>
</tr>
<tr>
<td>September 1986</td>
<td>Spring</td>
<td>0.32 (0.02)</td>
<td>0.28 (0.03)</td>
<td>0.40 (0.03)</td>
</tr>
<tr>
<td>April 1987</td>
<td>Autumn</td>
<td>0.25 (0.01)</td>
<td>ND</td>
<td>0.26 (0.02)</td>
</tr>
<tr>
<td>October 1987</td>
<td>Spring</td>
<td>0.40 (0.02)</td>
<td>ND</td>
<td>0.40 (0.03)</td>
</tr>
<tr>
<td>All data (mean)</td>
<td></td>
<td>0.35 [n = 85]b</td>
<td>0.37 [n = 43]</td>
<td>0.40 [n = 83]</td>
</tr>
</tbody>
</table>

a SE of the mean.
b Number of samples.
ND - Not determined.
The values varied with soil sampling time (i.e. season), being generally highest in winter and lowest in autumn (Table 5.5). Ross (1987) determined $k_N$ values of added organisms (2 bacteria and 2 fungi) in four pasture soils and reported that nearly all $k_N$ values varied significantly with time of sample collection. These authors attributed the variation to the seasonal changes in soil moisture content, and reported that the $k_N$ factors were significantly lower in smeared and compacted wet soil because of the impeded mineralization in fumigated soil.

In conclusion, the use of a $k_N$-factor is clearly necessary to convert Fl-N flush to microbial N estimated by the fumigation-extraction approach. This is supported by regressions calculated between the microbial biomass N values which were converted from Fl-N flush values using a $k_N$-factor of 0.57 (see Jenkinson, 1988) and the microbial N obtained by the fumigation-extraction methods (Table 5.6). The

<table>
<thead>
<tr>
<th></th>
<th>y</th>
<th>No. of samples</th>
<th>$y = a + b \times x$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-day Hexanol</td>
<td>y</td>
<td>86</td>
<td>$y = 22.3 + 0.99 \times x$</td>
<td>0.66</td>
</tr>
<tr>
<td>1-day CHCl$_3$</td>
<td>y</td>
<td>46</td>
<td>$y = 29.0 + 0.35 \times x$</td>
<td>0.23</td>
</tr>
<tr>
<td>5-day CHCl$_3$</td>
<td>y</td>
<td>86</td>
<td>$y = 14.8 + 1.05 \times x$</td>
<td>0.64</td>
</tr>
</tbody>
</table>

slopes of regression line between the 1-day hexanol or 5-day chloroform fumigation followed by immediate extraction and the biomass N estimated from Fl-N flush indicate that direct extraction yielded virtually the same estimate as obtained after incubation (Table 5.6). The slope of regression between 1-day chloroform fumigation-extraction and biomass N was much lower, and the relationship between the estimates was also poor compared to other fumigation-extraction procedures.
5.3 General conclusions from studies reported in this chapter

1) The duration of incubation after fumigation affected the FI-N flush. Generally, the maximum FI-N flush values were achieved after 10 days.

2) The maximum FI-N flush values were always less than the amounts of microbial N obtained by the fumigation-extraction methods, confirming that the FI method is not a suitable approach to determine microbial N in acid forest soils. However, there was a positive and significant relationship between them for 1-day hexanol and 5-day chloroform fumigation.

3) The ratio of FI-N flush-to-microbial N flush varied markedly with soil type and time of soil sampling. A correction factor of 0.57 ($k_N$) applied to the FI values made the two types of estimates equivalent. The relationship between the estimates based on 1-day chloroform fumigation was, however, poor.

4) Direct extraction of hexanol-fumigated soils with 0.5M $K_2SO_4$ can provide an alternative and simple procedure to either chloroform fumigation-extraction or fumigation-incubation methods for obtaining an estimate of microbial N in acid forest soils.

The following chapter examines seasonal changes and the effects of various forest management practices, such as fertilization and prescribed burning, on soil microbial N measured by the hexanol fumigation-extraction procedure. Effects of these treatments on soil microbial C are also studied using the Substrate-Induced Respiration (SIR) method.
CHAPTER SIX
CHAPTER SIX

EFFECTS OF TREATMENT AND SEASON ON MICROBIAL NITROGEN AND CARBON IN SEVERAL FOREST SOILS

6.1 Seasonal changes in microbial N in several untreated forest soils
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6.2 Effects of air-drying and rewetting before fumigation on soil microbial N
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6.3 Changes in soil microbial N and C to various forest management practices
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CHAPTER SIX

EFFECTS OF TREATMENT AND SEASON ON MICROBIAL NITROGEN AND CARBON IN SEVERAL FOREST SOILS

The microorganisms in forest soils are influenced by a number of factors, such as environmental conditions (e.g. temperature and soil moisture content) and soil management practices, for example fertilizer addition, liming, sewage sludge amendment and prescribed fire. Soil microorganisms play a vital role in the growth of plants and they are responsible for the decomposition of dead plant material and for nutrient transformations. Rates of decomposition of plant material would vary markedly with temporal and seasonal changes, and with the effects of various treatments. The effects of these treatments can evaluate whether the soil microorganisms will take up nutrients for growth or release nutrients through death or decay.

This chapter describes studies of the effects of season, wetting-drying cycles, fertilizer addition and low-intensity prescribed burning on microbial N and C in several acid forest soils.

6.1 Seasonal changes in microbial N in several untreated forest soils

6.1.1 Materials and Methods

Three untreated soils (YP, RP and RE) were collected in September 1986, April and October 1987, June and September 1988, and January, March, June and November 1989. The highly acid red earth (RE) soils from three contrasting sub-alpine eucalypt forest communities [stands dominated by *E. delegatensis* (PD), *E. dives - E. dalrympleana* (PDD) and *E. pauciflora* (PS)] were sampled on two
occasions (May and December 1988). Soil cores (total of 24, 6 cores bulked to form a replicate) were sectioned by depth increments (0 - 5 and 5 - 10 cm). Four replicates for each soil were used and the results are presented on an oven-dry soil basis. Descriptions of soils and analytical procedures are given in Chapter 3. The amount of microbial N was determined by the hexanol fumigation-extraction method, as described in Chapter 4. Final microbial N concentrations were calculated using a correction factor to account for incomplete extraction of microbial N and the contribution of non-microbial N as described in Chapter 4 (section 4.6).

6.1.2 Results and Discussion

(a) Seasonal changes in microbial N in different soil types

The seasonal changes in microbial N in three untreated soils are depicted in Fig. 6.1a. Seasonal change was very little. In the YP soil, the concentration of microbial N (mg N kg\(^{-1}\) soil) varied from 29.3 (October 1987, spring) to 34.8 (September 1986, spring). In the RP soil, the lowest microbial N (38.1 mg N kg\(^{-1}\) soil) was observed in October 1987 and the maximum in June 1986 (62.2 mg N kg\(^{-1}\) soil). In the RE soil, a much larger variation was observed and the minimum microbial N was found in January 1989 (summer) (91.9 mg N kg\(^{-1}\) soil) and the maximum in April 1987 (autumn) (142.2 mg N kg\(^{-1}\) soil). The results thus show that there is no clear seasonal changes or similarity in microbial N content between the soils. Bolton \textit{et al.} (1985) found lower soil microbial biomass N in the spring samples, which they attributed to a cold and wet climate. Ross \textit{et al.} (1981) found similar levels of biomass N (measured as mineral-N flush) during early spring, autumn and winter in four grassland soils from New Zealand. However, annual fluctuations of microbial N measured in spring were not significant, with the exception of the RP soil from 1987 to 1989 (Fig. 6.2). Recently, Patra \textit{et al.} (1990) also found very little annual changes in microbial N content in two soils under long-term field
Figure 6.1 Seasonal changes in microbial N (a) and field moisture content (b) of untreated yellow podzolic (YP), red podzolic (RP) and red earth (RE) soils (0 - 5 cm).
Figure 6.2 Annual variations of microbial N concentration of three untreated forest soils sampled during spring season.
management experiments which they explained were due to experimental and sampling error. However, Sarathchandra et al. (1988) observed a large variation in microbial N values over the 2-year period and attributed them to the fluctuations in temperature and moisture content.

Generally, soil moisture content variation was greater than that of microbial N (Fig. 6.1b). The pattern of soil gravimetric moisture contents in three soils was very similar.

The microbial N content was poorly correlated with field moisture content for individual soils (Table 6.1). This is probably because only single point measures of soil moisture were taken, not measures which integrated the effect of moisture over time on the microbial activity of soils. Ritz and Robinson (1988) also found no relationship between microbial biomass N and soil moisture content. The surprising result was that the relationship between the microbial N concentration and the soil moisture content was significant ($r^2 = 0.65$, $P<0.001$) when data for all three soils and all seasons were combined (Table 6.1), indicating that overall soil moisture may be an important factor in determining seasonal pattern of soil microbial N. However, a controlled experiment is needed to verify this point.

<table>
<thead>
<tr>
<th>Soil</th>
<th>No. of samples</th>
<th>$r^2$</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>YP</td>
<td>19</td>
<td>0.18</td>
<td>NS</td>
</tr>
<tr>
<td>RP</td>
<td>21</td>
<td>0.18</td>
<td>NS</td>
</tr>
<tr>
<td>RE</td>
<td>32</td>
<td>0.12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>All soils</td>
<td>72</td>
<td>0.65</td>
<td>0.001</td>
</tr>
</tbody>
</table>

NS - Not significant.
In general, microbial N was relatively greater in June (winter) samples, possibly because of low microbial and fungal activity. Seasonal effects of temperature and plant growth are also responsible for the storage and release of nutrients by the soil microbial biomass (Sarathchandra et al., 1989).

(b) Seasonal changes in microbial N in the red earth soil under different eucalypt forests

Soil microbial N varied slightly between the sampling times for each depth or between the depths for each sampling time (Fig. 6.3). A small increase in microbial N was observed in May (late autumn) than in December (early summer) for the soils under *E. delegatensis* and *E. pauciflora*, while microbial N was about 8% higher in early summer compared to late autumn in soil under *E. devis- E. dalrympleana*, although the increase was not significant. Generally, microbial N was greater in the surface 5 cm and decreased with depth. The soil under *E. pauciflora* had relatively higher microbial N than the other two soils, but differences for wet sclerophyll (*E. delegatensis*) compared to dry sclerophyll (*E. dives - E. dalrympleana*) forests were very small.

Virtually, no significant changes in soil gravimetric moisture content was observed for the three soils studied, irrespective of soil sampling time and depths, except for the soil under *E. pauciflora* (0 - 5 cm) (Fig. 6.3).

