The effect of restoration plantings and plant genus on soil biota

Daniela Carnovale

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

of the Australian National University

March 2019

Australian National University

Cover images (clockwise from left): mixed species shelterbelt in Murrumbateman New South Wales, © Daniela Carnovale; *Acacia* shelterbelt in Murrumbateman New South Wales, © Daniela Carnovale; Litter bags in the field ready for deployment under litter layer © Daniela Carnovale

Candidate's Declaration

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

Daniela Carnovale Date:13th March 2019

Preface

This thesis is structured as a compilation of connected papers that have been published, have been submitted for publication or are in preparation for submission in scientific journals. Each paper is a stand-alone body of work. However, there is unavoidable repetition of content and methodology between papers. Published chapters and those submitted appear in the thesis as per publication, as such, some formatting, for example references may differ between chapters.

The formatting and content of my thesis complies with The Australian National University's College of Medicine, Biology and Environment Thesis by Compilation Guidelines. An Extended Context Statement has been provided at the beginning of the thesis, which provides a framework for understanding the relationship between the different components of my research and succinctly identifies broad themes that may be especially relevant for practitioners and applicable to other studies further afield. The Extended Context Statement is not intended to be a comprehensive literature review.

I completed the majority of the work, including: study design, data collection, laboratory work, data analysis and write-up. For all papers, I received advice from my supervisors: Dr John Field, Dr Peter Thrall, Dr Andrew Bissett, Dr Geoff Baker, Associate Professor Philip Gibbons and Dr Alan Richardson.

All co-authors peer-reviewed written content and agreed to the submission of each paper. The author contribution statements below have been agreed to in writing by all authors listed. Detailed acknowledgments are provided at the end of each paper.

Paper I. Published

Carnovale, D., Baker, G., Bissett, A., Thrall, P., 2015. Earthworm composition, diversity and biomass under three land use systems in south-eastern Australia. Applied Soil Ecology 88, 32-40.

Conceptualisation and design: DC, GB, AB, PT; Data collection: DC, GB; Data analysis: DC; Manuscript drafting: DC; Manuscript editing: GB, AB, PT.

Paper II. Ready for submission

Carnovale, D., Thrall, P.H., Bissett, A., Baker, G., Shelterbelt influence on soil microbial community structure.

Conceptualisation and design: DC, PT; AB, GB, Data collection: DC; Data analysis: DC; Manuscript drafting: DC; Manuscript editing: PT, AB, GB.

Paper III: Published

Carnovale, D., Bissett, A., Thrall, P.H., and Baker, G. (2019). Plant genus (*Acacia* and *Eucalyptus*) alters soil microbial community structure and relative abundance within revegetated shelterbelts. Applied Soil Ecology, 133, 1-11.

Conceptualisation and design: DC, AB, PT GB; Data collection: DC; Data analysis: DC, AB; Manuscript drafting: DC; Manuscript editing: AB, PT, GB.

Paper IV: In review

Carnovale, D., Richardson, A.E., Thrall, P.H., Bissett, A., Baker, G., The effect of litter type on decomposition rates and soil microbial communities in a shelterbelt system. Pedobiologia

Conceptualisation and design: DC, AR, PT, GB, AB; Data collection: DC, AR; Data analysis: DC, AR; Manuscript drafting: DC; Manuscript editing: AR, AB, PT GB.

Acknowledgements

First of all I would like to thank my fantastic supervisory panel: Dr Peter Thrall, Dr Andrew Bissett, Dr Geoff Baker and Dr Philip Gibbons. I would like to especially thank Peter, Andrew and Geoff who were always available for a chat and offered advice and guidance throughout the project from its start through to submission. Thanks for taking the long journey with me, including during two periods of maternity leave. I would also like to thank Dr John Field for his assistance early in the planning process, and his role as Chair in the early stages; and Dr Gibbons for assuming the role as Chair upon Dr Field's retirement.

I sincerely thank the Australian National University. I am greatly appreciative of all the wonderful support staff at Fenner School for help with all things administration as well the many staff who offered support and guidance over the years. A special thanks to Piers Bairstow, Mauro Davanzo and Andrew Higgins for help with field and laboratory work.

I am grateful for the support from CSIRO, without which this work could not have been carried out. In particular I would like to thank Shamsul Hoque for his assistance in processing DNA extract samples. A very special thankyou to Alan Richardson for all his guidance and assistance with enzyme assay sampling. Thanks to all those in the "Green Machine" for assisting in laboratory work and answering my many questions.

A special thankyou to Greening Australia and the many landholders who provided me with access to their properties to obtain soil samples and take measurements. This research could not have been undertaken without their generosity and support.

A huge thankyou to all those who came and assisted in field sampling, James Tyrell, Linda Grevinga, Tim Goodwin, and Kathleen Anderson. Thanks to all my fellow PhD room buddies including Helen King, Jess Drake, Zoe Read, Marwan, Nathan Webb for the laughs and tears. It was a joy working beside you all sharing the crazy PhD journey.

Finally I am grateful to my friends and family, who stuck by me and supported me over the whole process. A big thankyou to my parents, Pauline and Michael Carnovale who always supported and encourage me to pursue my dreams. Thanks for helping make mesh bags full of litter. A huge thanks my husband, Timothy Goodwin for helping my with field work and setting up experiments as well as being so patient and offering not so subtle nudges to get it finished. You have been a great support to which I am so grateful. And last but not least I would like to

thank my two children Matilda, and Oliver, you do not understand at this stage what I have been working towards but thanks for the many hours of “typing with mummy”.

Abstract

In south-eastern Australia, linear strips of planted trees and shrubs (shelterbelts) are often established to restore ecosystem services that have been altered due to agriculture. Despite their widespread use, little is known about the effect of shelterbelts on soil biota and how they alter aboveground-belowground interactions. In this thesis I aim to: 1) improve understanding of how shelterbelts affect soil biotic communities and how time since establishment may influence soil biota, 2) investigate how dominant shelterbelt tree genera (*Acacia* and *Eucalyptus*) influence soil biota, and 3) explore how changes in dominant tree genera affect key ecosystem process such as decomposition rates, microbial community structure and enzyme activities associated with carbon degradation. This thesis is structured as a compilation of connected papers: a literature review, two papers that have been published, and a further two papers, one in review and, one ready for submission. Each paper is a stand-alone body of work.

In the first paper I assessed earthworm composition, diversity and biomass in three land use systems: native shelterbelts dominated by *Acacia* and *Eucalyptus* species, agricultural pastures and native remnant woodland fragments dominated by *E. blakelyi* and/or *E. melliodora*. Earthworm communities differed significantly among systems; with abundance, biomass and diversity greatest under pasture. Within shelterbelts I saw a shift from high earthworm biomass and density to low values with increasing time after establishment. Soil edaphic variables did not correlate strongly with earthworm biomass or density but were correlated with earthworm community composition. Overall, the introduction of native woody vegetation was associated with a decline in density and biomass of earthworms, including a decrease in the relative abundance of exotic species.

In a second paper I investigated how the presence of shelterbelts and time since their establishment affects soil biotic communities. I compared the abundance and structure of fungal and bacterial communities using quantitative polymerase chain reaction (qPCR) and terminal restriction fragment length polymorphism (T-RFLP) between shelterbelts, adjacent pastures and native remnants. Fungal:bacterial (F:B) ratios (copy number) were higher under pasture and old shelterbelts, however, there was no significant effect of site type on the F:B ratio, MBC, or on MBN. Fungal community composition in shelterbelts, regardless of age, was significantly different to pasture and native remnants. There was no significant difference in bacterial community composition between young shelterbelts (<5 years) and pastures, while middle-aged (5-15 years) and old (15-20 years) shelterbelts significantly differed to pasture communities.

There was a significant difference in fungal and bacterial community composition between all shelterbelts and native remnants. Evaluation of the effect of time since shelterbelt establishment did not provide clear evidence that microbial communities in shelterbelts show trajectories that converge towards those observed in natural remnant patches. This indicates that the effects of agriculture are long-lasting and that revegetated sites are different to remnant patches and may not provide the same functions as remnant patches, even after 17 years post-establishment of a shelterbelt.

In a third paper I compared soil microbial communities beneath the dominant tree genera used in shelterbelts in south-eastern Australia (*Acacia*, *Eucalyptus*) and those found in adjacent pastures. The quantity and structure of bacterial and fungal communities was analysed using quantitative PCR (qPCR) and T-RFLP. The ratio of fungi to bacteria was highest under *Eucalyptus* trees but did not differ between *Acacia* and pasture. Both fungal and bacterial communities varied at different depths in the soil profile and across sampling periods. The dominant tree genus was a significant factor in observed differences in bulk soil bacterial and fungal communities compared to the surrounding pasture. The effect of tree genus on fungal communities was more pronounced than on bacterial communities. Fungal communities not only differed between shelterbelts and pastures, but also between *Acacia* and *Eucalyptus* dominated shelterbelts. These patterns were consistent at different soil depths.

In a fourth paper, I examined decomposition in shelterbelts. Decomposition of plant material in soil is largely mediated by microbes. An increasing number of studies have assessed extracellular enzyme activities as an indicator of microbial metabolism in soils and litter. In particular, various studies have examined the effect of litter type and litter mixing on decomposition rates, largely focusing on deciduous and northern hemisphere species. *Acacia* and *Eucalyptus* species are commonly used for revegetation in Australia and other parts of the world, yet little is known regarding how their litter might differentially impact on decomposition rates and extracellular enzyme activities. To investigate this, I established a litter decomposition experiment using a reciprocal design (litter x dominant overstory genus) in revegetation shelterbelts to establish if litter type affects decomposition rates, microbial community and enzyme activities associated with carbon degradation. Litter bags with two mesh sizes (≤2mm and >2mm) were used to exclude or allow access by soil macrofauna respectively. Bags were filled with either *Eucalyptus*, *Acacia* or an equal mix of *Acacia* and *Eucalyptus* litter. Litter decomposition rates, enzyme activities and bacterial and fungal

community profiles were determined following recovery of surface-placed bags at 40, 96, 187, 307 days and 395 days and a buried control at day 395. The abundance and structure of bacterial and fungal communities were assessed by T-RFLP and quantified by qPCR. After 395 days, decomposition of *Acacia* litter was faster than *Eucalyptus* or mixed litter treatments, irrespective of the overstory genus. Exclusion of macrofauna had no significant effect on most of the variables measured, other than chitin-degrading N-acetylglucosamine (NAG) activity. Distinct fungal and bacterial communities occupied each litter type and this differed between dominant overstory genera. However, these changes did not coincide with short term differences in litter decomposition rate.

Collectively, the research described in this thesis provides quantitative information on the aboveground-belowground linkages in native shelterbelts established on agricultural land. It is apparent that the effects of agriculture are long-lasting. There was little evidence that the earthworm, bacterial and fungal communities in shelterbelts converge towards those observed in natural remnant patches within 17 years. Shelterbelts can, however, be used to promote native earthworm abundance, which may be important for local diversity, soil function and landscape connectivity. Shelterbelt systems which contain a mixture of different plant genera (*Acacia* and *Eucalyptus* spp.) may contribute to conserving important bacterial and fungal diversity in agricultural landscapes. However, there was no significant effect of differing litter types on decomposition within the decomposition time frames studied (395 days).