6.1.3 Conclusions

Concentration of microbial N varied seasonally, depending on soil type with greatest variability shown by the RE soil and least by the YP soil. Microbial N was not related with the field moisture content when individual soils were considered. However, significant relationship was observed when pooled data for each soil was
Figure 6.3 Seasonal changes in microbial N and field moisture content of a red earth soil under different forest types.
used, suggesting that soil moisture content may be an important factor regulating microbial N contents in soils. On the other hand, no major effect of soil sampling time was apparent for the soils under different forest types, although these soils were sampled only twice.

The following experiments examined the effect on soil microbial N of simulated environmental condition, particularly drying and rewetting of soils. Experiments were conducted under laboratory conditions by subjecting the RE soils from three different eucalypt forest sites (i.e. PD, PDD and PS) to various wetting and drying cycles.

6.2 Effects of air-drying and rewetting before fumigation on soil microbial N

The experiments examined the effects of air-drying over three days under laboratory conditions, and the effect of rewetting air-dried soils before fumigation on the measured soil microbial N concentrations.

6.2.1 Materials and Methods

Soils were collected in May, 1988. They were slowly air-dried at room temperature (ca. 20 °C) over 3 days. Some of the air-dried soils (e.g. 5 - 10 and 10 - 20 cm) were rewetted before fumigation. Rewetted air-dried soils were not incubated prior to fumigation. To achieve rewetting different volumes (0, 1, 2, 3, 4, and 5 ml) of distilled water was added to a 10 g portion of sample of the air-dried soil followed by mixing with a glass rod.

Soil microbial N contents were determined in both field moist and air-dried soils at the corresponding moisture contents using the hexanol fumigation-extraction method, as described in Chapter 4. It is sometimes suggested that air-dried soils
should be rewetted before fumigation to allow greater extractibility of nitrogen in 0.5M K₂SO₄ (Ross, 1989; Sparling and West, 1989). Microbial N was calculated using the values of total N of rewetted-fumigated soil and mineral N of air-dried unfumigated soil, as suggested by Sparling and West (1989), except that the authors subtracted total N in unfumigated air-dried soil from total N in fumigated-rewetted soils. These workers preferred this approach because greater amounts of N were extracted from rewetted fumigated soils which suggested a better recovery of microbial N and the use of air-dry unfumigated controls avoided the immobilization of N in the rewetted soils. All results are expressed on an oven-dry (105 °C) soil basis.

6.2.2 Results and Discussion

(a) Changes in microbial N caused by air-drying

Air-drying of field moist samples for three days significantly decreased the level of microbial N in all three soils at all depths (Fig. 6.4). This effect was expected because the microbial biomass is generally decreased on air-drying (Bottner, 1985; Sparling et al., 1986). However, the results indicate that the fumigation might not be complete in the air-dry soils because of changes in enzymic activities. When all soils and depths were considered, the decrease in microbial N on air-drying ranged from 14 to 57 (mean 33) mg N kg⁻¹ soil which corresponded to 27 to 58% (mean 45%) of field moist soils (Table 6.2). The decrease was greater in the surface horizons compared to lower depths. Reduced microbial N in the air-dried soils has also been reported by many workers (e.g. Sparling and Ross, 1988; West et al., 1988a,b). West et al. (1988a,b) determined microbial N by the chloroform fumigation-extraction method of several pasture soils gradually dried from field moist to air-dryness and found a much larger decrease (by 93 and 87%) in microbial N in the air-dried soils than in the field moist soils. The drop in the estimates of microbial N following air-dryness was caused largely by the decrease (20 to 50%) in K₂SO₄-extractable total N of the fumigated soils (Table 6.3). Extractable mineral N in the air-dried fumigated
Figure 6.4 Effect of air-drying on microbial N (mg N kg⁻¹ soil) concentrations of a red earth soil under different eucalypt forest types.
soils were also decreased, ranging from 33 to 52%. The reason is probably because less efficient enzymic hydrolysis of cell components for air-dried fumigated soils (Sparling and West, 1989). The involvement of hydrolytic enzymes in increasing the extractability of microbial N has been indicated by the chloroform fumigation experiments (e.g. Brookes et al., 1985a and Amato and Ladd, 1988). In contrast to fumigated soils, extractable total and mineral N in the unfumigated soils (i.e. before fumigation) increased on air-drying by 18 to 58% and 16 to 97%, respectively (Table 6.3). This suggests that the increased soil N in the K$_2$SO$_4$-extracts of unfumigated air-dried soils came from microbial sources which was killed by air-drying treatment (Powlson and Jenkinson, 1976; Seneviratne and Wild, 1985). This is partly supported by the relationship ($r^2 = 0.33$) between the decreased amounts of microbial N upon air-drying and increased amounts of K$_2$SO$_4$-extractable total N in air-dried unfumigated soils (Fig. 6.5).
Table 6.3 Changes on air-drying in K<sub>2</sub>SO<sub>4</sub>-extractable total and mineral N (mg kg<sup>-1</sup> soil) of hexanol-fumigated and unfumigated samples.

<table>
<thead>
<tr>
<th>Soil Depth (cm)</th>
<th>Total N</th>
<th>Mineral N</th>
<th>Change&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total N</th>
<th>Mineral N</th>
<th>Change&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moist soil (mg N kg&lt;sup&gt;-1&lt;/sup&gt; soil)</td>
<td>Air-dried</td>
<td>(%)</td>
<td>Moist soil (mg N kg&lt;sup&gt;-1&lt;/sup&gt; soil)</td>
<td>Air-dried</td>
<td>(%)</td>
</tr>
<tr>
<td>(a) After fumigation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD 0-5</td>
<td>65.3 (1.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.7 (3.7)</td>
<td>-37</td>
<td>13.6 (2.0)</td>
<td>8.1 (1.3)</td>
<td>-41</td>
</tr>
<tr>
<td>5-10</td>
<td>55.8 (3.2)</td>
<td>39.8 (4.1)</td>
<td>-34</td>
<td>11.5 (0.8)</td>
<td>7.6 (0.4)</td>
<td>-34</td>
</tr>
<tr>
<td>10-20</td>
<td>44.5 (2.0)</td>
<td>38.3 (12)</td>
<td>-20</td>
<td>14.0 (3.0)</td>
<td>9.3 (2.2)</td>
<td>-33</td>
</tr>
<tr>
<td>PDD 0-5</td>
<td>92.7 (6.0)</td>
<td>50.6 (2.8)</td>
<td>-50</td>
<td>19.4 (2.2)</td>
<td>9.2 (0.2)</td>
<td>-52</td>
</tr>
<tr>
<td>5-10</td>
<td>70.2 (3.0)</td>
<td>44.7 (2.7)</td>
<td>-42</td>
<td>14.0 (1.1)</td>
<td>8.8 (0.7)</td>
<td>-37</td>
</tr>
<tr>
<td>10-20</td>
<td>58.2 (4.9)</td>
<td>39.8 (3.4)</td>
<td>-38</td>
<td>10.7 (1.1)</td>
<td>7.0 (0.5)</td>
<td>-34</td>
</tr>
<tr>
<td>PS 0-2.5</td>
<td>96.3 (3.4)</td>
<td>57.0 (1.9)</td>
<td>-45</td>
<td>21.9 (4.7)</td>
<td>10.9 (1.5)</td>
<td>-50</td>
</tr>
<tr>
<td>2.5-5</td>
<td>79.1 (2.6)</td>
<td>52.9 (4.2)</td>
<td>-39</td>
<td>17.9 (1.9)</td>
<td>10.1 (1.2)</td>
<td>-44</td>
</tr>
<tr>
<td>5-10</td>
<td>66.5 (1.3)</td>
<td>49.5 (4.0)</td>
<td>-33</td>
<td>12.4 (4.4)</td>
<td>8.1 (1.8)</td>
<td>-34</td>
</tr>
<tr>
<td>10-20</td>
<td>57.3 (0.8)</td>
<td>43.8 (4.2)</td>
<td>-31</td>
<td>12.0 (2.5)</td>
<td>7.4 (1.8)</td>
<td>-38</td>
</tr>
<tr>
<td>(b) Before fumigation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD 0-5</td>
<td>24.5 (1.7)</td>
<td>34.4 (1.0)</td>
<td>+29</td>
<td>4.9 (1.5)</td>
<td>7.5 (1.9)</td>
<td>+53</td>
</tr>
<tr>
<td>5-10</td>
<td>23.5 (1.0)</td>
<td>30.5 (4.3)</td>
<td>+19</td>
<td>4.3 (0.2)</td>
<td>7.6 (0.8)</td>
<td>+76</td>
</tr>
<tr>
<td>10-20</td>
<td>23.7 (2.0)</td>
<td>30.3 (2.1)</td>
<td>+18</td>
<td>7.7 (2.6)</td>
<td>9.0 (2.6)</td>
<td>+16</td>
</tr>
<tr>
<td>PDD 0-5</td>
<td>27.6 (1.8)</td>
<td>40.7 (3.2)</td>
<td>+34</td>
<td>5.4 (0.5)</td>
<td>10.1 (0.7)</td>
<td>+86</td>
</tr>
<tr>
<td>5-10</td>
<td>28.6 (1.6)</td>
<td>37.7 (2.8)</td>
<td>+21</td>
<td>7.4 (0.9)</td>
<td>8.9 (0.8)</td>
<td>+20</td>
</tr>
<tr>
<td>10-20</td>
<td>22.7 (1.6)</td>
<td>31.5 (2.3)</td>
<td>+27</td>
<td>5.4 (0.8)</td>
<td>7.3 (0.6)</td>
<td>+34</td>
</tr>
<tr>
<td>PS 0-2.5</td>
<td>34.6 (4.9)</td>
<td>51.3 (0.4)</td>
<td>+33</td>
<td>5.2 (0.9)</td>
<td>10.2 (0.2)</td>
<td>+97</td>
</tr>
<tr>
<td>2.5-5</td>
<td>29.0 (3.8)</td>
<td>50.6 (3.2)</td>
<td>+59</td>
<td>6.2 (0.9)</td>
<td>11.1 (0.7)</td>
<td>+79</td>
</tr>
<tr>
<td>5-10</td>
<td>31.4 (6.9)</td>
<td>42.6 (1.5)</td>
<td>+23</td>
<td>5.5 (0.6)</td>
<td>7.5 (0.2)</td>
<td>+37</td>
</tr>
<tr>
<td>10-20</td>
<td>28.7 (2.3)</td>
<td>39.5 (1.9)</td>
<td>+25</td>
<td>5.8 (0.9)</td>
<td>7.1 (0.5)</td>
<td>+21</td>
</tr>
</tbody>
</table>

<sup>a</sup> - and + signs denote decrease and increase on air-drying, respectively.