Table of Contents

Candidate's Declaration ..... **iii**

Preface ..... **iv**

Acknowledgements ..... **vi**

Abstract ..... **viii**

Table of Contents ..... **xi**

Extended context statement **1**

Introduction 2

Research aims and objectives 3

Study area 6

Summary of outcomes 9

Synthesis and concluding remarks 12

Time since establishment of shelterbelts *12*

Spatial heterogeneity within shelterbelts *13*

Limited clear links between edaphic properties and soil biota *14*

Concluding remarks *15*

References 16

Literature review: Soil biota in restored agricultural landscapes **20**

Abstract 21

Introduction 21

Defining soil biotic communities in restoration ecology 24

Soil biota in agricultural settings 25

Restoration of agricultural land 26

Soil biota as bio indicators *28*

Trajectories of change *30*

Effect of the restoration of native vegetation in agricultural landscapes on soil biota . 31

Macrofauna *34*

Mesofauna *35*

Microbes *36*

Biota as agents of restoration 39
Implications for restoration of degraded sites: Where to now? 43
Taking advantage of molecular advances *43*
Restoring function *44*
Time constraints *44*
Conclusion 45
References 46
Paper I: Earthworm composition, diversity and biomass under three land use systems in south-eastern Australia 65
Abstract 66
Introduction 66
Methods 68
Study site and design *68*
Earthworm sampling *70*
Soil sampling and analysis *71*
Statistical analysis *71*
Results 73
Density, biomass and species diversity *73*
Earthworm species and community composition *75*
Soil edaphic properties *78*
Discussion 82
Conclusions 85
Acknowledgements 85
References 86
Paper II: Shelterbelt influence on soil microbial community structure. 93
Abstract 94
Introduction 94
Methods 97
Study site description and experimental design *97*
Physical and chemical Soil Analysis *98*
Microbial biomass *98*
DNA extraction *98*
Real time qPCR for Fungi and Bacteria Abundance *99*
T-RFLP *99*
Statistical analysis *100*

Results 101
Soil edaphic properties *101*
Soil edaphic Properties and soil biological community *106*
Discussion 110
Acknowledgements 113
References 114

Paper III: Plant genus (*Acacia* and *Eucalyptus*) alters soil microbial community structure and abundance within revegetated shelterbelts 124

Abstract 125
Introduction 125
Methods 127
Study sites *127*
Soil sampling *128*
Soil analysis *128*
DNA extraction *129*
Real time qPCR for fungi and bacteria abundance *129*
T-RFLP *129*
Statistical analysis *130*
Results 132
Soil edaphic properties *132*
Fungal:bacteria ratio *134*
Bacterial and fungal T-RFLP community profiles *135*
Soil edaphic properties and bacterial and fungal communities *139*
Discussion 143
Acknowledgements 147
Appendix A. Supplementary data 147
References 148

Paper IV The effect of litter type on decomposition rates and soil microbial communities in a shelterbelt system. 157

Abstract 158
Introduction 158
Methods 161
Site description *161*
Decomposition experiment *162*
DNA extraction *163*

Real time qPCR for fungi and bacteria abundance *163*
T-RFLP *164*
Litter extracellular enzyme activity *165*
Statistical analysis *165*
Results 167
Litter decomposition *169*
Enzyme activity *170*
Fungi to bacteria ratio *173*
Bacterial and fungal T-RFLP community profiles *174*
Discussion 180
Litter decomposition *180*
Enzyme activities *181*
Microbial community profiles and fungal to bacterial ratios *182*
Conclusions 183
Acknowledgements 184
References 185
Appendix 1: Paper II supplementary data 192
Appendix 2: Paper III supplementary figures 196
Appendix 3: Paper IV supplementary table 199

Extended context statement

At the beginning of the thesis I present an Extended context statement, which provides a framework for understanding the relationship between the different components of my research and succinctly identifies broad themes and conclusion from my research that may be especially relevant for practitioners and applicable to restoration of agricultural lands. This context statement is consistent with The Australian National University's College of Medicine, Biology and Environment Thesis by Compilation Guidelines. The Extended context statement is not intended to be a comprehensive literature review.

Introduction

Agriculture is one of the main causes of deforestation globally, often resulting in land degradation, carbon dioxide (CO_2) emissions and landscape fragmentation (Hobbs, 1993, 2005). Agricultural practices can potentially alter decomposition, nutrient cycling and production due to changes in vegetation cover and composition, litter quality and fertilisers (Matson et al., 1997). Despite the impacts of agriculture on the land there is still a need to manage landscapes for food production. In Australia, many agricultural areas have been subject to extensive clearing of native vegetation, which has contributed to reduced levels of native biodiversity (Yates and Hobbs, 1998). In response to a growing awareness of the need to reduce land degradation and conserve local biodiversity, the use of native plant species for ecological restoration has been widely implemented in Australian agricultural landscapes (Cleugh et al., 2002; Hobbs, 1993; Yunusa et al., 2002). For the purpose of this thesis, ecological restoration is defined as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (Clewell et al., 2004).

The method used for ecological restoration of agricultural land varies depending on the objective. One method of re-introducing woody species into agricultural land is through the planting of native shelterbelts which are strips, typically single or multiple rows of planted native trees and shrubs, or a strip of retained natural vegetation (Cleugh et al., 2002). Native shelterbelts in Australia are usually established using native species and local seed dominated by a mix of *Acacia* and *Eucalyptus* species and including understorey plants. Shelterbelts allow for ecological objectives to be met while maintaining high agricultural output. Shelterbelts require minimal land, and can take advantage of existing fence lines, as well as local topography. The addition of shelterbelts to agricultural landscapes provides various ecosystem services including, but not limited to, improved pasture quality, shelter for stock, supplementary feed sources, improved soil health and reduced erosion (Kavanagh et al., 2005), improved paddock tree health, increased wildlife (Lindenmayer and Hobbs, 2004) and bird diversity (Barrett et al., 2008; Cunningham et al., 2008; Munro et al., 2011), increased landscape connectivity and productivity, and reduced erosion (Bird, 1998; Bird et al., 1993; Cleugh et al., 2002; Yunusa et al., 2002).

In order to restore an ecosystem, we need to understand how it functioned prior to modification, and then use this knowledge to implement management practices that promote essential ecosystem services that may have been lost (Hobbs et al., 2007). The effectiveness of

ecological restoration can be defined using indices, the most common of which include vegetation characteristics, species diversity and specific ecosystem processes such as nutrient cycling. A key point is that ecological restoration has almost entirely focused on re-establishing aboveground plant communities, with much less attention given to other biota, particularly soil communities. This is despite increasing recognition that soil biota drive many ecosystem services and processes, including biogeochemical cycling of most nutrients essential to plant growth, decomposition, symbiosis with plants, and soil physical characteristics (Wardle and Giller, 1996). As agriculture has the capacity to greatly alter soils, the possibility of restoring native flora (and fauna) may diminish if soil biota are not taken into account. In the last decade, the importance of these organisms in ecological theory has been recognized (Ball et al., 2009; Coleman, 2008). There is also a growing body of literature on how the restoration of agricultural land alters soil biota or the functions they perform, and the extent to which it is possible to restore the community to that seen in native ecosystems. However, there are still many knowledge gaps. For example, shelterbelts and their potential to alter soil biota can potentially affect carbon dynamics as soil biota are known to be essential in biogeochemical cycling (Coleman, 2008). Aboveground-belowground linkages represent a growing area of research (Bardgett and Wardle, 2003; Van Der Heijden et al., 2008; Van Der Putten et al., 2009; Wardle et al., 2004), although relatively few studies have focused on such interactions in the context of restoration ecology.

In order to better manage restored systems, we must better understand belowground processes and what drives changes in these systems. Overall there is little information on the effect of landscape restoration on soil biotic diversity and function. The role soil biota play in ecosystem function within shelterbelts is also poorly understood.

Research aims and objectives

The objective of my research is to identify changes in soil biota community and the effect on ecosystem function associated with native revegetation (shelterbelts) of agricultural landscapes within Australia.

The main aims of this thesis are:

1. To establish if soil microbial and macrofauna communities differ under restored agricultural land (shelterbelts), compared to pasture and native remnant vegetation and if differences become greater with shelterbelt age.

2. To determine if plant genus affects the ecological dynamics of soil microbial communities and edaphic properties in revegetated sites and whether changes in microbial community composition correspond to changes in soil chemical properties driven by differences in plant type.
3. To explore the effect of litter type (*Acacia*, *Eucalyptus* and mixed) on decomposition enzyme activities and soil microbial communities under an *Acacia* or *Eucalyptus* dominant overstory.

This thesis has been constructed as five papers/chapters (literature review chapter plus four research papers) that are standalone papers, however, when combined address the overall aims of this thesis (Fig.1).

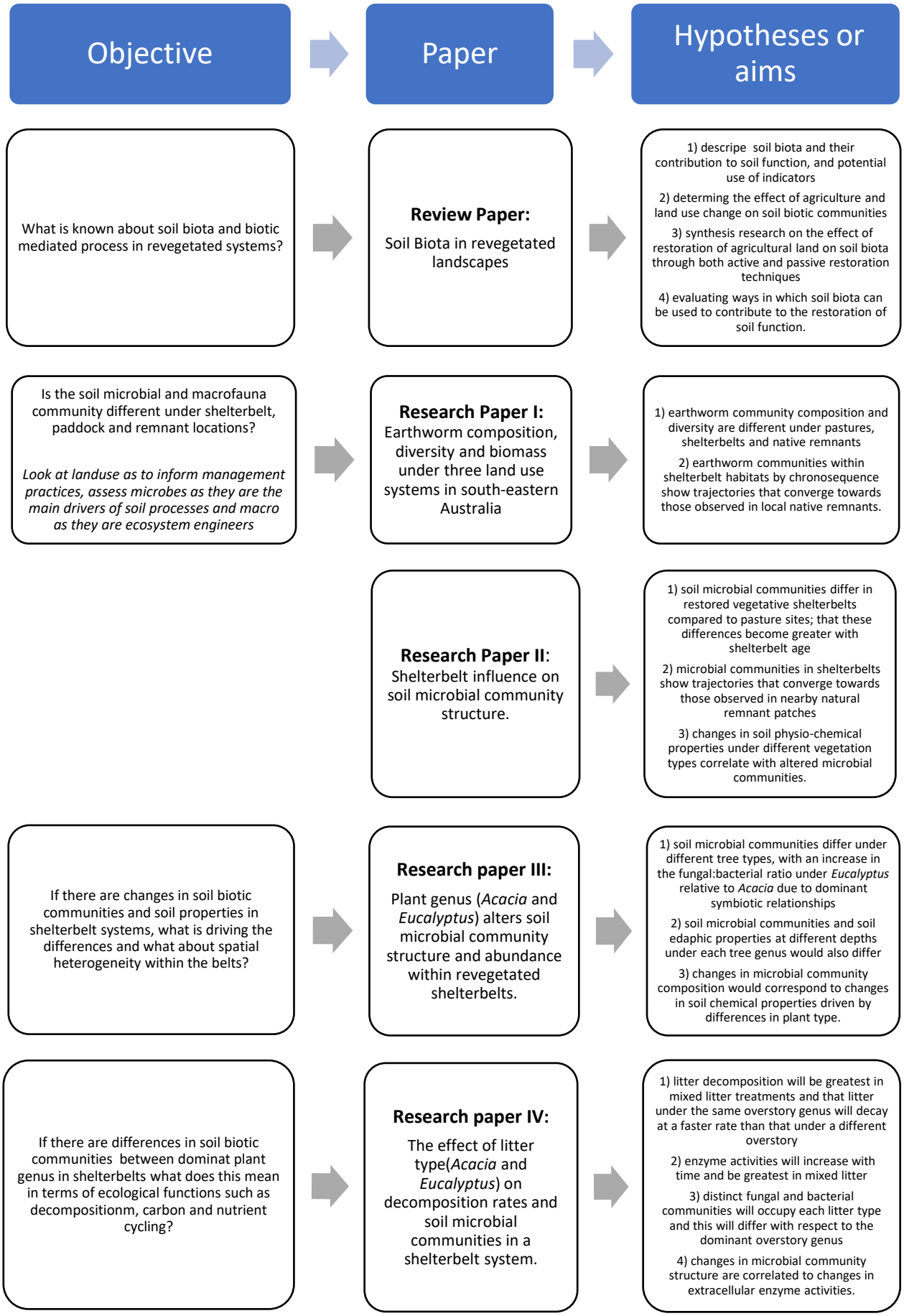


Figure 1. Reasoning and links between the chapters

Study area

All research was conducted on sites located near Murrumbateman in New South Wales, Australia (34°57′0″S, 149°01′0″E). The region is predominantly agricultural land dominated by livestock grazing. The climate is temperate, with warm to hot summers and cool winters. The average annual rainfall is 927 mm (Australian Bureau of Meteorology, 2014). Rainfall is higher in spring to summer with October and November being slightly wetter than other times. Rainfall is lowest within the winter months of June and July.