<sup>b</sup> SE of the mean values in parentheses.
Figure 6.5 Relationship between decrease in microbial N ($\Delta$ Microbial N) upon air-drying and increase in $K_2SO_4$-extractable total N ($\Delta$ Total N) in air-dried samples of a red earth soil under different eucalypt forests.
(b) Effects of rewetting air-dried soils before fumigation on measured microbial N

Varying degrees of rewetting air-dried soils before fumigation linearly increased the amount of measured microbial N in all three soils at both depths (Fig. 6.6). The pattern of microbial N released in rewetted air-dried soils was very similar for the three soils studied, irrespective of depths. The results indicate that the presence of water during fumigation enhances the extractibility of soil N, probably because of increased enzyme activity in the rewetted fumigated soils. Recently, an effect of soil moisture content during chloroform fumigation has, however, been shown for air-dried pasture samples by Sparling and West (1989). These authors reported that the rewetting air-dried soils (with 0.5 ml g⁻¹ soil) before chloroform fumigation significantly increased the K₂SO₄-extractable total N and C compared with soils fumigated while air-dry. They observed that the effect of adding different volumes of water on extractable organic C was not consistent for those soils, but soils fumigated when rewetted to 20 to 50% (by volume) water had significantly greater amount of extractable organic C. They suggested that the soil microbial N and C could be measured on air-dried samples provided the soils were remoistened before fumigation with adequate water for greater extractibility of the compounds in 0.5M K₂SO₄.

6.2.3 Conclusions

Air-drying for three days under laboratory conditions reduced soil microbial N significantly, possibly because of reduced enzymic activity in air-dried fumigated samples. The increased levels of extractable soil N in the unfumigated air-dried soils appear to be primarily derived from the microbial biomass killed by desiccation. Rewetting air-dried soils before fumigation increased the concentration of soil microbial N compared to air-dried soils, possibly indicating that moisture content
Figure 6.6 Changes in measured microbial N after rewetting air-dried soils before hexanol fumigation. Soils were not incubate prior to fumigation.
affects the efficiency of fumigation. The degree of water addition, however, did appear crucial, as the microbial N increased gradually up to 50% (by volume) water.

The overall conclusion is that the estimation of microbial N in air-dried soils could be possible only when the soils are rewetted before fumigation for better recovery of microbial N. The volume of water is to be determined for each soils.

6.3 Changes in soil microbial N and C to various forest management practices

6.3.1 Introduction

The main objective of the following studies was to compare quantitative changes in microbial N and C due to different forest management practices, for instance, application of inorganic fertilizer and other soil amendments (e.g. liming, sewage sludge and sugar addition), irrigation and repeated low-intensity prescribed burning. Seasonal fluctuations in microbial N were also measured in the differently treated forest stands. Microbial C in these soils was determined by the Substrate-Induced Respiration (SIR) method on only one occasion for comparison with soil microbial N. Distribution of microbial N and C down the soil profile was also examined.

6.3.2 Materials and Methods

The effects of fertilizer and irrigation (alone or in combination) and sewage sludge amendment were examined in the YP soil under *Pinus radiata*. Soils (0 - 5 cm) were collected in September 1986 from the BFG site (Site 1, Fig. 3.1).

The effects of fertilizer and other soil amendments as well as low-intensity prescribed fire on microbial N and C were examined using the RE soil under eucalypt
forest (Site 4, Fig. 3.1). Description of these sites was given in Chapter 3 (section 3.2). Soils (up to 10 cm) were collected from RE site on four occasions (June and September 1988, and January and March 1989). Microbial C was measured only in the March 1989 samples. Soil cores (12 for each treatment) were sectioned by depth (0 - 2.5, 2.5 - 5 and 5 - 10 cm) before sieving (<5 mm).

Microbial N was measured by the hexanol fumigation-extraction method and calculated as described in Chapter 4 (section 4.6). Microbial C was estimated by the Substrate-Induced Respiration (SIR) method of Anderson and Domsch (1978b) with the modification of West and Sparling (1986), as described in Chapter 3 (section 3.7). In the SIR method, the rate of soil respiration was converted to microbial C using a factor of 50.4, as suggested by Sparling et al. (1990).

Analysis of variance (ANOVA) was used to determine the significance of the main treatment factors with time of soil sampling and depth using GENSTAT. The statistical analysis of the treatment, season and depth for the effects of fertilization and low-intensity prescribed fire on microbial N in the RE soil was based on the data from September 1988 to March 1989. All samples of June 1988 were excluded in the ANOVA, because different depths were sampled on some treatments. Differences between means were determined using the least-significant difference (LSD) test at P<0.05. For the microbial C, the differences between the treated soils were compared using the Students' t-test at P<0.05.

6.3.3 Results and Discussion

(a) Effects of fertilizer, irrigation and sewage sludge amendment on microbial N in the YP soil

Microbial N in differently fertilized YP soil is shown in Fig. 6.7. The amount of
Figure 6.7 Effects of fertilizer and irrigation alone or in combination (IL, IF, I and F), and sewage sludge amendment on microbial N in the YP soil (0 - 5 cm) under *Pinus radiata*. Soils were sampled in September 1986. Bars are SE of the mean values.
microbial N was significantly (P<0.05) greater in soil treated with sludge than in untreated control soil, probably because of the liming effect of sludge (e.g. Sommers et al., 1976). Liming increased pH (in KCl from 4.34 to 7.67). In addition, sewage added organic N and much P (Bekunda, 1987). Bekunda (1987) also found higher microbial N (FI method) and greater microbial activity (CO₂ evolution and O₂ consumption) in the same sludge treated soil as used here three years before. Boyle and Paul (1985) measured increased biomass N (estimated by FI method) in sludge treated soil (over an eight-year period) than in the untreated control soil.

Control soil had significantly higher microbial N than the IF soil. Soil microbial N concentration in the IL, I and F treatments were 24.4, 24.1 and 24.3 mg N kg⁻¹ soil, respectively and were not significantly different from control. The possible reason is that the pH of these fertilized soils were lower than the unfertilized soil (Khanna et al., 1991). Bekunda (1987) also found depressed biomass N contents in the fertilized soils than in the control soil. Singh et al. (1989) reported that microbial biomass C and nutrient pools declined as N-mineralization increased, during periods when plant growth was rapid. Raison et al. (1990), who studied this site measured two to three times greater in situ N mineralization rates in the fertilized soils than in the unfertilized control soil. Many other studies (e.g. Baath et al., 1981; Söderström et al., 1983) have also shown that the number and biomass of microbes is reduced over time to levels below those in unfertilized soils. Decreased biomass N in N-fertilized soil compared with control soil was also found by Christie and Beattie (1989). These authors reported that the lower biomass N in the fertilized soils was because of the acidifying effect of fertilizer-N. By contrast, several other studies (e.g. Schnurer et al., 1985; Shen et al., 1989) showed that inorganic fertilizer increased the level of microbial biomass N. Thus the effects of fertilizer are not universal.

The results also show that the rate of fertilizer addition did not affect the concentration of microbial N. In the IL treatment, 300 kg N ha⁻¹ was added, whilst
400 kg N ha\(^{-1}\) was introduced in either IF and F treatment. These results accord with those reported by Ritz and Robinson (1988), who found an inconsistent effect of fertilizer rate on the biomass N contents. Shen et al. (1989) observed relatively little differences in total microbial biomass N between plots receiving 48, 96, 144 and 196 kg N ha\(^{-1}\) yr\(^{-1}\).

Overall, microbial N accounted for 4.4% of the soil total N averaged over all treatments, which is within the range (1 - 5%) of a large group of soils across different ecosystems (Jenkinson and Ladd, 1981; Smith and Paul, 1990) and is very similar (4.3%) to that measured in a dry tropical forest soil (Srivastava et al., 1989).

In conclusion, the effects of inorganic fertilizer and irrigation, alone or in combination tended to slightly decreased microbial N, but the decrease was only significant for the irrigation + solid fertilized (IF) treatment. By contrast, the plot which received sewage sludge had a much higher microbial N.

(b) Effects of fertilizer and other soil amendments on microbial N and C in the RE soil

Soil microbial N

Distribution of microbial N in differently treated RE soils sampled on four occasions is shown in Fig. 6.8. The effects of treatments, time and depths were examined using ANOVA for the three samplings from September 1988 to March 1989. The results for June 1988 samples were not included in the analysis because different depths were sampled on some treatments (see Fig. 6.8). The P treatment was not sampled in January 1989, but estimated values were used in the analysis.
Figure 6.8 Distribution of microbial N with sampling time in differently treated RE soil. Bars are SE of the mean values (n = 6 for June 1988 and n = 3 for other seasons). For June 1988, soils from N, N+P and sugar treatments were sampled from 0 - 5 and 5 - 10 cm depths, and soils from P was not sampled in January 1989.
Analysis of variance (Table 6.4) showed a significant ($P = 0.025$) effect of treatment on soil microbial N concentrations, and the effects of seasons and depths were highly significant ($P<0.001$). Treatment x season was also significant ($P<0.001$), but other interactions were not.

Table 6.4 Three-way ANOVA for soil microbial N at the differently fertilized RE soil.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>f value</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main effects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>4.24</td>
<td>0.025</td>
</tr>
<tr>
<td>Depth</td>
<td>2</td>
<td>38.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Season</td>
<td>2</td>
<td>33.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Two-way interactions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment x depth</td>
<td>10</td>
<td>1.66</td>
<td>0.150</td>
</tr>
<tr>
<td>Treatment x season</td>
<td>10</td>
<td>5.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Depth x season</td>
<td>4</td>
<td>0.79</td>
<td>0.533</td>
</tr>
<tr>
<td><strong>Three-way interaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment x depth x season</td>
<td>20</td>
<td>0.61</td>
<td>0.879</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>152</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Three seasons, e.g. spring (September, 1988), summer (January, 1989) and autumn (March, 1989), were used in the analysis (see Fig. 6.8).

Figure 6.9 summarizes the main effects of treatment, season and depth on the amount of soil microbial N. The P treatment was less than the others which were similar. The lime + P treatment did not reduce microbial N as much as P alone. Liming increases the soil pH, thus favouring microbial activity (Bekunda, 1987). Using the same soils three years before this experiment, Bekunda (1987) found that liming resulted in a short-term (up to 157 days) reduction in biomass N (FI method). The effects of liming are not consistent as reported in the literature. For instance, Carter (1986) reported that liming alone either increased or maintained a constant level of soil biomass N compared to the unlimed soil. Many workers (e.g. Adams
Figure 6.9 Main effects of treatment (a), season (b) and depth (c) on microbial N concentration in a RE soil. Vertical bars indicate LSD (0.05) for determining differences between treatments (T), seasons (S) and depths (D).
and Adams, 1983; Haines, 1988) found greater biomass N due to lime addition in agricultural soils. Adams and Adams (1983) measured a 30% increase in biomass N from a range of acidic grassland soils following liming at 8 t ha\(^{-1}\) three years prior to sampling. Haines (1988) reported that biomass N (FI method) increased by 38 and 48% due to liming acid soils at rates of 2.5 and 5.0 t ha\(^{-1}\), respectively.