Field sites for research **Papers I and II** consisted of six sites, each with a paired design of parallel transects, one within a shelterbelt established on former pastoral land and the other within adjacent pasture (Table 1). The shelterbelt transects were separated into three age classes: 0-5 years since establishment (young), 5-15 years (middle-aged), 15-20 years (old), with two transects in each age class. While these age class represent the original plan, actual ages (5,14 or 17 years) reflect the reality of trying to locate shelterbelts in similar topographic and spatial locations. In **Paper II** another two transects were selected within two native remnant woodlands, while in **Paper I** three transects were selected within three native remnant woodlands. These native remnants were small (approximately 1 hectare) fragmented patches which had experienced various levels of disturbance. Two native remnant woodlands selected were in close proximity to all sites (about 5 km), whereas one native remnant was located about 15 km away. The region has been extensively cleared and often the only native remnant patches are those along road reserves on land that was deemed not suitable for farming. The native remnant woodlands selected were representative of general topography and vegetation communities that would have been present prior to clearing and revegetation. At the time of establishment, each shelterbelt had been direct sown with a seed mix of tree and shrub species endemic to the Murrumbateman area. All shelterbelts had fencing around them. The dominant overstory trees were a mix of *Acacia* and *Eucalyptus* species, the dominant understory species included *Themeda australis* and *Austrodanthonia spp*. All transects were situated within a 12 km radius, thus limiting climatic variability between sites. The pasture sites in the study comprised of sheep, goat and cattle grazed pasture containing a mix of native and exotic pasture species. While ideally in **Paper I** and **Paper II** a negative control site with two paired pastures would have allowed for exploration of within site variation, due to time and funding constraints it was decided that replication of sites was of greater benefit.

The study area for research **Papers III and IV** was located on one property, Harwood, within Murrumbateman. Two shelterbelts, one dominated by *Acacia* species and the other by *Eucalyptus* species were selected. The *Eucalyptus* shelterbelt was comprised mostly of *E. macarthurii, E. blakelyi, E. viminalis* and *E. melliodora*. The *Acacia* shelterbelt included *A. mearnsii, A. baileyana, A. decurrens, A. cardiophylla* and *A. cultriformis*. The two shelterbelts ran parallel to each other (approximately 100m apart). The soils at all sites are described as chromosol, yellow, eutrophic (Australian Soil Classification: order, sub-order, great-group; Isbell, 2016).

Table 1. Study sites used in each of the research papers.

Site ID	Property name	Latitude Longitude	Grazed [1] Y/N	No. of Rows	Year Established	Age Classification*
Paper I and II study sites						
Shaw A	Shaw's Winery	-34.979915, 149.010151	N	4	2007	0-5
Shaw B	Shaw's Winery	-34.978946, 149.012465	N	3	2007	0-5
Annagrove	Annangrove	-35.032800, 149.040264	N	3	1998	10-15
Kerralee	Kerralee	-35.054269, 149.022351	N	3	1998	10-15
Harwood A	Harwood	-35.040281, 149.015775	N	4	1995	15-20
Harwood B	Harwood	-35.043305, 149.019608	N	3	1995	15-20
Native ref 1	-	-35.037436, 149.021165	Y	Na	Na	Na
Native Ref 2	-	-35.040718, 149.024528	Y	Na	Na	Na
Native Ref 3[2]	-	-35.157660, 149.060538	Y	Na	Na	Na
Paper III and IV study sites						
Harwood *Acacia*		-35.040477, 149.017013	N	4	1995	15-20
Harwood *Eucalyptus*		-35.040281, 149.015775	N	4	1995	15-20

*at time of sampling

[1] Grazing by livestock such as cattle and sheep (grazing by wild animals such as rabbits and kangaroos was possible at all sites but did not appear to be a major issue).

[2]Native ref 3 only sampled in paper 1.

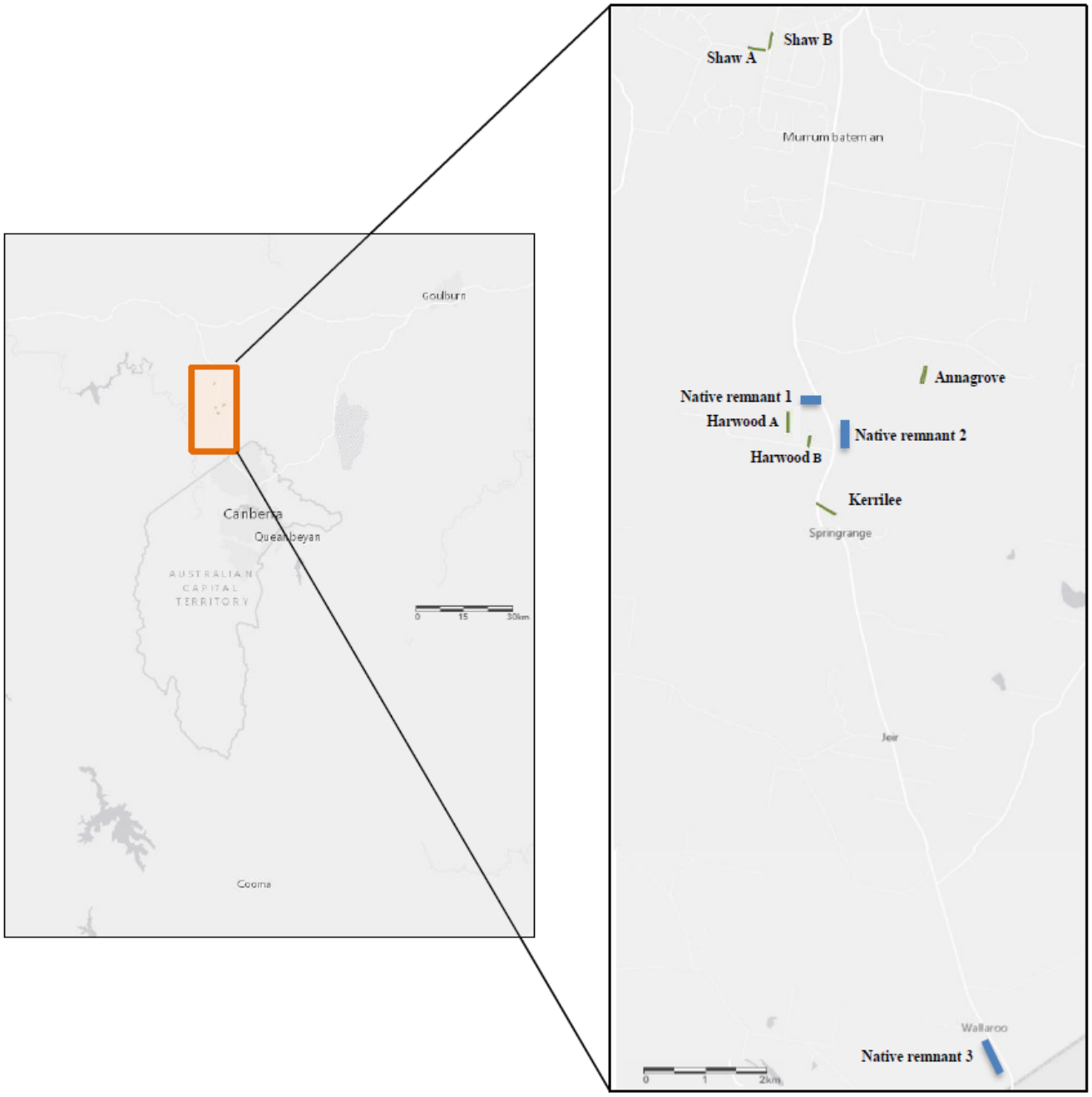


Figure 2. Study sites for **Papers I** and **II** located with the Murrumbateman NSW region. Shelterbelts and paired pasture sites are shown in green and native remnant sites are shown in blue. Study sites for **Papers III** and **IV** were conducted near Harwood A.

Summary of outcomes

Literature review

In this chapter, I review the current understanding of how ecological restoration of agricultural systems affects soil biota and make recommendations for the direction of future research. Relatively little is known about how restoration of agricultural land alters soil biota or the functions they perform, and the extent to which it is possible to restore the community to that seen in native ecosystems. I identified three main areas for future development. These were: 1) the use of recent molecular advances to further understand microbial communities in restored ecosystems; 2) developing understanding of how variation in soil communities alters ecosystem function; and 3) strengthening knowledge regarding trajectories of change in restored systems. These areas are particularly important for native woody systems which have received relatively limited attention from researchers in this context.

Research Paper I

The experimental work in this paper aimed to add to current knowledge on trajectories of change in earthworm communities in restored systems, as earthworms are an ecologically important component of the soil macrofauna. I examined earthworm composition, diversity and biomass in three land use systems: native shelterbelts dominated by *Acacia* and *Eucalyptus* species, agricultural pastures and native remnant woodland fragments dominated by *Eucalyptus blakelyi* and/or *Eucalyptus melliodora*. Earthworm communities differed significantly among systems, with abundance, biomass and diversity greatest under pasture. Within shelterbelts I saw a shift from high earthworm biomass and density to low values with increasing time after establishment. There was no evidence for convergence of earthworm communities towards those in remnant native vegetation, although this comparison is potentially confounded by various levels of disturbance within remnants of native vegetation in the study area. Soil edaphic variables did not correlate strongly with earthworm biomass or density but were correlated with earthworm community composition. Overall, the introduction of native woody vegetation was associated with a decline in density and biomass of earthworms, including a decrease in the relative abundance of exotic species. As such, shelterbelts can be used to promote native earthworm relative abundance which may be important for local diversity and soil function.

Research Paper II

In this paper I investigated how the presence of shelterbelts, and time since their establishment, affects soil microbial communities. This research complemented the research undertaken in **Paper I** by further characterising trajectories of change, in this case focusing on the microbial community. I used quantitative PCR (qPCR) and T-RFLP to compare the abundance and structure of fungal and bacterial communities between shelterbelts, adjacent pastures and native remnants. Fungal community composition in shelterbelts, regardless of age, was significantly different to pasture and native remnants. There was no significant difference in bacterial community composition between young shelterbelts (<5 years) and pastures, while middle-aged (5-15 years) and old (15-20 years) shelterbelts were significantly different to pasture communities. There was a significant difference in fungal and bacterial community composition between all shelterbelts and native remnants. Evaluation of the effect of time since shelterbelt establishment did not provide clear evidence that microbial communities in shelterbelts show trajectories that converge towards those observed in natural remnant patches, which may indicate that our native remnant sites may not represent true natural remnants. This indicates that the effects of agriculture are long-lasting, even after 17 years post-establishment of a shelterbelt. Research **Papers I and II** thus jointly raised the question about drivers of change within soil biotic communities in restored systems.

Research Paper III

I then conducted research to determine if potential drivers such as plant genus affect the ecological dynamics of soil microbial communities in shelterbelts in south-eastern Australia. In this paper I investigated the effects of two commonly used overstory genera (*Acacia, Eucalyptus*) on soil microbial community structure compared with those under adjacent pastures. This research was conducted to obtain insight into potential drivers of change in soil microbial communities. The quantity and structure of bacterial and fungal communities was analysed using quantitative PCR (qPCR) and terminal restriction fragment length polymorphism (T-RFLP). The ratio of fungi to bacteria was highest under *Eucalyptus* trees but did not differ between *Acacia* and pasture. Both fungal and bacterial communities varied at different depths in the soil profile and across sampling periods. The dominant tree genus was a significant factor in observed differences in bulk soil bacterial and fungal communities compared to the surrounding pasture. The effect of tree type on fungal communities was more pronounced than on bacterial communities. Fungal communities not only differed between

shelterbelts and pastures, but also between *Acacia* and *Eucalyptus* dominated shelterbelts and these patterns were consistently observed at different soil depths. Shelterbelt systems which contain a mixture of different plant genera thus have the potential to exhibit greater microbial community heterogeneity than single species shelterbelts. Maintaining a variety of tree species during afforestation may therefore contribute to conserving important bacterial and fungal diversity in agricultural landscapes.