There were significant (P<0.001) changes in microbial N concentration with time of sampling (i.e. season) (Fig. 6.9b). Microbial N was highest in September 1988 (spring) and then decreased significantly to similar values in summer and early autumn. The microbial N was 15 to 20% higher in spring than in summer and autumn. The decrease in microbial N in summer may have resulted from low soil moisture. A previous experiment (see Fig. 6.4) showed that drying decreased the concentration of soil microbial N.

Microbial N concentration decreased with depth (Fig. 6.9c). Microbial N was 14 and 19% higher (P<0.001) at the 0 - 2.5 cm depth than at the 2.5 - 5 and 5 - 10 cm, respectively. Other studies (e.g. Carter and Rennie, 1982; Christie and Beattie, 1989; Hart et al., 1988) have also shown decreased microbial N with increasing soil depth.

The effect of treatments averaged across depths varied significantly (P<0.001) with season (Fig. 6.10). Treatment effects were greatest in spring and less in summer and autumn. The control soil contained significantly (P<0.001) higher microbial N in spring than the L+P and P treatments. The other treatments were similar to the control. In summer, the L+P treatment increased whilst all others declined. Overall, the seasonal pattern of microbial N was not similar to that found in other studies. For example, Singh et al. (1989) found highest soil microbial biomass N in samples collected in summer, followed by those sampled in winter and lowest in samples collected in rainy season in dry tropical forest. Sarathchandra et al. (1988)
Figure 6.10 Changes in microbial N by treatment and season in the RE soil. Vertical bars indicate LSD (0.05) for determining treatment x season interaction (T x S) and changes with season (S) within each treatment.
also noted higher microbial N in dry summer months and lower values in winter months in a grazed pasture soil. Whilst Ross et al. (1981) and Ritz and Robinson (1988) found no seasonal variation in soil microbial biomass N in pasture and agricultural soils. Microbial N concentration was 3.2% of the soil total N, which is very similar (3.1%) to that found for variously fertilized grassland soil (Christie and Beattie, 1989).

Microbial N values did show significant but poor relationship with soil gravimetric moisture content in most cases (Table 6.5). The correlation was highest in spring \( (r^2 = 0.68, P<0.001) \), but when all treatments, seasons and depths were considered, the \( r^2 \) was only 21%.

Table 6.5 Simple linear regression relationship \( (r^2) \) between soil microbial N and field moisture content of differently treated red earth soil under eucalypt forest sampled at different seasons. The range of soil soil moisture content for winter (30.0 - 59.1%), spring (29.1 - 57.6%), summer (36.2 - 57.1%) and autumn (32.0 - 57.2%).

<table>
<thead>
<tr>
<th>Month of sample collection</th>
<th>Season</th>
<th>No. of samples</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>June, 1988</td>
<td>Winter</td>
<td>64</td>
<td>0.26***</td>
</tr>
<tr>
<td>September, 1988</td>
<td>Spring</td>
<td>50</td>
<td>0.68***</td>
</tr>
<tr>
<td>January, 1989</td>
<td>Summer</td>
<td>42</td>
<td>0.00 NS</td>
</tr>
<tr>
<td>March, 1989</td>
<td>Autumn</td>
<td>46</td>
<td>0.13*</td>
</tr>
<tr>
<td>All seasons</td>
<td></td>
<td>202</td>
<td>0.21***</td>
</tr>
</tbody>
</table>

*** and * Significant at 0.1 and 5% level, respectively; NS - Not significant.

Soil microbial C

Different soil amendments had different effects on soil microbial C concentration which also varied with depths (Fig. 6.11). Compared with untreated control (C) soil, the plot which received lime and phosphorus (L+P) had higher
Figure 6.11 Microbial C concentration measured by the SIR method of differently treated RE soil. Soils sampled in March 1989. Bars are SE of the mean values.
microbial C at all three depths, whereas N and P treatments had lower microbial C in the 0 - 2.5 and 2.5 - 5 cm soil depths, but no difference at the 5 - 10 cm depth. Microbial C was significantly higher at the 0 - 2.5 and 5 - 10 cm depths in the N+P treatment than in the respective depths of the control soil, but showed no significant difference at 2.5 - 5 cm depth. Sugar addition had no effect on microbial C, except for the 2.5 - 5 cm depth where microbial C was significantly lower than the control soil.

Averaged across depths, microbial C was highest in the L+P treatment (1205 mg C kg⁻¹ soil) and least in N the treatment (342 mg C kg⁻¹ soil). These were significantly different from control which had 455 mg microbial C kg⁻¹ soil. Other treatments (i.e. P, N+P and sugar which contained 346, 566 and 442 mg microbial C kg⁻¹ soil, respectively) did not differ significantly from the control. The increase in microbial C in the L+P treatment is possibly because of solubilization of organic matter. In their modified method, West and Sparling (1986) pointed out that the SIR method would not give reliable biomass C values in near neutral soil where pH exceeds 6.0, because of retention of evolved CO₂ in the soil solution. A change in the microbial population due to liming may also be responsible for higher microbial activity. The use of conversion factor of 50.4, as suggested by Sparling et al., 1990) may not be appropriate for all the differently treated soils. However, many workers (e.g. Adams and Adams, 1983; Haines, 1988) have found higher (about 30%) biomass C in limed soil than unlimed soil using the FI method. Overall, the effects of fertilizer addition on soil microbial C can not be generalized because these treatments have variable effects. For example, fertilization increases carbon input into soil thereby increasing the size of the soil microbial C. On the other hand, long-term application of acidifying fertilizers will impair the survival capability of many soil microorganisms as a result of a reduction in soil pH and thus reduce concentration of soil microbial C. Many studies (e.g. Eiland, 1981; Alef et al., 1988; Nohrstedt et al.; 1989) have shown decreased biomass C due to inorganic fertilizer addition. Whilst
Lynch and Panting (1982) found that N-fertilizer significantly increased biomass C in the soil supporting winter wheat, but only when the crop was direct drilled.

Generally, microbial biomass C represents 2 - 5% of the total soil C over a wide range of locations and soil types (Jenkinson and Ladd, 1981; Smith and Paul, 1990). Srivastava et al. (1989) reported that the biomass C accounted for 2.9% of the soil organic carbon in a mixed dry deciduous forest soil. These literature values are much greater than the value of 0.7% (averaged across treatments and depths, but excluding soils the from L+P treatment) in this study.

Microbial C showed a very poor relationship ($r^2 = 0.32$) with soil moisture content, excluding soils from the L+P treatment.

(c) Effects of low-intensity prescribed burning on soil microbial N and C

**Soil microbial N**

The effects of fire treatments, seasons and depths on soil microbial N measurement are shown in Fig. 6.12. The ANOVA of these data is given in Table 6.6. All main effects were significant and no significant interactions occurred. Microbial N was greater in soils from the long unburned (UB) site than at the other two sites (Fig. 6.13). When data for all four seasons and three depths were combined, the concentrations of microbial N were 102.6, 85.7 and 85.9 mg N kg$^{-1}$ soil for the UB, RB and FB treatments, respectively. The decrease in microbial N concentration at the burnt sites is probably because of volatilisation of C and N in fires. Frequent fire also decreased N mineralization at this site (H. Keith, personal communication). Matson et al. (1987) also found higher microbial biomass N in unburned (secondary forest) than in slash burned (successional and bare) plots. Haines and Uren (1990) found an insignificant effect of stubble burning in autumn on
Figure 6.12 Distribution of microbial N with sampling time in unburnt (UB), regularly burnt (RB) and frequently burnt (FB) sites. Bars are SE of the mean values (n = 6 for June 1988 and n = 3 for other seasons). For June 1988, soils from UB treatment were sampled from 0 - 5 and 5 - 10 cm depths.
Table 6.6 Three-way ANOVA for soil microbial N at the unburnt and burnt sites.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>f value</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main effects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fire</td>
<td>2</td>
<td>5.47</td>
<td>0.072</td>
</tr>
<tr>
<td>Depth</td>
<td>2</td>
<td>19.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Season</td>
<td>2</td>
<td>38.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Two-way interactions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fire x depth</td>
<td>4</td>
<td>2.03</td>
<td>0.155</td>
</tr>
<tr>
<td>Fire x season</td>
<td>4</td>
<td>0.52</td>
<td>0.725</td>
</tr>
<tr>
<td>Depth x season</td>
<td>4</td>
<td>0.53</td>
<td>0.712</td>
</tr>
<tr>
<td><strong>Three-way interaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burning x depth x season</td>
<td>8</td>
<td>0.27</td>
<td>0.973</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Three seasons, e.g. spring (September, 1988), summer (January, 1989) and autumn (March, 1989), were used in the analysis (see Fig. 6.12).

Biomass N in an agricultural soil in Australia, but Powlson et al. (1987) found significantly lower biomass N values in Danish soils where cereal straw had been burnt rather than incorporated. The amount of total soil N in the microbial biomass averaged over fire treatments, depths and seasons was 3.3 (range 3.1 - 3.7) which is within the range (1.4 - 4.7%, mean 2.8) for stubble unburnt and burnt sites (Haines and Uren, 1990), but higher (0.9 - 2.0%, mean 1.5) than Powlson's study.

Season (i.e. time of soil sampling) had a significant (P<0.001) effect on soil microbial N (Fig. 6.13). Microbial N was highest in spring (September, 1988) and decreased in summer (January, 1989), with no significant change in autumn (March, 1989) as seen before for fertilizer treatments. Soil microbial N concentrations decreased with increasing soil depths (Fig. 6.14) as found previously.
Figure 6.13 Effects of season on microbial N at the UB, RB and FB sites. Data are pooled for depths.
Microbial N (mg kg\textsuperscript{-1} soil)

Figure 6.14 Effects of sampling depth on microbial N at the UB, RB and FB sites. Data are pooled for September 1988, January and March 1989.
The correlations between soil microbial N and field moisture content were low ($r^2 = <0.20$), except for spring samples ($r^2 = 0.37$).

**Soil microbial C**

The quantities of soil microbial C were determined by the SIR method in March, 1989 (autumn) and the results are summarized in Fig. 6.15. The RB and FB sites were last burnt two years prior to sampling (in autumn 1987).

For all soil depths, microbial C was highest at the RB site, less at the UB site and lowest at the FB site. The corresponding microbial C values (averaged over depths) for these three sites were 981, 646, and 406 mg C kg$^{-1}$ soil, respectively. The reason why microbial C increased at the RB site and decreased at the FB site compared to the UB site can only be speculated upon. The FB site had been subjected to six fires since 1973 and 2 to 3 years intervals which would have reduced litter C inputs to the soil and also decreased N mineralization (H. Keith, personal communication). The RB site apparently stimulated litter incorporation and decomposition rates (see Raison et al., 1986). Tateishi et al. (1989) measured biomass C by the FI method in Japanese red pine forest soil, and found no significant difference between unburned and burned treatments. Haines and Uren (1990) also reported no significant effect of stubble burning compared with stubble retention on soil microbial biomass C. On the other hand, Powlison et al. (1987), who studied the long-term effects of straw incorporation and straw burning at sites, reported that microbial biomass C significantly decreased due to straw burning. When averaged across treatments and depths, microbial C as a proportion of total soil organic C was only 0.7%. Microbial C was greatest in the surface 2.5 cm layer and decreased with soil depth at all three sites. This trend followed that of soil organic carbon content. However, microbial C did not show any relationship with field moisture content ($r^2 = 0.40$).
Figure 6.15  Microbial C concentration measured by the SIR method of the UB, RB and FB sites. Soils sampled in March 1989. Bars are SE of the mean values.
(d) Comparison of estimates of soil microbial N and C in differently treated RE soil, and unburnt and burnt sites

Comparison between microbial N and C in differently treated RE soil, and unburnt and burnt sites was made on soils sampled in March 1989. The correlation between the microbial N and C was significant ($r^2 = 0.78$), excluding soil samples from the L+P and RB treatments (Fig. 6.16). Many studies also show highly significant correlation between the two measurements. For example, Srivastava et al. (1989) found a coefficient of regression ($r^2$) of 97% in mine spoils, native forest and deforested soils in a dry tropical environment. Christie and Beattie (1989) reported that the correlation ($r$) between biomass C and N in differently treated grassland soil was 0.96. Ocio and Brookes (1990a) found a very close ($r = 0.98$) relationship between biomass N and C (fumigation-extraction methods) in straw amended and unamended soils.

The ratios of microbial C-to-microbial N in different soils are presented in Table 6.7. The ratios were significantly higher in the 0 - 2.5 cm layer compared to those obtained for other depths. This perhaps suggests that more of the biomass is metabolically active in the surface soil and that this declines with increasing depth in the soil (i.e. SIR measures the active biomass component, but FE measures microbial N contributed by both active and resting microorganisms). Excluding data for L+P treatment, the mean ratio for the fertilized soils was 5.0 (range 3.0 - 8.1) which is same as the value found for other forest soils (see Smith and Paul, 1990) and comparable (6.2, range 4.5 - 9.0) to values obtained for a wide range of English forest soils (Vance et al., 1987c). However, the ratios in this present study were much lower than in virgin grassland soils (8.7 - 13.2) (Dalal and Mayer, 1986) or in dry tropical forest soil (11.6) (Srivastava et al., 1989). Schnurer et al. (1985) reported that the C:N ratio of the soil microbial biomass was almost constant (5.9 - 6.1), regardless of treatment or sampling time. The ratios for the unburnt and burnt sites
Figure 6.16 Relationship between soil microbial N and C estimated from SIR for differently treated RE soil, and the UB and FB sites. Soils were sampled in March 1989. Samples from L+P and RB were excluded, because of variable microbial C contents.
Table 6.7 The ratios of estimated soil microbial C to microbial N at various sampling depths of differently treated red earth soil under eucalypt forest (a), and unburnt and burnt sites (b).

<table>
<thead>
<tr>
<th>Soila</th>
<th>Depth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 2.5</td>
</tr>
<tr>
<td>(a) Treatment</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.1 (0.2)b</td>
</tr>
<tr>
<td>L+P</td>
<td>19.7 (4.3)</td>
</tr>
<tr>
<td>P</td>
<td>5.7 (1.0)</td>
</tr>
<tr>
<td>N</td>
<td>5.1 (0.3)</td>
</tr>
<tr>
<td>N+P</td>
<td>8.1 (0.7)</td>
</tr>
<tr>
<td>Sugar</td>
<td>7.7 (1.7)</td>
</tr>
<tr>
<td>(b) Fire</td>
<td></td>
</tr>
<tr>
<td>UB</td>
<td>9.3 (0.9)</td>
</tr>
<tr>
<td>RB</td>
<td>14.8 (1.6)</td>
</tr>
<tr>
<td>FB</td>
<td>6.7 (1.6)</td>
</tr>
</tbody>
</table>

a Soils sampled in March, 1989 (autumn).
b SE of the mean values in parentheses (n = 3).

varied from 3.5 to 14.8 (mean 8.3). Overall, the ratios were higher at the RB site, less at the UB and lowest at the FB site, the pattern similar to microbial C. Powlson et al. (1987) found slightly higher biomass C-to-biomass N ratio in the straw-burnt plots than straw-incorporated plots.

6.4 Summary

The studies reported in this section investigated the effects of different soil amendments as well as the influence of different prescribed burning regimes on soil microbial N and C. The distribution of these measurements with depths and the seasonal nature of soil microbial N was also studied.
Generally, soil amendment did not change microbial N concentrations in YP soils, but microbial N contents varied with the treatments in RE soil. Similarly, regularly (6 - 7 yearly) or frequently (2 - 3 yearly) burning lowered the microbial N values compared with those of the long unburned soil, probably because of volatilisation of N in fires and less N mineralization, particularly in frequently burnt site. By contrast, microbial C was affected by soil amendment or fire, e.g. N+P addition increased microbial C in the 0 - 2.5 cm soil, whilst addition of N and P decreased microbial C at all depths compared to control soil. Regularly burnt soils contained more microbial C, long unburnt soils less and frequently burnt soils least.

Both microbial N and C concentrations were higher at the upper layer and decreased with increasing depths. Microbial N was generally greater in spring and lower in summer probably because of dry conditions. Summer and autumn values were similar. Effects of the fertilizer were more pronounced in spring than in the other seasons. However, the amounts of microbial N and C were approximately correlated.

The overall results suggest the need for soil sampling at regular intervals to monitor the effects of these various management practices on the dynamics of soil microbial N and C.

The following chapter examines the relationships between soil microbial N and N mineralization under both laboratory (controlled) conditions and under undisturbed field conditions in a range of acid forest soils.
CHAPTER SEVEN
CHAPTER SEVEN

SOIL NITROGEN MINERALIZATION AND ITS RELATIONSHIP WITH MICROBIAL NITROGEN

7.1 Introduction

7.2 Materials and Methods

7.3 Results and Discussions
   (a) Comparison of N mineralization ($N_{\text{min}}$) under laboratory and field (in situ) conditions at similar times or at different time
   (b) Relationship between N mineralization and soil microbial N

7.4 Conclusions
CHAPTER SEVEN

SOIL NITROGEN MINERALIZATION AND ITS RELATIONSHIP WITH MICROBIAL NITROGEN

7.1 Introduction

Mineralization of nitrogen is a critical component of the N cycle in forest soils and influences other N cycling processes, such as plant uptake, leaching and denitrification. Management-induced changes in soil N availability can be detected by measuring N mineralization rates in situ (Raison et al., 1987; 1990).

It is likely that the N mineralized during incubation of unfumigated soils is derived from both microbial and non-microbial sources. In aerobic incubations, Shen et al. (1984) reported that only 68% of microbial N was mineralized over 10 days after soils were fumigated with chloroform. Myrold (1987) hypothesized that the N mineralized during anaerobic incubation came mainly from the killed microbial biomass and suggested that anaerobic incubation and fumigation-incubation resulted in mineralization of the same pool of soil N.

The microbial biomass is both the labile fraction of soil organic matter and the agent of biochemical change in soil (Jenkinson, 1988). Microbial biomass is considered as a potential source of readily-mineralized N (Jenkinson and Ladd, 1981). Thus the amount of N contained in the soil microbial biomass may be a useful index of N availability. Moreover, biomass measurements can reveal management-induced changes in soil fertility before they become apparent in total C and N reserves (e.g. Powlson and Jenkinson, 1976; Powlson et al., 1987). Methods for early detection of change in soil fertility are especially important in forest environments because clear trends in alteration to growth of trees may take many
years to establish. Early detection of long-term adverse trends can allow change to management practices and thus lessen soil damage in the long term. The nitrogen content of soil microbial biomass may be an important fraction contributing to N turnover in forest soils.

Microbial biomass C and N contents in various ecosystems have been found to be positively correlated with indices of mineralizable N using both aerobic and anaerobic incubations under laboratory conditions (Hesebe et al., 1985; Hart et al., 1986; Myrold, 1987; Williams and Sparling, 1988). The possible reason for the significant relationships of microbial biomass C and N with anaerobic N mineralization is that much of the NH$_4^+$ released during the incubation comes from mineralization of N from dead aerobic microorganisms killed by the anaerobic conditions (Adams and Attiwill, 1986). Several other studies (e.g. Carter, 1986; Carter and Rennie, 1982; Carter and McLeod, 1987) also reported strong correlation ($r^2 = 0.8$ to 0.9) between biomass N and nitrogen mineralization potential ($N_0$) values for several agricultural and grassland soils. Ross and Cairns (1981) and Azam et al. (1986) found no relationship between microbial biomass N and mineralized N in grassland and agricultural soils.

Estimation of microbial biomass N as an index of the pool of mineralizable organic N in forest soils must apply to undisturbed soils because soil mixing can markedly affect both the magnitude and direction of soil N mineralization (Raison et al., 1987). The in situ core method uses relatively undisturbed soils and also captures the effects of fluctuating environmental conditions (i.e. temperature and moisture) on N mineralization. Binkley and Hart (1989) emphasized the need for further studies to evaluate seasonal change in the size of forest soil microbial N and how they relate to seasonal change in in situ N mineralization. Soil microbial biomass N represents a pool of N, but not necessarily the rate of flux through it and thus net N mineralization.
As an alternative to the measurement of soil N mineralization *in situ*, which is a laborious and expensive exercise, direct measurement of changes in the labile microbial biomass N would be advantageous if it proved to be a good index of N mineralization. Measurement of microbial biomass can be done quickly using the fumigation-extraction method, as described in Chapter 4 (see also Jenkinson, 1988), and its determination is thus much less resource demanding than is the measurement of N mineralization *in situ*. This chapter describes several tests of the usefulness of microbial biomass N as a measure of labile organic matter and N mineralization in forest soils.