Research Paper IV

Once I established that the dominant plant genus (*Acacia* or *Eucalyptus*) resulted in differing soil bacterial and fungal communities, this raised the question as to how such changes in soil communities alter ecosystem function. To explore this, I examined the effect of litter type and litter mixing on decomposition rates and extracellular enzyme activities. In particular, I explored the effects of *Acacia, Eucalyptus* litter and mixed litter in a litter bag experiment in the field. I established a litter decomposition experiment using a reciprocal design (litter x dominant overstory genus) in revegetation shelterbelts to establish if litter type affects decomposition rates, microbial community and enzyme activities associated with carbon degradation. Litter bags with two mesh sizes were used to either exclude (≤2mm) or allow access (>2mm) by soil macrofauna. Bags were filled with either *Eucalyptus*, *Acacia* or a mix of *Acacia* and *Eucalyptus* litter. Decomposition, enzyme activity and bacterial and fungal community profiles were determined following recovery of bags at 40, 96, 187, 307, and 395 days. The abundance and structure of bacterial and fungal communities were assessed by T-RFLP and quantified by qPCR. After 395 days, decomposition of *Acacia* litter was greater than *Eucalyptus* or mixed litter treatments, irrespective of the overstory genus. There was no evidence that litter under the same overstory genus (home field advantage) decayed at a faster rate than that under a different overstory, which may be due to the litter types used being chemically similar. Litter mesh size had no significant effect on most of the variables measured, other than N-acetylglucosamine (NAG) activity. Enzyme activity first increased until 96 days then decreased at 395 days. Across most sampling points, NAG, glucosidase (βG) and cellobiohydrolase (CBH) were highest in *Eucalyptus* litter and lowest in *Acacia* litter. Acid phosphatase (AP) activity was highest in *Acacia* litter with *Eucalyptus* and mixed litter showing similar AP activity levels.

We were able to demonstrate that distinct fungal and bacterial communities occupied each litter type and these also differed between dominant overstory genus. However, we still lack a

predictive framework for understanding more generally the impact of different plant species mixes on revegetation outcomes, including rates of return of contribution to ecosystem function.

Synthesis and concluding remarks

My research was conducted to provide quantitative information on the aboveground and belowground linkages in native shelterbelts established on agricultural land in south-eastern Australia. This research provided information on the soil biotic communities (earthworms and microbial communities) and demonstrated the complex nature of aboveground and belowground linkages in these systems. In this section, I synthesise the key findings and themes from my collective research and provide a summary of recommendations and further areas of research for the restoration of agricultural landscapes in south-eastern Australia.

Time since establishment of shelterbelts

Chronosequences are an important and often necessary tool for studying the temporal dynamics of plant communities and soil development across multiple timescales (Walker et al., 2010). By exploring a chronosequence of shelterbelts, inferences regarding the projected outcomes of restoration in the form of shelterbelts systems can be made where long term, monitoring is not possible. Chronosequences are most appropriate for studying communities that are following convergent successional trajectories and have low biodiversity, rapid species turnover and low frequency and severity of disturbance (Walker et al., 2010).

It was evident from my research that with the reintroduction of native vegetation in the form of shelterbelts, there were differences in the soil biotic communities and, to a lesser extent, soil physiochemical properties. In **Papers I and II**, I identified differences in both the earthworm and microbial communities between shelterbelts, pasture and native remnants. Evaluation of the effect of time since shelterbelt establishment did not provide clear evidence that microbial communities in shelterbelts show trajectories that converge towards those observed in natural remnant patches. In fact, it was clear that the effects of agriculture were long-lasting, even after 17 years post-establishment of a shelterbelt. However, due to the lack of pristine native remnants within the study area I was unable to determine whether there is general convergence of earthworm and microbial communities in shelterbelts towards undisturbed native communities, as other factors (e.g. exotic pasture plants) could potentially influence the trajectory. I saw a shift from high earthworm biomass and density to low with increasing time since establishment of shelterbelts. Overall the introduction of native woody

vegetation was associated with a decline in density and biomass of earthworms, including a decrease in the relative abundance of exotic species, possibly as a result of reduced soil moisture within shelterbelts.

Following restoration of woody vegetation on agricultural land, one expectation is that soil properties such as soil carbon will increase due to higher organic matter inputs, whilst soil nutrients such as phosphorus and nitrogen may decrease in shelterbelts due to the removal of fertiliser and manure inputs (Sauer et al., 2007). Such trends are likely to continue with time since establishment of shelterbelts. However, within my study, middle-aged (5-15 years) shelterbelts had higher total carbon, nitrogen and phosphorus in the top 10 cm of soil than both old (15-20 years) and young (0-5 years) shelterbelts. This may reflect optimum resource availability (e.g. nutrients and water) for growth and organic matter production in middle-aged belts. Many other soil edaphic properties did not differ significantly between pasture and shelterbelts. This is consistent with Hoogmoed et al. (2012) who, through a meta-analysis of published data, found no evidence for substantial changes in soil C, N or the C:N ratio over 30 years of afforestation, however the meta-analysis was climate zone restricted (Mediterranean). This is further supported by Cunningham et al. (2015a) who showed that soil C was slower to accumulate in native mixed-species plantings, with increases relative to adjacent pastures observed only after 45 years. My results indicate that monitoring of shelterbelts older than 17 years since establishment is required to make further comment on the trajectory of soil microbial and earthworm communities in these systems. This slow transition of community structure supports some previous findings that establishment of a diverse, native soil assemblage in restored agricultural land may be slow following reforestation (Cunningham et al., 2015b).

Spatial heterogeneity within shelterbelts

Soil development is influenced by the dominant tree species through inputs such as the quantity and quality of litter (Šnajdr et al., 2013). I was able to demonstrate that in shelterbelt systems, spatial heterogeneity is altered by the dominant overstory genus (**Paper III**). The effect of tree genus on soil microbial communities was stronger than that of individual soil properties. This was consistent with the finding that land use (i.e. pasture, shelterbelt and native remnant) also has a strong effect on soil biota (**Papers I and II**). This heterogeneity was also apparent within fungal and bacterial communities in differing litter types (*Acacia*, *Eucalyptus*) (**Paper IV**). Shelterbelt systems which contain a mixture of different plant genera have the

potential to exhibit greater microbial community heterogeneity than single species plantations. Maintaining a variety of tree species during afforestation may therefore contribute to conserving important bacterial and fungal diversity in agricultural landscapes. The effects of overstory genus within shelterbelts were most apparent within the first 0-10 centimetres of the soil profile, below which microbial communities were influenced to a greater extent by edaphic properties. This is consistent with other studies showing that organic inputs and plant effects are greatest in the upper soil stratum (Hoogmoed et al., 2012; Li et al., 2018). My results demonstrate that microbial community composition can be significantly influenced by the presence of different dominant tree genera in revegetated shelterbelt systems, and that these communities in turn differ from those occurring in adjacent pastures.

Limited clear links between edaphic properties and soil biota

As part of my thesis research, I evaluated the use of soil edaphic properties (e.g. % Carbon, Nitrogen, Phosphorus, and bulk density) as predictors of soil biotic properties. My results indicate that, at least in these shelterbelt systems, edaphic properties are not strong predictors of soil biotic community structure and function. There were no strong correlations between earthworm density and biomass for any of the soil chemical properties measured (**Paper I**). Soil edaphic properties were not strongly correlated with the composition of either fungal (24% variation) or bacterial (28% variation) communities **(Paper II)**. This is consistent with many studies in which there is a large component of unexplained variation in fungal and bacterial communities (Banning et al., 2011; Bissett et al., 2011). These results suggest that vegetation and land use are strong drivers of changes in the composition of soil biota and that their influence is not mediated through their effects on soil edaphic factors, but through other mechanisms.

Despite changes in soil microbial community structure between differing litter types (*Acacia*, *Eucalyptus* or mixed) the response in decomposition rates was not as clear. Litter decomposition (up to 395 days) was influenced by litter genus, with the greatest effects being observed after 96 days, when mass loss was greatest for *Acacia* and least for *Eucalyptus* litter, which was most likely due to differences in litter quality (i.e., higher N in the *Acacia* litter) This could indicate that, despite changes in community composition during the initial stage of decomposition, these changes are having little effect on decomposition between the differing litter types. However, as the study duration was relatively short (13 months) differentiation in

community composition may be significant for later stages of decomposition and thus cannot be disregarded.

Concluding remarks

Collectively, this research provides quantitative information on aboveground-belowground linkages in native shelterbelts established on agricultural land. The earthworm, bacterial and fungal communities did not converge towards those observed in natural remnant patches over this time-frame. However, this may be indicative of the remnant sites not being representative of true reference native sites. Despite this, it was often clear that the effects of agriculture are long-lasting, even after 17 years post-establishment of a shelterbelt. This further highlights the need to study changes in soil edaphic properties and microbial communities at sites that have been reforested for a longer time.

Shelterbelts can, however, promote native earthworm abundance, which may be important for local diversity, soil function and landscape connectivity. Shelterbelt systems which contain a mixture of different plant genera (*Acacia* and *Eucalyptus* spp.) may contribute to conserving important bacterial and fungal diversity in agricultural landscapes. However, there was no significant effect of differing litter on decomposition within the decomposition timeframes studied (395 days). Further areas of research for the restoration of agricultural landscapes in south-eastern Australia should include trying to better understand how the initial mix of revegetation species influences microbial community function, with the aim of ultimately being able to provide guidelines that help improve the effectiveness of restoration efforts.

Overall my research has shown that the maintenance of species diverse woody plantings in agricultural landscapes has the potential to alter and conserve soil communities which may support some key ecosystem functions.

References

Ball, B.A., Bradford, M.A., Coleman, D.C., Hunter, M.D., 2009. Linkages between below and aboveground communities: Decomposer responses to simulated tree species loss are largely additive. Soil Biology and Biochemistry 41, 1155-1163.

Banning, N.C., Gleeson, D.B., Grigg, A.H., Grant, C.D., Andersen, G.L., Brodie, E.L., Murphy, D., 2011. Soil microbial community successional patterns during forest ecosystem restoration. Applied and Environmental Microbiology 77, 6158-6164.

Bardgett, R.D., Wardle, D.A., 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84, 2258-2268.

Barrett, G.W., Freudenberger, D., Drew, A., Stol, J., Nicholls, A., Cawsey, E., 2008. Colonisation of native tree and shrub plantings by woodland birds in an agricultural landscape. Wildlife Research 35, 19-32.

Bird, P., 1998. Tree windbreaks and shelter benefits to pasture in temperate grazing systems. Agroforest Systems 41, 35-54.

Bird, P., Bicknell, D., Bulman, P., Burke, S., Leys, J., Parker, J., Van der Sommen, F., Voller, P., 1993. The role of shelter in Australia for protecting soils, plants and livestock, The Role of Trees in Sustainable Agriculture. Springer, 59-86.

Bissett, A., Richardson, A.E., Baker, G., Thrall, P.H., 2011. Long-term land use effects on soil microbial community structure and function. Applied Soil Ecology 51, 66-78.

Cleugh, H.A., Prinsley, R., Bird, P.R., Brooks, S.J., Carberry, P.S., Crawford, M.C., Jackson, T.T., Meinke, H., Mylius, S.J., Nuberg, I.K., Sudmeyer, R.A., Wright, A.J., 2002. The Australian National Windbreaks Program: overview and summary of results. Australian Journal of Experimental Agriculture 42, 649-664.

Clewell, A., Aronson, J., Winterhalder, K., 2004. The SER international primer on ecological restoration.

Coleman, D.C., 2008. From peds to paradoxes: Linkages between soil biota and their influences on ecological processes. Soil Biology and Biochemistry 40, 271-289.

Cunningham, R.B., Lindenmayer, D.B., Crane, M., Michael, D., Macgregor, C., Montague-Drake, R., Fischer, J., 2008. The combined effects of remnant vegetation and tree planting on farmland birds. Conservation Biology 22, 742-752.

Cunningham, S.C., Cavagnaro, T.R., Mac Nally, R., Paul, K.I., Baker, P.J., Beringer, J., Thomson, J.R., Thompson, R.M., 2015a. Reforestation with native mixed-species plantings in a temperate continental climate effectively sequesters and stabilizes carbon within decades. Global Change Biology 21, 1552-1566.

Cunningham, S.C., Mac Nally, R., Baker, P.J., Cavagnaro, T.R., Beringer, J., Thomson, J.R., Thompson, R.M., 2015b. Balancing the environmental benefits of reforestation in agricultural regions. Perspectives in Plant Ecology, Evolution and Systematics 17, 301-317.

Hobbs, P.R., Sayre, K., Gupta, R., 2007. The role of conservation agriculture in sustainable agriculture. Philosophical Transactions of the Royal Society B: Biological Sciences 363, 543-555.

Hobbs, R.J., 1993. Can revegetation assist in the conservation of biodiversity in agricultural areas? Pacific Conservation Biology 1, 29.

Hoogmoed, M., Cunningham, S., Thomson, J., Baker, P., Beringer, J., Cavagnaro, T., 2012. Does afforestation of pastures increase sequestration of soil carbon in Mediterranean climates? Agriculture, Ecosystems and Environment 159, 176-183.

Kavanagh, R., Law, B., Lemckert, F., Stanton, M., Chidel, M., Brassil, T., Towerton, A., Herring, M., 2005. Biodiversity in eucalypt plantings established to reduce salinity. A report for the RIRDC/Land and Water Australia FWPRDC/MDBC Joint Venture Agroforestry Program. (Rural Industries Research and Development Corporation: Canberra.).

Li, D., Wen, L., Jiang, S., Song, T., Wang, K., 2018. Responses of soil nutrients and microbial communities to three restoration strategies in a karst area, southwest China. Journal of Environmental Management 207, 456-464.

Lindenmayer, D., Hobbs, R., 2004. Fauna conservation in Australian plantation forests–a review. Biological Conservation 119, 151-168.

Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural intensification and ecosystem properties. Science 277, 504-509.

Munro, N.T., Fischer, J., Barrett, G., Wood, J., Leavesley, A., Lindenmayer, D.B., 2011. Bird's response to revegetation of different structure and floristics—are “restoration plantings” restoring bird communities? Restoration Ecology 19, 223-235.

Sauer, T., Cambardella, C., Brandle, J., 2007. Soil carbon and tree litter dynamics in a red cedar–scotch pine shelterbelt. Agroforest Systems 71, 163-174.

Šnajdr, J., Dobiášová, P., Urbanová, M., Petránková, M., Cajthaml, T., Frouz, J., Baldrian, P., 2013. Dominant trees affect microbial community composition and activity in post-mining afforested soils. Soil Biology and Biochemistry 56, 105-115.

Van Der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296-310.

Van Der Putten, W.H., Bardgett, R.D., De Ruiter, P., Hol, W., Meyer, K., Bezemer, T., Bradford, M., Christensen, S., Eppinga, M., Fukami, T., 2009. Empirical and theoretical challenges in aboveground–belowground ecology. Oecologia 161, 1-14.

Walker, L.R., Wardle, D.A., Bardgett, R.D., Clarkson, B.D., 2010. The use of chronosequences in studies of ecological succession and soil development. Journal of Ecology 98, 725-736.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629-1633.

Wardle, D.A., Giller, K.E., 1996. The quest for a contemporary ecological dimension to soil biology. Soil Biology and Biochemistry 28, 1549-1554.

Yates, C.J., Hobbs, R.J., 1998. Temperate eucalypt woodlands: a review of their status, processes threatening their persistence and techniques for restoration. Australian Journal of Botany 45, 949-973.

Yunusa, I.A., Brown, G.W., Kwong, R.M., Ronnfeldt, G.R., Slater, T., Crouch, A., Unkovich, M., 2002. Integrating biodiversity and productivity on intensive farms: A potential role for shelter-belts in the Victorian Riverina, Agriculture for the Australian Environment. Proceedings of the 2002 Australian Academy of Science Fenner Conference on the Environment', 255-277.

Literature review: Soil biota in restored agricultural landscapes

In this chapter I present a literature review on the effect of restoration of native species in agricultural areas. This review provides background information on our understanding of the effects of restoration on soil biology. From this, several key knowledge gaps are highlighted. These in turn form the basis of further study within this thesis.

Abstract

Many areas around the world have been subject to land degradation as a result of agricultural practices and land clearing. There is an increased awareness of the need to rehabilitate and restore some degraded agricultural landscapes. Generally ecological restoration has focused on establishing aboveground plant communities, largely disregarding belowground soil biotic assemblages despite increasing recognition that soil communities are major drivers of many critical ecosystem services. As agriculture has the capacity to greatly alter native soil systems, the potential to restore native flora and fauna in these landscapes may diminish if soil biota are not taken into account, particularly given that soil biota can also drive the trajectory of aboveground communities. There is a growing body of literature on the restoration of agricultural land through active (e.g. reintroduction of native species) or passive (secondary succession/land abandonment) pathways, and how it alters soil biota or the functions they perform. While trajectories of change in restored systems has been a common area of research, there are large gaps which still need to be explored. In this chapter I review current understanding of how ecological restoration of agricultural systems affects soil biota; highlighting knowledge gaps and make some recommendations for the direction of future research. I have identified three main areas for future development: 1) use of recent molecular advances to further understand microbial communities in restored ecosystems; 2) developing understanding of how variation in soil communities alters ecosystem function; and 3) strengthening knowledge on trajectories of change in restored systems. These areas are particularly important for native woody systems which have received relatively limited attention from researchers in this context.

Introduction

Agricultural ecosystems can be defined as ecosystems that have been modified for the purpose of production of goods that are of value to humans (Swift et al., 2004). Throughout the world agriculture has altered native landscapes, resulting in highly fragmented landscapes (Hobbs, 1993, 2005). Agricultural practices can potentially alter decomposition, nutrient cycling and production due to changes in vegetation cover and composition, litter quality and fertilisers (Matson et al., 1997). Agricultural practices also alter soil biotic communities (Allison and Jastrow, 2006; Andersen and Sparling, 1997; Ekschmitt and Griffiths, 1998). In addition to variation in soil community composition, changes in ecosystem processes regulated and carried out by soil biota can significantly alter soil physical and chemical structure. Owing to these potential detrimental effects of agriculture on ecosystem function, many land

management practices have been implemented to increase or maintain productivity and diversity within these landscapes.

Restoration science is a relatively new discipline in ecology and as such, the definition of restoration ecology has been highly variable within the literature. For the purpose of this review restoration ecology is defined as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (Clewell et al., 2004). Due to the varied contexts in which restoration has been used, it may be more useful to focus on the reasons for restoration. In a review of restoration ecology, motivations for undertaking restoration include: 1) restoration of highly degraded systems; 2) improving productivity; and 3) enhancement of conservation values in protected or production landscapes (Hobbs and Norton, 1996). These have resulted in a focus on establishing aboveground plant communities. As a consequence, considerable effort has gone into the re-introduction of native plant species into agro-ecological systems.

In order to restore an ecosystem, we need to understand how it functioned prior to modification and use this knowledge to implement management practices that promote essential ecosystem services that may have been lost (Hobbs and Norton, 1996). The effectiveness of ecological restoration can be defined using indices, the most common of which include vegetation characteristics, species diversity and specific ecosystem processes such as nutrient cycling.

Soil biota underpin many essential ecosystem services and processes, including: biogeochemical cycling of most nutrients essential to plant growth, decomposition, symbioses with plants (e.g. legumes and rhizobia), and soil physical characteristics (Wardle and Giller, 1996). Land management practices, including agricultural restoration, affect biologically mediated processes through land use change such as the reduction of fertiliser and herbicide inputs (Roper and Gupta, 1995). We have little quantitative understanding of how and at what spatial and temporal scales restoration of agricultural land, particularly with woody species, affects soil function through changes in soil community structure and dynamics (Andersen and Sparling, 1997; Osborne et al., 2011). Soil biota are of fundamental importance in ecosystem function. As such, they play a major role in various biogeochemical cycles and are vital in determining nutrient cycling, decomposition and energy flow (Wardle and Giller, 1996). Below I propose a conceptual diagram which highlights the interactions and effect of agricultural restoration on soil biota and key ecosystem services (Fig. 1). The essential role of soil biota in

ecosystem function highlights the importance of integrating soil ecological knowledge in restoration management (Callaham et al., 2008; Eviner and Hawkes, 2008; Pavao-Zuckerman, 2008).

In some contexts, such as mine site rehabilitation, considerable information is available on the role of soil biota (Anderson et al., 2008; Andrés and Mateos, 2006; Courtney et al., 2010; Dunger and Voigtländer, 2005; Dunger et al., 2001; Šnajdr et al., 2013). The large amount of literature on this topic will not be addressed within this review, rather the focus is on the rehabilitation of agricultural land and the role of soil biota in this context. It is evident that there is a lack of consistency regarding drivers of soil biotic communities in restored systems. The objective of this paper is to present a synthesis of the effects that restoration, in particular revegetation of native species in agricultural landscapes, may have on soil biotic communities. I explore this by: 1) presenting a description of soil biota and their contribution to soil function, and potential use of soil biota as indicators of restoration success; 2) describing the effect of agriculture and land use change on soil biotic communities; 3) providing a synthesis of research on the effect of restoration of agricultural land on soil biota through both active and passive restoration techniques; and 4) evaluating ways in which soil biota can be used to contribute to the restoration of soil function in revegetated agricultural landscapes. I conclude by outlining current knowledge gaps and further priority areas of research and development.