### 7.2 Materials and Methods

The soils used in these studies are listed in Table 7.1. Differently treated yellow podzolic (YP) soils were sampled in September 1986, while untreated YP and

<table>
<thead>
<tr>
<th>Soils and treatments&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Month of sampling</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) YP soil C, IL, IF, I, F and Sludge</td>
<td>September 1986</td>
<td>Laboratory and <em>in situ</em> N mineralization at similar time</td>
</tr>
<tr>
<td>(b) Untreated YP and RE soils</td>
<td>September 1986, April and October 1987</td>
<td>Seasonal changes in <em>in situ</em> N mineralization</td>
</tr>
<tr>
<td>(c) RE soil C, L+P, P, N, N+P and sugar, and prescribed fire (e.g. UB, RB and FB treatments)</td>
<td>June and September 1988, and January 1989</td>
<td>Microbial N and <em>in situ</em> N mineralization</td>
</tr>
</tbody>
</table>

Forest types

| *E. delegatensis*, *E. dives* - *E. dalrympleana* and *E. pauciflora* | June and December 1988 | Microbial N and *in situ* N mineralization |

<sup>a</sup> For detailed description of soils and treatments, see Chapter 3 (section 3.2).
red earth (RE) soils for the seasonal changes in N mineralization were collected in September 1986, April and October, 1987 from the 0 - 5 cm soil depth. Soils from differently fertilized RE (i.e. control, L+P, P, N, N+P and sugar treatments), and from long unburned (UB), regularly burned (RB) and frequently burned (FB) were sampled at three different times (i.e. June and September, 1988 and January, 1989) from 0-2.5, 2.5 - 5, and 5 - 10 cm depths. Soils under different forest types (i.e. *E. delegatensis*, *E. dives* - *E. dalrympleana* and *E. pauciflora*) were sampled in May and December 1988 from 0 - 5 and 5 - 10 cm depths.

Mineralization of N in the laboratory was measured on sieved (< 2mm) soils during 10 days of aerobic conditions, as described in Chapter 5 (section 5.1). The sequential *in situ* soil-core technique described by Raison et al. (1987) was used to estimate net N mineralization. Hereafter the term 'net' has been omitted when referring to mineralization. Nitrogen mineralization was calculated as the changes in mineral-N content in confined, covered (no N uptake by roots) soils during field exposure (Raison et al., 1990). At the start of each measurement period, either 24 (for YP) or 36 (for RE) soil cores (5 cm diameter) per plot were pushed into the soil to a depth of 20 cm, eight or twelve cores were removed immediately with intact soil. Of the 16 or 24 cores left *in situ*, eight or twelve were covered to prevent leaching. Field exposures varied from 30 to 60 days during the main growing-season to about 110 days during winter (Raison et al., 1990). In the laboratory, soils were removed from sampling tubes, bulked by depth increments, sieved (<5 mm) and the fine earth fraction weighed. Mineral N was extracted from fine earth by shaking 10 g soil in 50 ml 0.5M K₂SO₄ for 1 hour, followed by filtering. Analyses of NH₄ and NO₃-N were same as described earlier (Chapter 3). The mass of fine earth was used to convert both N mineralization and microbial N estimates on an area basis and the results are expressed as kg N ha⁻¹ 10 d⁻¹ and kg N ha⁻¹, respectively.
Microbial N was measured by the hexanol fumigation-extraction procedure and the final calculation was done as described in Chapter 4 (section 4.6). The relationships between either laboratory and in situ mineralization, or between N mineralization and microbial N were done using replicated values of each plot as individual observations.

7.3 Results and Discussions

(a) Comparison of N mineralization (N\text{min}) under laboratory and field (in situ) conditions at similar times or at different times

The amount of N mineralized under laboratory conditions were higher than those mineralized under field conditions at all treatments (Fig. 7.1). However, the average rates of N\text{min} (kg N ha\textsuperscript{-1} 10 d\textsuperscript{-1}) ranged from 0.74 to 4.84 (mean 2.73) and from 0.03 to 0.35 (mean 0.11) for the laboratory and in situ incubation, respectively. Thus in situ N mineralization was only 4% (range 2 to 10%) of that obtained under laboratory conditions when averaged over all treatments. The increase in laboratory mineralization results from soil mixing and more optimal soil moisture and temperature (e.g. Kenney, 1980; Kladivko and Kenney, 1987; Raison et al., 1987).

Table 7.2 shows the percentage of mineralized N as total soil N and microbial N in the differently treated YP soil. Laboratory and in situ N mineralization accounted for 0.25 to 1.65% (mean 0.94) and from 0.01 to 0.12% (mean 0.04) of total soil N, respectively. This is only a small amount. When expressed as the percentage of soil microbial N, N mineralization during laboratory and field incubations varied from 8.06 to 41.30% (mean 22.84) and from 0.17 to 3.47% (mean 1.02), respectively.

Results of the time course study on N mineralization under both laboratory and field conditions also show that the level of N mineralization in the upper 5 cm of
Figure 7.1 Mineralization of N in differently treated yellow podzolic (YP) soil (0 - 5 cm depth) under laboratory and field (in situ) conditions.
Table 7.2 Mineralization of N ($N_{\text{min}}$) under laboratory and field (in situ method) conditions, expressed as percentage of either total soil N or soil microbial N for differently treated yellow podzolic (YP) soil (0-5 cm depth).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Total soil N</th>
<th>Microbial N</th>
<th>Total soil N</th>
<th>Microbial N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.87 (0.20)a</td>
<td>17.56 (2.59)</td>
<td>0.02 (0.01)</td>
<td>0.48 (0.14)</td>
</tr>
<tr>
<td>IL</td>
<td>1.27 (0.16)</td>
<td>35.79 (3.32)</td>
<td>0.12 (0.02)</td>
<td>3.47 (0.29)</td>
</tr>
<tr>
<td>IF</td>
<td>0.74 (0.18)</td>
<td>23.31 (5.18)</td>
<td>0.02 (0.01)</td>
<td>0.71 (0.17)</td>
</tr>
<tr>
<td>I</td>
<td>0.25 (0.08)</td>
<td>8.06 (2.96)</td>
<td>0.01 (0.01)</td>
<td>0.33 (0.16)</td>
</tr>
<tr>
<td>F</td>
<td>1.65 (0.22)</td>
<td>41.30 (3.32)</td>
<td>0.04 (0.01)</td>
<td>0.98 (0.25)</td>
</tr>
<tr>
<td>Sludge</td>
<td>0.85 (0.12)</td>
<td>11.04 (0.50)</td>
<td>0.01 (0.01)</td>
<td>0.17 (0.04)</td>
</tr>
</tbody>
</table>

*a* SE of the mean in parentheses.

the untreated YP and RE soils was significantly higher under laboratory conditions than those obtained under field conditions (Fig. 7.2). The *in situ* N mineralization for the two soils was only 3% of that obtained under laboratory conditions when averaged across all sampling times. However, the pattern of N mineralization varied with time of soil sampling, being higher in September 1986.

When expressed as the percentage of total soil N, the amount of mineralized N under laboratory and *in situ* conditions varied from 0.14 to 0.87% (mean 0.43%) and from 0.01 to 0.05% (mean 0.2%), respectively (Table 7.3). Similarly, laboratory and *in situ* N mineralization was 2.73 to 17.56% (mean 8.80%) and 0.11 to 0.55% (mean 0.29%) of the soil microbial N, respectively.

The relationship between N mineralization in the laboratory and *in situ* incubations was evaluated using differently treated YP soil (Fig. 7.1) and untreated YP and RE soils sampled at different times (Fig. 7.2) and is shown in Fig. 7.3. The correlation between these two measurements was very poor ($r^2 = 0.32$, $P<0.01$). The poor relationship between field and *in situ* N mineralization can be due to both
Figure 7.2 Seasonal variation of laboratory and *in situ* N mineralization of untreated YP and RE soils.
Figure 7.3 Relationship between field and laboratory N mineralization.

\[ y = 1.5 + 8.32 x \]

\[ r^2 = 0.32 \ (n = 44) \]
Table 7.3 Seasonal mineralization of N ($N_{\text{min}}$) under laboratory and field (in situ method) conditions, expressed as percentage of either total soil N or microbial N of untreated yellow podzolic (YP) and red earth (RE) soils (0-5 cm depth).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Month of sample collection</th>
<th>Laboratory N$_{\text{min}}$ as %</th>
<th>Field N$_{\text{min}}$ as %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total soil N</td>
<td>Microbial N</td>
</tr>
<tr>
<td>YP</td>
<td>September 86</td>
<td>0.87 (0.20)$^a$</td>
<td>17.56 (2.59)</td>
</tr>
<tr>
<td></td>
<td>April 87</td>
<td>0.47 (0.09)</td>
<td>10.21 (2.73)</td>
</tr>
<tr>
<td></td>
<td>October 87</td>
<td>0.45 (0.18)</td>
<td>10.73 (1.15)</td>
</tr>
<tr>
<td>RE</td>
<td>September 86</td>
<td>0.44 (0.05)</td>
<td>6.21 (0.67)</td>
</tr>
<tr>
<td></td>
<td>April 87</td>
<td>0.14 (0.04)</td>
<td>2.73 (0.69)</td>
</tr>
<tr>
<td></td>
<td>October 87</td>
<td>0.22 (0.03)</td>
<td>5.36 (0.77)</td>
</tr>
</tbody>
</table>

$^a$ SE of the mean in parentheses.

Differing environmental conditions and soil disturbance (Raison et al., 1987). Gosz and White (1986) reported that field N mineralization and nitrification rates were positively correlated with those measured in laboratory incubations in four forest ecosystems (ponderosa-pine, mixed conifer, aspen and spruce-fir) in New Mexico. Rice et al. (1987) also found a good positive correlation for plowed and no-till soils. However, these workers used intact soil cores to estimate N mineralization under laboratory conditions.

(b) Relationship between N mineralization and soil microbial N

There was no relationship ($r^2 = 0.05$) between laboratory N mineralization and soil microbial N (Fig. 7.4). Similarly, in situ N mineralization also showed no relationship with microbial N for either YP soil (Fig. 7.5a) ($r^2 = 0.017$, Table 7.4) or differently treated RE soil when pooled data used (Fig. 7.5b) ($r^2 = 0.020$, Table 7.4). The only positive significant but poor correlation ($r^2 = 0.266$; Table 7.3) was observed
Figure 7.4 Relationship between soil microbial N and laboratory N mineralization.

\[ y = 2.2 + 0.02 \times \]
\[ r^2 = 0.005 \ (n = 60) \]
Figure 7.5 Relationships between microbial N and \textit{in situ} N mineralization for YP soil (a) and differently treated RE soil (b).
Table 7.4  Simple linear regression and coefficient of determination ($r^2$) between *in situ* N mineralization (N$_{\text{min}}$) and soil microbial N in (a) YP soil and (b) RE soil. Form of equation is $y = $ Field N$_{\text{min}}$ (kg N ha$^{-1}$ 10$^{-1}$ days) and $x =$ Microbial N (kg N ha$^{-1}$).