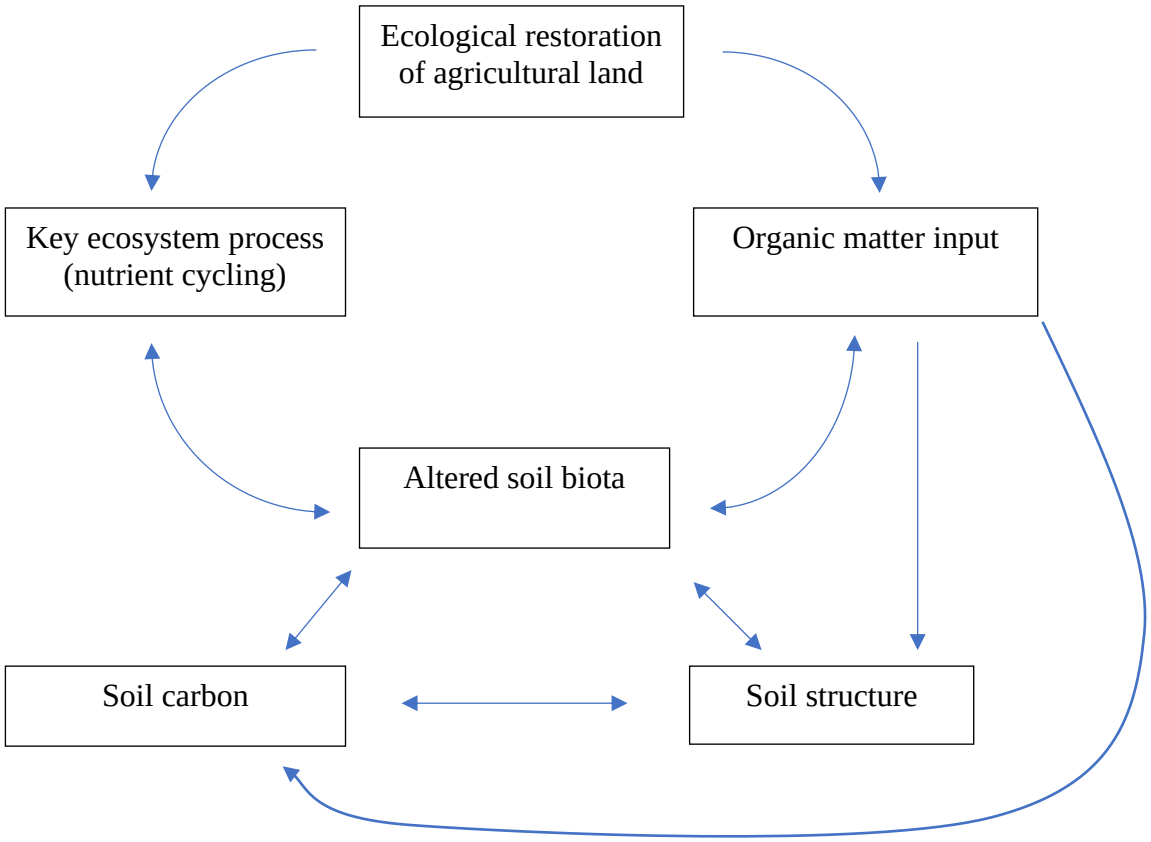


Figure 1. Conceptual diagram of the effect of restoration practices in agricultural landscape on soil biota and key ecosystem processes. Arrows indicate direction of influence. Influence can be positive or negative.

Defining soil biotic communities in restoration ecology

The term 'soil biota' generally refers to four broad groups based on size: the macrofauna (e.g. earthworms and beetles, 2mm-2m); mesofauna (e.g. collembolla, 100µm-2mm); microfauna (e.g. nematodes, 10µm-2mm; protozoa, 5µm-200µm) and the microflora (e.g. bacteria and fungi, <5 µm). The way in which we describe the soil biological community differs greatly depending on the objective of the research. The most common ways in which the soil biotic community are broadly described are in terms of functional groups or taxonomic diversity (Brussaard, 1998; Swift et al., 2004). The ecological function of soil biota has been extensively studied, with authors taking a range of approaches to functional classification (Brussaard, 1998; Giller et al., 1997). For a detailed review of the role of diversity and functional groups in agricultural landscapes, see Swift et al. (2004). It is generally believed that changes at higher taxonomic levels (i.e. families or orders) are more likely to affect ecosystem function than

changes at species level (Swift et al., 2004). The relationship between taxa and soil function has been established for macrofauna. For example, reduction in earthworm abundance decreases the occurrence of soil pores and thus decreases soil structure and water infiltration (Blouin et al., 2013; Fischer et al., 2014). Such physical effects are generally easier to detect than those mediated by microfauna and microbes (Emmerling et al., 2001).

Soil decomposer communities are very diverse even though only a minority of the species of fungi, bacteria or invertebrates may participate in the decomposition process at a given time and place (Banerjee et al, 2016). This implies significant functional redundancy within soil communities (Brussaard, 1998). Nonetheless, this may not be the case in all systems and communities. Strickland et al. (2009) found that the effects of different microbial inocula explained between 22% and 86% of the variation in litter carbon mineralisation, supporting the view that there is some functional dissimilarity among distinct microbial communities. Other studies support these results which suggest that there is great value in studying soil communities in terms of diversity and keystone organisms (Kemmitt et al., 2008; Rousk et al., 2009). The concept of certain biota having a disproportionate effect on other soil components has long been accepted by soil biologists (Wardle and Giller, 1996). There may be keystone species which are critical determinants of soil function (e.g. nitrogen fixers). These keystone species have the ability to affect process rates disproportionately to their abundance (Wardle and Giller, 1996). The effects of agricultural restoration on ecosystem function will thus be dependent on the functional role of the soil biota in the system.

Soil biota in agricultural settings

Before addressing the effect of restoration on soil biology, I need to briefly consider the effects that agriculture may have on soil biological communities and soil function. Agricultural systems are significantly different from natural ecosystems in terms of composition, abundance, and activity of the soil community (Carnovale et al., 2015; Osborne et al., 2011). Agricultural management can decrease soil biodiversity and alter soil processes from those observed in native forest or grassland communities (Andersen and Sparling, 1997). These changes have been attributed to: 1) increased frequency of disturbance; 2) reduction/change in the quality and quantity of organic matter input; or 3) the use or synthetic compounds such as fertilisers (Beare et al., 1997).

The effect of agricultural practices on soil biota and community structure is very much dependent on the type of agricultural practice (Stark et al., 2008). Agricultural systems are

essentially maintained at an early succession stage due to the high frequency of disturbance (i.e. grazing and tillage). This disturbance, combined with land management practices, such as fertiliser and pesticide inputs, crop types, or water regimes, has profound impacts on the soil biotic community that are distinct from native ecosystems (Chan and Barchia, 2007; Haines and Uren, 1990; Hernández-Hernández and López-Hernández, 2002; Li et al., 2018; Pankhurst et al., 2002). For example, within frequently disturbed agricultural soils endogeic earthworms are better adapted than other behavioural groups such as epigeic and anecic earthworms (Chan, 2001; Crittenden et al., 2014; Ekschmitt and Griffiths, 1998). Land cultivation such as tillage management has large effects on the microbial community. Under conventional tillage bacteria dominate, but as management practices decrease in intensity (e.g. reduced till or no till) fungi dominate (Allison et al., 2005). Soil pH also changes under different tillage management scenarios, and fertiliser application is an important regulator of the soil biotic community and soil respiration (Cookson et al., 2008; Osborne et al., 2011). The lack of substrate for specialised saprotrophic basidiomycetes can also drive decreased levels of fungi in agricultural systems (Carlile et al., 2001; Maharning et al., 2009). Management practices such as no-till and stubble retention can increase total soil organic matter (Haines and Uren, 1990; Helgason et al., 2010) and fungi (Oehl et al., 2004; Ryan et al., 1994). The community structure profiles of microbes are also correlated with total soil organic matter (SOM), light fraction organic matter and dissolved organic matter (DOM) in agricultural systems (Cookson et al., 2008).

The change in land use from forest or natural grasslands to agricultural land may alter soil biota, for example through effects on keystone species, functional groups with low dispersal rates, species-poor functional groups, narrow-physiology microorganisms (e.g. nitrogen-fixers, nitrifiers), or by altering biotic interactions such as predation, mutualism or rhizovory (Andrén et al., 1999). As such, the size, composition and activity of soil microbial communities differ greatly between systems and between management strategies (Allison et al., 2005; Giller et al., 1997; Hartmann et al., 2015).

Restoration of agricultural land

As noted earlier, the methods applied in agricultural restoration vary considerably. Methods of reintroducing native species in restoration can be quite varied and range from land abandonment and subsequent secondary succession to plantation forests or native plantings. Other restoration practices focus on providing a habitat favourable to the development of native

species. These include reduced fertiliser application and organic matter amendments and will not be considered further in this review.

The restoration of agricultural land can have multiple aims, including improving visual appeal, increasing biodiversity, and improving ecological processes (Wade et al., 2008). As such, there is considerable interest in developing easily observable measures of success (e.g. establishment of vegetative communities). This is problematic when considering soil biotic communities, as often such measures have not been established. This coupled with the labour intensity of soil biological studies hinders their ability to be an indicator of restoration success. In a review by Ruiz-Jaen and Aide (2005), plant (79%) and arthropod (35%) richness were the most common measures of diversity used in restored sites. Attempts to measure changes in ecological processes are far less common than assessments of diversity or vegetation structure, leaving a large gap in our current understanding of restoration trajectories, or which restoration approaches are likely to most effectively lead to self-sustaining communities.

It is important to consider the importance of both aboveground and belowground interactions in the functioning of ecosystems (Bach et al., 2010; Bardgett et al., 2005; Wardle et al., 2004). The maintenance of system diversity and ecosystem services is at least partly determined by the nature of the plant community. The composition and structure of plant communities will greatly affect the heterogeneity of soil biota (Beare et al., 1997). The role that soil biodiversity plays in ecosystem function will depend on the quality and quantity of the litter input (Beare et al., 1997). There is a growing body of literature addressing the effect of plants on belowground processes. For example, it is well known that changes in vegetation composition can influence decomposition rates by altering the quality and quantity of litter inputs to soil (Bardgett, 2005; Hobbie, 1992). Trinder et al. (2009) found that in cutover peatlands it was litter quality that drove decomposition rates and litter fungal communities, and not plant species directly. Further to this, measures of microbe-mediated processes such as soil enzyme activity, were shown to change with respect to both season and litter type (Mukhopadhyay and Joy, 2010).

Not only does litter quality affect soil populations, plant rhizospheres significantly influence the microbial community (Berg and Smalla, 2009; Grayston et al., 1998; Singh et al., 2007). Root exudates are carbon rich compounds which provide readily available nutrients and energy sources. These exudates have a pronounced selective and promoting effect on specific microbial communities. For a detailed review on plant driven selection of microbes see

Hartmann et al. (2009). Understanding the relationship between plant types and soil biological function in restored agricultural landscapes has the potential to help improve rehabilitation methods and achieve increased ecosystem services such as carbon-sequestration and improved nutrient cycling (Post and Kwon 2000).

Overall, soil systems are highly complex, and this, coupled with the varied methods of restoration used, has meant that the effects of restoration on soil biota remain poorly understood. This complexity also means that there are relatively few guidelines for how to most effectively restore soil biotic diversity and function. Further to this, restoration of agricultural land is often in areas that have no reasonable natural analogue against which to evaluate results of the revegetation process.

Soil biota as bio indicators

Many papers have focused on indices that can be used to assess the success of restoration (Ritz et al., 2009). For example, the use of biological indicators has been applied to assess soil health (e.g. as determined by physical, chemical, and biological properties) and function. Soil biological communities can be useful indicators of changes in biogeochemical cycles as the biotic components of soil change more rapidly than changes in soil physical and chemical properties (Pankhurst et al., 1995; Patra et al., 2008). In aquatic ecosystems, the role of invertebrates as bio-indicators has been well established as indicators of water quality (Hellawell, 1978; James and Evison, 1979). In terrestrial systems less is known, and this area of research began to feature within the early 1990's literature (Pankhurst et al., 1995).

Macrobiota have generally been used as bio-indicators in restoration due to the relative ease of collection and identification. For example, millipedes can be a useful indicator of restoration success (Decaëns et al., 2006; Redi et al., 2005). Ants have been the most widely used bio-indicators of restoration success (Andersen and Sparling, 1997), and are useful indicators as they show strong succession patterns in systems that have been subject to restoration. Ants are also highly abundant and diverse, relatively easy to sample and are ecologically important at all trophic levels (Andersen et al., 2002). The use of mites as indicators has potential, yet the taxonomic scale at which indicators are selected needs to be appropriate to the environment. Nakamura et al. (2003) looked at the abundance of both oribatid and non-oribatid mites as an indication of the success of rainforest restoration from pasture, finding this level of classification to be insufficient to detect changes.

Microbial community parameters such as microbial biomass, enzyme activities and respiration rate are often used as indicators of soil biological activity (Balota et al., 2014; Doran and Zeiss, 2000; Gonzalez-Quiñones et al., 2011; Raiesi and Salek-Gilani, 2018). However, this can prove to be more complex than the use of macroinvertebrates. The diversity of soil microbiota and the diversity of the enzymes they produce have been commonly used as indicators of the effect of agricultural management on soil microbial community function (Bowles et al., 2014; García-Ruiz et al., 2008; Gianfreda et al., 2005; Pajares et al., 2011). Soil enzyme activities are possible indicators of soil quality because of their relationship to soil biota, and ease of measurement compared to molecular profiling (Dick et al., 1994). Measures such as microbial biomass can provide the first evidence of improved soil function (Gonzalez-Quiñones et al., 2011).

When using a bio-indicator approach to assess the success of restoration activities, a suitable reference site for that ecosystem (i.e. determined by localised climate, soil type, and land use) is required. This is often the case for land restoration projects where either the characteristics of the site before disturbance have been well documented or a parallel undisturbed system can be identified (Hobbs and Harris, 2001; White and Walker, 1997). The inclusion of paired plots of native vegetation is common in research studies seeking to define indicators for soil quality (e.g. Sanchez-Maranon et al., 2002; Saviozzi et al., 2001). However, native reference ecosystems suitable for comparison are often hard to locate or have been altered either directly or indirectly by human pressure (Gil-Sotres et al., 2005). Where no suitable reference site exists, a chronosequence or measures over time can be used as a benchmark for future monitoring (Nielsen et al., 2002). In general, comparing changes in soil properties over time at a specific site rather than making comparisons with other sites is recommended as an appropriate dynamic assessment approach when only poor reference sites are available.

The use of soil biota as indicators of restoration needs further exploration, as soil biology is highly complex and no single biotic measure to assess the success of restoration on ecosystem function will fit all ecosystems. With further work on restored agricultural land a set of indicators for different environments would be of high value for monitoring the effect and overall success of restoration practices.

Trajectories of change

It is well established that soil biota have the capacity to quickly respond to, and change with, restoration but the current understanding of how restoration techniques affect the spatial and temporal recovery of soil biotic communities is limited. The restoration of agricultural land may or may not drive a system to a predictable state. The progression of soil biotic communities to states present prior to disturbance can take many years. Jangid et al. (2011) found that microbial communities in disturbed soils became similar to native vegetation after land abandonment if given enough time (>50 years). Conversely, alternative stable states (e.g. different soil biotic community profiles to that of target native vegetation) may develop and it has been recognised that some ecosystems may have multiple steady states which can be determined by both natural and human disturbance (Aronson et al., 1993).

Agricultural land abandonment and subsequent succession can be viewed as a form of restoration of agricultural lands (Zhang et al., 2013). The effect of succession on biota is a growing area of research (see Table 1) and the principles of succession can potentially be used to govern restoration techniques such as revegetation which accelerate and complement regeneration and subsequent restoration. Succession can indirectly affect soil communities through changes in the quality and diversity of litter. A link between certain soil biotic assemblages and increased rates of plant succession has been found (De Deyn et al., 2003; Reynolds et al., 2003). Behera and Sahani (2003) showed that spontaneous regeneration of agricultural land improved soil biotic characteristics more than *Eucalyptus* plantations although comparisons to native vegetation communities were not made. Natural regeneration through secondary succession of agricultural lands can however be slow. Active restoration of agricultural lands, for example through planting native woody vegetation is one method employed by practitioners to speed up restoration processes. When assessing trajectories of change in actively restored areas a chronosequence approach has been found to be useful for quantifying soil change over decadal time scales and predicting recovery to undisturbed conditions (Allison et al., 2005; Bach et al., 2010).

Effect of the restoration of native vegetation in agricultural landscapes on soil biota

While our understanding of the effects of agricultural restoration on soil biota is growing (see Table 1), often research efforts have focused on one group of soil organisms with little discussion of multiple groups. The following section will discuss the known effects of agricultural restoration on the three broad groups of soil biota based on size.

Table 1. Published studies on the effect of both active and passive restoration on soil biology in agricultural landscapes. Research from 2000-current has been synthesised.

Country	Restoration community	Biotic measure	Macro	Meso	Micro				Reference Author	Type of paper
					Taxa	CO_2	EA	MB		
Netherlands	grassland	isopoda, centipede, millipede	X						Berg and Hemerik (2004)	succession
England	grassland	ants	X						Fagan et al. (2010)	restoration
Netherlands	grassland	macrobiota and beetles	X						Hemerik and Brussaard (2002)	succession
Argentina	grassland	macrofauna	X						Thomas et al. (2004)	succession
USA	meadow	isopods, beetles, earthworms and ants	X						Riggins et al. (2009)	restoration
China	woodland/grassland	beetles	X						Liu et al. (2012)	restoration
Australia	woodland	earthworms	X						Carnovale et al. (2015)	restoration
Australia	woodland	beetles	X						Gibb and Cunningham (2010)	restoration
Australia	rainforest	litter arthropods	X	X					Nakamura et al. (2003)	restoration
Netherlands	grassland	predatory mites, earthworms, enchytraeids, nematodes and bacteria	X	X	X				Postma-Blaauw et al. (2012)	
USA	forest	earthworms	X						Szlávecz and Csuzdi, 2007	succession
USA	grassland	nematodes		X					Biederman et al. (2008)	restoration
Netherlands	grassland	nematodes, microarthropods		X					De Deyn et al. (2004)	succession
Germany	grassland	collembolan		X					Filser et al. (2002)	restoration
Netherlands	grassland and heathland	food web, fungal and bacterial, nematodes, micro arthropods		X	X				Holtkamp et al. (2008)	succession
Netherlands	grassland	nematodes		X					Kardol et al. (2009a)	restoration
Netherlands	grassland	mites, nematodes		X					Kardol et al. (2009b)	succession
Australia	rainforest	mites		X					Proctor et al. (2011)	restoration
USA	grassland	PLFA			X				Bach et al. (2010)	restoration
China	grassland	FAME, MB			X			X	Jia et al. (2010)	succession
Sweden	grassland	PLFA, NFLA			X	X		X	Hedlund (2002)	restoration
Netherlands	grassland and heathland	C and N mineralisation modelled			X				Holtkamp et al. (2011)	succession
USA	grassland and forest	16S rRNA			X				Jangid et al. (2011)	restoration
USA	grassland	PLFA, B hydroxbutrate			X				McKinley et al. (2005)	restoration

Country	Restoration community	Biotic measure	Macro	Meso	Micro				Reference Author	Type of paper
					Taxa	CO_2	EA	MB		
Netherlands	grassland	isopoda, centipede, millipede	X						Berg and Hemerik (2004)	succession
France	grassland	fungi, bacteria, Biolog			X	X	X	X	Plassart et al. (2008)	restoration
USA	grassland	PLFA			X				Potthoff et al. (2006)	restoration
china	grassland	MB and community profile			X			X	Jia et al. (2010)	succession
China	grassland	PCR-denaturing gradient gel electrophoresis (DGGE). nifH gen			X				Huhe et al. (2014)	succession
Czech Republic	grassland	fungal community			X				Pánková et al. (2018)	succession
USA	grassland					X			Allison and Jastrow (2006)	
Iran	grassland	EA					X	X	Raiesi and Salek-Gilani (2018)	succession
USA	prairie	fungi, bacteria, EA			X		X		Upton et al 2018	restoration
USA	prairie	MB and CO_2 efflux				X		X	Rosenzweig et al. (2016)	restoration
Italy Austria	grassland	MB, E_C/E_{NIN} ratio, ergosterol, and PLFA							Zeller et al. (2001)	
Australia	forest/woodland	bacteria			X				Osborne et al. (2011)	restoration
Australia	forest	fungi, bacteria			X				Kasel et al. (2008)	restoration
Australia	forest	MB. respiration, permanganate oxidation			X	X		X	Mendham et al. (2002b)	restoration
Spain	forest	PLFA			X	X		X	Zornoza et al. (2009b)	succession
China	forest	N functional genes			X				Wang et al. (2017)	restoration
India	forest	EA, MBC, respiration				X	X	X	Mukhopadhyay and Joy (2010)	restoration
Brazil	forest	MB, EA					X	X	Nunes et al. (2012)	restoration
Italy	native vegetation	MB, PCR-DGGE fingerprinting			X			X	Cavani et al. (2016)	restoration
USA	riparian	soil microbes,			X		X	X	Tian et al. (2004)	restoration
USA	wetlands	bacterial community			X				Kluber et al. (2014)	restoration
Australia	woodland	microbial DNA			X				Hamonts et al. (2017)	restoration
Australia	woodland	AMF			X				Prober et al. (2015)	restoration
Australia	woodland	microbial eDNA			X				Gellie et al (2017)	restoration
Australia	woodland	microbial eDNA			X				Yan et al (2018)	restoration

phospholipid fatty acids (PLFA), Enzyme activity (EA), microbial biomas carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass (MB), Fatty acid methyl esters (FAME), Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), Arbuscular mycorrhizal fungi (AMF).

Macrofauna

The general importance of macrofauna in agricultural systems, especially the role that earthworms play in productivity and improved soil structure, has received considerable attention (Baker, 1998, 2004; Baker et al., 2003; van Groenigen et al., 2014). However, the effect of ecological restoration of agricultural lands on earthworms and other macrofauna is less well understood (Carnovale et al., 2015). Studies of abandoned sites have shown that the macrofaunal community does not always return to that of native sites (Scheu (1992). For example, Thomas et al. (2004) found that at 15 years after abandonment, fields shared only 23.4% of macrofauna species with those of natural grasslands. This is consistent with other work which indicated that earthworm assemblages were different in successional forests compared to the mature native forests (Szlávecz and Csuzdi, 2007). Despite many studies in grassland systems, native woody systems have received limited research to date.

Changes in community composition of ground dwelling beetles in restored systems is well acknowledged within Australian landscapes (Gibb and Cunningham, 2010; Gibb et al., 2006). When comparing pasture, revegetated sites and native natural references sites, epigeic (ground-foraging) beetle biomass for all trophic groups was similar across sites. This suggests that beetle-driven functions operate similarly under different conditions and may reflect high functional resilience of epigeic beetles (Gibb and Cunningham, 2010). It has been shown that revegetation attracts beetle community assemblages that begin to resemble that within remnant areas (Gibb and Cunningham, 2010; Liu et al., 2012). For example, pasture sites have a more homogeneous assemblage of saprophages and predator species, while revegetated and native sites have more heterogeneous assemblages (Gibb and Cunningham, 2010).

Restoration of agricultural land through reduced fertiliser application to natural nutrient poor grasslands, resulted in low diversity in isopod, centipede, and millipede communities within both the highest and lowest nutrient status sites (Berg and Hemerik, 2004). Nutrient status could explain some of the variation within these communities, however it is hypothesised that carbon accumulation may have a larger influence on the density and diversity of macroinvertebrate communities.

As noted earlier, the use of ants as bio-indicators has been well established. For example, in the restoration of an English grassland, *Myrmica sabulieti* could be used as an indicator of the success of restoration, in that this species along with several others was found in natural sites but not modified sites (Fagan et al., 2010). In Australia, ant assemblages have also been

shown to be useful indicators of restored ecosystem services (Andersen and Majer, 2004). Ant species richness was positively correlated to microbial biomass, demonstrating a link between aboveground activities and decomposition processes (Andersen and Majer, 2004). This supports the use of ants as indicators of restoration success and restoration of ecological services (Andersen and Majer, 2004). Other studies such as Nakamura et al. (2003) found that ant genus could not differentiate pasture from rainforest but a broader taxonomic approach using ants, centipedes, millipedes, and isopods could collectively differentiate restored sites from pastures and native forest.

Few studies have looked at the overall macroinvertebrate community in agricultural restoration (Nakamura et al., 2003; Riggins et al., 2009; Thomas et al., 2004). Riggins et al. (2009) compared soil invertebrate biodiversity between restored and native wet meadows to assess the effectiveness of restoration practices. Native sites had higher Shannon and Simpson diversity values and contained greater invertebrate biomass than restored sites. Isopods, scarab beetles, click beetles, earthworms, and ants were surveyed and of these, only ants occurred more frequently in restored sites. This indicated that restoration practices in wet meadows may not be completely effective with regard to returning sites to native conditions. Often studies that combine multiple orders of invertebrates only identify the macroinvertebrate community to order and family. Characterisation of communities at this taxonomic level is only sensitive to short term varying abiotic factors within secondary succession (Hemerik and Brussaard, 2002). This highlights the importance of appropriate classification and identification of invertebrate communities when assessing the effect of restoration in agricultural landscapes.