<table>
<thead>
<tr>
<th>Soil and treatments</th>
<th>Regression line $y = a + bx$</th>
<th>$r^2$</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) YP</td>
<td>$y = 0.12 - 0.002 x$</td>
<td>0.017</td>
<td>NS</td>
</tr>
<tr>
<td>(b) RE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilized</td>
<td>$y = 0.20 - 0.001 x$</td>
<td>0.005</td>
<td>NS</td>
</tr>
<tr>
<td>Prescribed fire</td>
<td>$y = 0.03 + 0.005 x$</td>
<td>0.266</td>
<td>P$&lt;$0.01</td>
</tr>
<tr>
<td>Forest types</td>
<td>$y = 0.25 - 0.002 x$</td>
<td>0.004</td>
<td>NS</td>
</tr>
<tr>
<td>All RE soils</td>
<td>$y = 0.11 + 0.002 x$</td>
<td>0.020</td>
<td>NS</td>
</tr>
</tbody>
</table>

a YP soils 0 - 5 cm, whilst RE soils were 0 - 10 cm depth.

b Number of measured samples.

for two measurements on the differently burned RE soil. Clearly microbial N is not an index of the pool of mineralizable organic N in these soils. The results suggest that higher rates of N mineralization are not associated with greater soil microbial N contents but rather with higher microbial activity. The results are in accord with those of Powlson et al. (1987) who also found no relationship between the mineral N flush after fumigation and the N mineralized during 10 days of aerobic incubation in a range of agricultural soils. But these authors reported a close correlation with 10 to 60 days of incubation. Several other studies (e.g. Ross and Cairns, 1981; Azam et al., 1986) have also found no significant relationship between microbial biomass N and mineralized N during 56 days of aerobic incubation for pasture and agricultural soils. A few other studies (e.g. Carter, 1986; Carter and Rennie, 1982 and Carter and McLeod, 1987) have shown strong correlations ($r^2 = 0.8$ to 0.9) between
biomass N and N mineralization potential ($N_0$) values for several agricultural and grassland soils. Hart et al. (1986) found a positive significant correlation between biomass C measured by the SIR method and the amount of N mineralized aerobically in a range of plant litters, peats and soils of moderate to low pH.

7.4 Conclusions

In the soils studied, net N mineralization during aerobic laboratory incubations for 10 days was a poor predictor of field N mineralization. Laboratory N mineralization rates were many fold higher than the field rates. Only a small (< 1%) fraction of the soil N was mineralized.

Soil microbial N was not correlated with either laboratory or field N mineralization. This indicates that biomass N represents a pool size of N, not a measure of its turnover and thus net N mineralization.
CHAPTER EIGHT
CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSIONS

8.1 Introduction

8.2 Measurement of microbial biomass N in acid forest soils

8.3 Estimation of soil microbial N and C using a single K$_2$SO$_4$-extract of hexanol fumigated soil

8.4 Effects of season and treatment on soil microbial N and C in several forest soils

8.5 Soil microbial N as an index of N mineralization
8.1 Introduction

Measurement of soil microbial biomass C and N content is an important step in studying the flux of energy and nutrients through the microbial population and thus nutrient cycling in soils. None of the currently available methods that attempt to quantify soil microbial biomass is without assumptions or limitations. There are special problems associated with the estimation of microbial biomass in acid forest soils. These result from slow microbial growth due to low soil pH, and the high potential for immobilization of N because of the addition of large amounts of fresh organic materials having a wide C:N ratio. This thesis is mainly concerned with the development of a direct extraction method for estimating microbial biomass N in acid forest soils. The results of experiments described in Chapters 4 to 7 are integrated and discussed here.

8.2. Measurement of microbial biomass N in acid forest soils

A direct extraction method for measuring microbial biomass in acid forest soils was developed (Chapter 4) and compared with the chloroform fumigation-incubation (FI) method (Chapter 5). The new method involves the use of hexanol, a less hazardous fumigant than chloroform, direct extraction of total K$_2$SO$_4$-soluble N from fumigated soil, and use of a modified 'control'.

Exposure of the three acid forest soils studied to hexanol for 16 to 24 hours achieved complete fumigation in most cases, but a longer fumigation (up to 120
hours) yielded slightly higher (18% more than 24-hour value) FE-N flush in a highly acid red earth soil. Quantitatively, hexanol fumigation had a greater effect on \( \text{K}_2\text{SO}_4 \)-extractable organic N than on extractable mineral N. The proportion of total N in \( \text{K}_2\text{SO}_4 \)-extracts of fumigated soils present as organic N ranged from 63 to 88%, and there was a tendency for this to decline with increasing fumigation time. This suggests that enzymes may have continued to degrade soluble organic N compounds during fumigation. One-day chloroform fumigation followed by immediate extraction gave a slightly higher FE-N flush compared to 5-day chloroform fumigation. This contrasts with the findings of Brookes et al. (1985a) with agricultural soils. A possible reason for this disparity is that the soils used in this study were much more highly acid than those used by Brookes et al. (1985b). Davidson et al. (1989) found the maximum efficiency of chloroform fumigation was 24 hours for acid forest soils and 5 to 7 days for grassland soils.

Single and repeated fumigations released similar amounts of microbial N, but a single \( \text{K}_2\text{SO}_4 \) extraction was not sufficient to extract all microbial N released by fumigation. During a second extraction of fumigated soils, a further 26 to 44% of the N initially extracted was removed. This amount of N removed reached a nearly constant value (of about 10% of the initial quantity) after the third or fourth extraction. These findings suggest that at least three extractions are required to extract all the microbial N from fumigated soils. In unfumigated soils, there was a tendency for a reduction in \( \text{K}_2\text{SO}_4 \)-extractable N from the first extraction to the second and third extraction, indicating that a portion of total N in the first extraction comes from microbial sources.

Part of the biomass N in unfumigated soils appears to be solubilized (attacked) very quickly (in less than 15 min) by \( \text{K}_2\text{SO}_4 \) and thus extracted into the solution. Increasing the duration of exposure of soils from 15 min to 240 min to \( \text{K}_2\text{SO}_4 \) slightly increased the amount of total N extracted (Fig 4.15). The amounts of
total N removed during a second 60 min extraction showed a sharp drop due to the initial 15 min extraction. Thus pre-treatment with K$_2$SO$_4$ decreased the amount of microbial N released after subsequent fumigation. Widmer et al. (1989) also reported that longer exposure of soil to K$_2$SO$_4$ increased the amount of total N extracted from unfumigated soil and demonstrated this was derived from biomass N solubilized by K$_2$SO$_4$. Subtraction of K$_2$SO$_4$-extractable total N in unfumigated soils (as a control) from that in fumigated soils therefore yields underestimates (of 50 to 80%, depending on soil type; Table 4.11) of microbial N. Azam et al. (1989a) also showed that a significant portion (15 to 28%) of organic N in K$_2$SO$_4$-extracts of unfumigated soils was derived from biomass. To separate non-microbial and microbial fractions of total N in K$_2$SO$_4$-extracts of unfumigated and fumigated samples, soils were extracted sequentially eight times and a curve-splitting technique was used (Figs 4.18 to 4.21; Tables 4.6 to 4.9).

Cumulative total N extracted sequentially in K$_2$SO$_4$ from three unfumigated soils was approximately linear after the third extraction (Fig. 4.17). Linear regressions account for >99% of the variance in all cases. Assuming that only non-microbial N is extracted after the third extraction, then the microbial component of the total N removed in the first extraction is equal to the intercepts of the regressions (Table 4.6). Thus the microbial fraction of total N in the initial K$_2$SO$_4$ extract of unfumigated YP, RP and RE soils are 6.5, 6.4 and 18.9 mg N kg$^{-1}$ soil, respectively. Extractable-total N for these three soils was 11.2, 12.6 and 29.1 mg N kg$^{-1}$ soil. Therefore the non-microbial N contents of initial extracts from unfumigated samples of YP, RP and RE soils were 4.7, 6.2 and 10.2 mg N kg$^{-1}$ soil, respectively. Similarly, cumulative total N in the K$_2$SO$_4$-extracts of hexanol and chloroform fumigated samples showed a curvilinear relationship for the first three extractions and then the relationships were linear (Fig. 4.18). This indicates that the first three extractions contained both non-microbial and microbial fraction of N. The slopes and intercepts were comparable for the two fumigants, but varied among the soils. The microbial N
extracted in the fumigated soils was 32, 41 and 96 mg N kg\(^{-1}\) soil (averaged across hexanol and chloroform fumigants) for the YP, RP and RE soils, respectively.

Utilizing the above information, microbial N in different soils was calculated using different approaches (Table 4.10). These were: (i) by curve-splitting using a regression approach, (ii) as \(F_T - UF_M\), where \(F_T =\) Total N in K\(_2\)SO\(_4\) extracts of fumigated soil, and \(UF_M =\) Mineral N in K\(_2\)SO\(_4\) extracts of unfumigated soil, and (iii) as \(F_T - UF_T\), where \(UF_T =\) Total N in K\(_2\)SO\(_4\)-extracts of unfumigated (control) soil.

The microbial N estimated by curve-splitting (i.e. correcting for non-microbial N in the fumigated soil) is theoretically most correct, but this method is rather complicated for routine use. A similar range of 16% of the mean estimates of microbial N were obtained from approaches (i) and (ii) above. Approach (ii) is simple and practical but requires a small soil specific correction factor (ranging from 12 to 22%; Table 4.11). The microbial N estimates for several of the forest soils studied, were very low if the method of Brookes \textit{et al.} (1985b) was used (Table 4.10), and would require a much larger correction factor (ranging from 55 to 81%; Table 4.11).

Estimates of microbial N obtained by method (ii) above were well correlated with estimates obtained using chloroform fumigation-extraction methods (Fig. 4.22 and Table 4.12). A highly significant correlation between estimates of microbial N obtained by the 1-day hexanol and 5-day chloroform fumigation-extraction methods confirmed the reliability of the former method. The correlation between the estimates of microbial N obtained after 1-day hexanol and 1-day chloroform fumigation was, however, only reasonable. McLaughlin \textit{et al.} (1986) reported that the hexanol fumigation-extraction method was as good as chloroform fumigation-extraction for estimating biomass P.

For the soils studied, and the method proposed, it is unknown whether the use of a \(k_N\) is necessary to convert microbial N estimates data to biomass N estimates
and if so, what this factor should be. Brookes *et al.* (1985b) proposed a \( k_N \) factor of 0.54 for the chloroform fumigation-extraction method based on a calibration against the FI method using a \( k_N \) of 0.68 (Shen *et al.*, 1984) to convert from FI-N flush to biomass N. No other \( k_N \) factor has been proposed for conversion of microbial N determined by the direct extraction procedure to biomass N. The \( k_N \) factor for the FI method covers a wide range, from 0.28 (Paul and Voroney, 1984) to 0.68 (Shen *et al.*, 1984), and depends on a number of factors, such as soil type especially texture and organic matter content, age of microbial tissue, soil treatment, season of sample collection and soil pH. Moreover, the determination of \( k_N \) is rather complicated and also involves several assumptions and simplifications. Thus several workers (e.g. Adams and Laughlin, 1981; Carter and Rennie, 1984; McGill *et al.*, 1986) have expressed their results as a mineral-N flush (i.e. FI-N flush) rather than attempt to convert to biomass N. It is recognized that the factor to convert to biomass N is likely to be different for each soil studied and possibly for different treatments imposed on them (e.g. fertilizer, liming and fire regimes). In this thesis, the estimate of microbial N obtained from direct extraction procedures is considered as an index, and no attempt has been made to convert it to microbial biomass N.

The microbial N calculated by the proposed hexanol fumigation-extraction method was significantly correlated \((r^2 = 0.66)\) with the FI-N flush obtained after chloroform fumigation-incubation (FI) (Fig. 5.3). This indicates that the microbial N estimated by the extraction and incubation methods originates from the same source. The slope of the regression was greater than 1.7; thus the direct extraction gives higher estimates of microbial N possibly because of immobilization of mineral N during the incubation step. The correlation between the estimates obtained after 5-day chloroform fumigation-extraction and fumigation-incubation, and the slope of the regression were very similar \((r^2 = 0.64, \text{ slope } 1.84)\). For the 1-day chloroform fumigation-extraction and fumigation-incubation, the relationship was poor \((r^2 = 0.23)\) and the slope much less (0.6). Brookes *et al.* (1985b) found a 1:1 relationship
between Fl-N flush and FE-N flush (following 5 days of chloroform fumigation), indicating that the extractable and mineralizable biomass fraction are largely the same. In a recent study, Martikainen and Palojarvi (1990) found a close correlation between biomass N estimated from chloroform fumigation-extraction and those derived from the amount of microbial C determined by microscopic counting in several acid forest soils, if they assumed that the C-to-N ratio of biomass released by fumigation was the same as that of the general microbial population. The FI method has several limitations, such as immobilization and denitrification during incubation and requires more time. The direct extraction methods are faster and do not suffer from these other problems.

In conclusion, any of the fumigation and direct extraction procedure can be used for estimating microbial N in the acid forest soils studied, but care must be taken to use an appropriate control. Definition of an appropriate $k_N$-factor is an unresolved issue. The hexanol fumigation-extraction method is safer and simpler than the chloroform methods.

**8.3 Estimation of soil microbial N and C using a single K$_2$SO$_4$-extract of hexanol fumigated soil**

Soil microbial C can be measured simultaneously using the hexanol fumigation-extraction method which was developed for microbial N. To do this, residual hexanol-C must be removed from the fumigated extracts prior to determination of carbon content by oxidation. This is easily done by heating extracts in an oven at 70 °C for about 30 minutes. After removal of residual hexanol-C from the fumigated solution, there was a highly significant correlation ($r^2 = 0.99$) between the estimates of microbial C determined by the hexanol and chloroform fumigation-extraction methods (Fig. 3.12). Thus microbial N and C can be measured on the same extracts. Sparling and West (1988b) reported that both microbial biomass C
and N could be measured simultaneously from a single extractant after chloroform fumigation. The advantages of using the same extract for both microbial N and C determination include major savings in time, labour, and cost of analytical reagents.

8.4 Effects of season and treatment on soil microbial N and C in several forest soils

Repeated measurements at several sites (Chapter 6) show that the concentrations of microbial N varied with season. The changes in soil microbial N from lowest to highest values for the untreated YP, RP and RE soils were 19, 63 and 55%, respectively. Soil microbial N concentration was generally highest in winter and lower in summer, possibly because of low soil moisture contents. Many other factors may also determine the reasons for seasonal variation in biomass content. These factors are temperature, litterfall and fine root substrate inputs, and these could not be adequately investigated in this study.

Under laboratory conditions, air-drying over 2 to 3 days lowered microbial N content by about 45% (range 27 to 58%). A partial reason for this result may be incomplete fumigation in air-dried soils, thus giving less extractable total N (20 to 50%) than in field moist soils probably because of less efficient enzymic hydrolysis of cell components (Sparling and West, 1989). The involvement of hydrolytic enzymes in increasing the extractability of microbial N has been indicated by the fumigation experiments of Brookes et al. (1985a,b) and Amato and Ladd (1988). However, in a similar study, West and Sparling (1988a,b) found a much larger (87 to 90%) decrease in microbial N after air-drying in several pasture soils. It was shown that rewetting air-dried soils immediately prior to fumigation drastically increased the estimate of soil microbial N. The effect of rewetting air-dried soils is thus to increase the extractibility of total N in the fumigated samples, possibly due to increased enzyme activities (Sparling and West, 1989) because of the breakdown of
aggregates which may cause an increase accessibility of enzymes to the substrate (Tabatabai and Bremner, 1970). Thus the results suggest that prior to fumigation dry soils require rewetting in order to obtain reliable biomass estimates. However, an increase of 18 to 58% in K$_2$SO$_4$-extractable total N in unfumigated air-dried soils suggests that some of the additional N is derived from microbial biomass killed by desiccation. The low correlation ($r^2 = 0.33$) between decreased amounts of measured soil microbial N upon air-drying and the increased amount of extractable total N in air-dried unfumigated soils, however, only partly supports this conclusion.

Several forest management practices (fertilization, liming, sewage sludge addition, irrigation, and low-intensity prescribed burning) affected soil microbial N and C content. Sewage sludge addition increased microbial N, whilst inorganic fertilization or irrigation decreased it in a yellow podzolic soil under *Pinus radiata*. The increase in microbial N in the sludge treatment was probably due to a combined liming effect (sludge contained a large amount of CaCO$_3$) and the slow release of N (Sommers, 1977) and P (McLaughlin and Champion, 1987). Sludge-treated soil also had higher microbial activity (Bekunda, 1987). The decrease in microbial N in the N-fertilized or irrigated soils might be attributed to an increased N mineralization (Raison et al., 1990) and soil acidification due to nitrification and nitrate leaching. Singh et al. (1989) found depressed biomass N during periods when N mineralization increased. Another possible cause may be the acidifying effect of fertilizer-N (Christie and Beattie, 1989), and Khanna et al. (1991) found lower soil pH in these N-fertilized soils. However, several studies (e.g. Schnurer et al., 1985; Shen et al., 1989) have shown higher microbial N content in inorganic fertilized soils compared to unfertilized soils. Thus the effects of N-fertilizer on soil microbial N are not universal.

In the red earth soil under *Eucalyptus pauciflora*, effects of fertilizer-N were very similar, but liming did not increase soil microbial N content. The reason is not clear, although liming increased soil pH (Nyborg and Hoyt, 1978) and favours
microbial activity (Bekunda, 1987). Carter (1986) found either similar or slightly higher microbial biomass N after liming. The P fertilized treatment had the lowest soil microbial N compared to other treatments. Long unburned soil contained about 20% more microbial N than soil from regularly or very frequently burned forest. This decrease in burnt forests is probably because of volatilization of N in fires. Frequent fire also decreased N mineralization at this site. This result accords with other findings (e.g. Matson et al., 1987; Powlson et al., 1987). However, Haines and Uren (1990) found no significant difference in microbial biomass N caused by stubble burning. From these results, it is however difficult to explain simply the effects of these various management practices on soil microbial N.

In the variously treated red earth soil, microbial N varied with time of soil sampling, being generally highest in spring and lower in summer, with no significant change in autumn compared to summer. The effect of season on soil microbial N varies for a range of ecosystems studied. For instance, many studies (e.g. Sarathchandra et al., 1988; Singh et al., 1989) have shown higher microbial N in the dry summer months and lower values in winter or rainy seasons. Others (e.g. Ross et al., 1981 and Ritz and Robinson, 1988) found no seasonal pattern in microbial N in pasture and agricultural soils.

Soil microbial C in fertilized and burned treatments was determined by the SIR method, but only at one sampling time. Treatment affected microbial C. For example, microbial C was high in soil which received combination of lime and phosphorus (i.e. L+P), possibly because of solubilization of organic matter. West and Sparling (1986) mentioned that the SIR method could give an overestimation of biomass C in near neutral soils (pH > 6.0) because of dissolution of CO₂ in the soil water. Changes in microbial activity due to liming may also be responsible for the higher respiration and thus microbial biomass C estimate. On the other hand, none of the fertilized soils contained more microbial C than the unfertilized control soil,
while N-fertilized soil contained significantly lower (about 25%) microbial C compared to control. Microbial C in the burnt and unburnt red earth soils showed a different pattern to that of microbial N. Compared to the long-unburnt plot, soil microbial C was significantly higher (about 50%) in the regularly burnt plot, but lower (about 37%) in the very frequently burnt plot, and showed no parallel pattern with microbial N. The reasons for these changes can only be speculated upon, based on other research at this site. Regular fire stimulates litter incorporation and decomposition rates (Raison et al., 1986), whereas the very frequently burning (fires at 2 to 3 years intervals) reduces litter C inputs and decreases N mineralization (H. Keith, personal communication). In contrast, Tateishi et al. (1989) found no significant difference in microbial biomass C (measured by the FI method) in burned and unburned forest soils. The effects of different treatments are obviously not universal, and depend on a number of factors, including soil type, rate and time of fertilizer addition, nature of fertilizer, pattern of litter and root turnover and the intensity of fire. It is very hard to explain the reasons for, or infer pattern of changes in soil microbial C from a once only measurement. Seasonal study would have given a better indication of trends.

Soil microbial N was higher in the surface soil layer than in the lower horizons, possibly because of the greater substrate C inputs (litter and root turnover) to this layer. Generally, microbial N represents 1 to 5% of the total soil N over a wide range of locations and soil types (e.g. Jenkinson and Ladd, 1981; Smith and Paul, 1990). In this study the microbial N was within this range. Like microbial N, soil microbial C was also higher in the upper soil layer and decreased with increasing soil depth.

The seasonal changes in microbial N and the effects of different forest management practices on soil microbial N and C can be monitored by the hexanol fumigation-extraction method. In order to explain patterns, measurements must be done at a regular intervals over several years and much supporting data on microbial substrate dynamics and environmental conditions must also be calculated.
8.5 Soil microbial N as an index of N mineralization

Laboratory rates of N mineralization were generally higher than those measured in situ. Rates of N mineralization under laboratory conditions are insensitive to changing field soil moisture content and temperature (Kenney, 1980; Raison et al., 1987). Seasonal changes in laboratory and in situ N mineralization were apparent, reflecting changes in environmental conditions and the availability of microbial substrates. Although microbial biomass is considered as a pool of readily mineralized N (Jenkinson and Ladd, 1981), soil microbial N and N mineralization rates were very poorly correlated. This suggests that N mineralization depends on the availability of substrates and the activity of soil microorganisms, not on the size of the pool of microbial N. Thus the use of microbial N as an additional tool for assessing soil N availability may not be very productive. In forest soils, N availability depends upon a number of factors such as environmental conditions and substrate (litter and root) 'quality'.

Microbial N estimates may afford no great advantage relative to other methods of assessing N availability for applied assessments of forest nutrition. A similar conclusion was drawn by Binkley and Hart (1989).
REFERENCES