Mesofauna

A large component of the literature on mesofauna is focused on succession (Table 1). For example, Kardol et al. (2009b) found that, in the case of oribatid mites, restoration of soil diversity after cessation of agricultural practices depended predominantly on colonization by new taxa from surrounding habitats, while diversity patterns of nematodes mainly depended on within-site spatial segregation and shifts in dominance patterns. Moreover, successional patterns of oribatid mite and nematode diversity differed across levels of scale (α-, β- or γ-diversity). Both the α- and γ diversity of oribatid mites increased with time since abandonment but, while β-diversity was initially high, this decreased thereafter. This is similar to previous observations from a 55 year chronosequence of temperate grassland sites (Zaitsev et al., 2006) and a 120 year chronosequence going from agricultural land to beech wood (Scheu and Schulz,

1996). Low oribatid mite abundance in early-successional sites observed by Zaitsev et al., (2006) and Scheu and Schulz (1996) may be attributed to the legacy of former agricultural practices, i.e. physical and chemical disturbance (Adl et al., 2006). Density and diversity of oribatid mites, are generally strongly reduced in arable soils (Minor and Cianciolo, 2007; Nielsen et al., 2010) due to the mites having long life cycles of more than one year and little capacity to respond to mechanical perturbations (Maraun et al., 2003). In contrast, nematode density can be high in arable soils (Freckman and Ettema, 1993). Most nematode taxa have fast reproduction cycles and hence may be less sensitive to agricultural disturbances than oribatid mites. Restoration of grassland resulted in establishment of a species-rich community of nematodes, while predatory mite species were less successful in re-establishing and negative effects on enchytraeid species persisted (Postma-Blaauw et al., 2012). It has been shown that collembolans can decrease with restoration as competition and predation increased due to reductions in disturbance (Filser et al., 2002). Similar to ants, it has been found that collembolan abundance also correlates with microbial biomass in restored landscapes (Andersen and Majer, 2004; Filser et al., 2002).

The mesofauna have received a fair amount of attention in restoration as indicators of the success of restoration (Nakamura et al., 2003; Neher, 2001; Proctor et al., 2011). Collembolans have received some attention as indicators because of their importance in ecological function and sensitivity to environmental change (Greenslade, 2007). However, individual taxa are not always good indicators of restoration. For example, progress in the restoration of rainforests on pastoral land within Australia could not be monitored using mites classified as oribatid or other, as the mites present had a high abundance in both pasture and restored sites (Nakamura et al., 2003). Further to this, Proctor et al. (2011) found that although mite assemblages clearly distinguished between rainforest and pasture sites, they did not identify any of the four revegetation methods as producing results consistently similar to native rainforest.

Microbes

Due to the complexity of the soil microbial community, the effect of restoration on soil microbes has received much attention (Harris, 2003; Potthoff et al., 2005; Potthoff et al., 2006). Due to the relative ease of quantification indirect measures of the microbial community such as microbial biomass and enzyme activity which rapidly responds to restoration have largely been used (Gonzalez-Quiñones et al., 2011; Wu et al., 1990). Microbial biomass provides an early indication of changes in soil organic matter, more so than measures of total carbon (Allison et

al., 2010; Anderson and Domsch, 1989; Carter, 1986). Microbial biomass is higher in old restored grassland sites and virgin remnant sites compared to agricultural fields or recently restored sites (McKinley et al., 2005; Rosenzweig et al., 2016). Other studies have found that microbial biomass may be a poor indicator of land use change (Mendham et al., 2002a; Zeller et al., 2001). For example, soil microbial biomass carbon was similar in abandoned and managed grasslands, whereas soil microbial nitrogen was lower and ergosterol contents (a measure of fungal biomass) were higher in abandoned grasslands as compared to managed meadows (Zeller et al., 2001). It was concluded by Zeller et al. (2001) that the impact of management abandonment on soil microbial biomass was of relatively less importance than the effects of site and sampling time.

Nitrification rates and nitrifier numbers are other measures which have been studied to assess the success of restoration in restoring ecosystem function. Restoration of degraded riparian zones led to a reduction in nitrifier numbers which has important implications for water and air quality (Tian et al., 2004). Nitrification rates increased with reforestation and reached levels similar to rainforest sites in at least one reforestation pathway (Paul et al., 2010). These indirect measures of the microbial community provide valid evidence of improved soil function but little insight into the taxa, species or community assemblages that are responsible for the function.

A rapidly growing body of work has examined processes of secondary succession on abandoned agricultural land (Holtkamp et al., 2008; Jia et al., 2010). During succession, microbial activity is dependent on, and responds positively to, vegetation development. Succession on agricultural land in the semi-arid Loess Plateau of China led to increasing organic Ca, total N, and microbial biomass C and N (Jia et al., 2010). In the same study, total fatty acid methyl esters (FAME) biomass and the ratio of fungal to bacterial biomass were useful indicators of soil quality during the restoration period. Soil organic matter showed a positive influence on the size and composition of microbial community. Additionally, during succession, Phospholipid Fatty Acid Analysis (PLFA) fungal to bacterial ratio was higher in abandoned agricultural soils, whereas the relative abundance of bacteria was higher in agricultural soils (Jia et al., 2010). Actinomycetes were generally lower in abandoned agricultural soils, while the proportions of vesicular-arbuscular mycorrhizal fungi were, as a general trend, higher in agricultural and abandoned agricultural soils than in forests (Zornoza et al., 2009b).

In terms of active restoration, as the intensity of land use decreases to more closely resemble a more natural state, there is an increase in the fungal to bacterial ratio. This increases further with the development of woody vegetation and forest conversion (Fierer et al., 2009). Recently, Cavagnaro (2016) found that reforestation of agricultural land supported a greater microbial PLFA, a higher fungal:bacterial PLFA ratio and a different microbial community (based on PLFA profiles) from that of the adjacent pasture.

Time since restoration plays a significant role in microbial community composition. Using terminal restriction fragment length polymorphism (T-RFLP), Osborne et al. (2011) found that soil bacterial communities in a revegetated site were more similar to those in a remnant native woodland than an adjacent pasture. However, time since establishment was not considered. Recent studies using high-throughput environmental DNA (eDNA) monitoring of fungi (Yan et al. 2018) and bacteria (Gellie et al. 2017) to follow the restoration of woody vegetation on cleared land, found that after just 10 (fungi) and 8 years (bacteria) of active native plant revegetation, shifts in the respective microbial communities towards that of the natural communities, could be observed. This is consistent with other findings where microbial biomass (Nunes et al., 2012) and PLFA profiles in revegetated sites most closely resemble those for native vegetation (Bach et al., 2010; Potthoff et al., 2006). Increases in microbial biomass and diversity (PLFA) in restored sites compared to cultivated land have also been observed in some studies where no remnant site was available as a reference (Zornoza et al., 2009a). Estimates of microbial biomass show that bacteria and saprophytic fungi increase in soil where seed mixtures have been sown compared to naturally colonised plots. Respiration measurements showed higher microbial activity and biomass in sown plots compared to naturally colonised plots (Hedlund, 2002).

The response of soil fungal communities in terms of composition depends on suitable habitat, and the ability to easily disperse after land use change within the environment studied (Kasel et al., 2008). As such, changes in community composition may not be evident in all restoration projects. There is some evidence that bacterial communities are more weakly affected by agricultural practices than fungi (Plassart et al., 2008). Plassart et al. (2008) found no significant differences in bacterial genetic diversity between cultivated and restored grassland sites. The lack of clear bacterial responses to plant community shifts could be a consequence of past land management having a stronger influence over microbial communities than plant community shifts. In contrast, other studies have found a strong relationship between

fungal genetic diversity and the age of grasslands (Jangid et al., 2011). An increase in microbial activity (i.e. % mineralization) with increasing time since restoration has also been observed (Plassart et al., 2008).

The response of microbes to shifts in plant communities has been shown to be dependent on both abiotic and biotic changes in local environmental conditions during plant establishment (Fierer and Jackson, 2006; Fierer et al., 2009; Rousk et al., 2010). The shift in plant communities from annual grassland to perennial grassland can result in minor responses of the microbe community (Porazinska et al., 2003; Potthoff et al., 2006). However, others found shifts from annual grassland to perennial woody vegetation can see larger changes in microbe communities, at least during the early stages of restoration (Cavagnaro et al., 2016). Bach et al. (2010) and Jangid et al. (2010) found that the ability of the microbial community to be restored is dependent on the structural recovery of the soil. For example, Bach et al. (2010) found that microbial community recovery from long-term cultivation through grassland establishment is moderated by soil texture. In silty clay loam soils, increases in total PLFA, biomass, richness, fungi, arbuscular mycorrhizal fungi, gram-positive bacteria, gram-negative bacteria, and actinomycetes occurred across the restoration chronosequence. However, recovery as indicated by microbial PLFA profiles was suppressed on loamy fine soil. The increase in PLFA was suggested to be a result of the clay component of soils having a greater capacity to store and make available C substrates compared to the loamy fine soil. Often the change in community composition is related to soil chemical properties rather than restoration. For example, pH has been found to be a key determinant of community similarity across agriculture, restored land and native sites in studies conducted on a variety of soil types and spatial scales, using a range of methods (Fierer and Jackson, 2006; Högberg et al., 2007; Lauber et al., 2009; Rousk et al., 2010).

Biota as agents of restoration

Symbiotic interactions between plants and mutualistic soil organisms are important components of all plant communities and therefore may drive the success of many restoration endeavours. It has been shown that soil biota are able to strongly affect both the composition of vegetation and soil function, therefore knowledge about these interactions has the potential to improve restoration success in agricultural areas (Bradford et al., 2002; De Deyn et al., 2003; Wardle et al., 2002).

There have been only limited attempts to inoculate agricultural sites with macrofauna and mesofauna as part of the restoration process (Table 2). In a microcosm experiment, Fraser et al. (2003) looked at the use of earthworms and the effect of crop residues on the restoration of degraded arable soils, finding that worms were important regulators of soil aggregates, increasing aggregate size. However, the effectiveness of earthworms as tools for soil restoration is varied and depends on the species of worm, climate, soil type and fertility (see Snyder and Hendrix (2008). De Deyn et al. (2003) inoculated microfauna (nematodes), mesofauna (micro-arthropods) and macrofauna (beetle larvae) into abandoned production grassland and found that the addition of all three successional communities of soil fauna resulted in significant shifts in the plant communities towards the dominance of the plant species representing the late succession (target) community, compared to control soils where plant communities were dominated by mid-succession plant species.

Broadly speaking, in terms of success, revegetation efforts have met with mixed results, however, it is evident that in some environments the use of beneficial microorganisms can significantly increase the success of restoration (Bourne et al., 2008; Thrall et al., 2005). A mesocosm experiment exploring the effect of the soil biota (soil freezing treatment −80°C for 3 days) on plant growth saw significantly retarded growth in *Eucalyptus* seedlings with a reduction in soil biota (Bourne et al., 2008). The inoculation of indigenous arbuscular mycorrhizal fungi and rhizobial nitrogen-fixing bacteria can enhance the establishment of plant species as well as increase soil fertility and quality in restored ecosystems (Herrera et al., 1993; Requena et al., 2001). In agricultural sites undergoing restoration, Thrall et al. (2005) found that inoculation of *Acacia* spp. with rhizobial strains led to a 118% increase in establishment of *Acacia* seedlings, and the effect was greatest in more marginal environments. Inoculation of ex-arable land has shown that soil inocula not only promote ecosystem restoration, but that different origins (e.g. heathland versus grassland) of soil inocula can steer plant community development towards different target communities (Wubs et al., 2016). The impact of soil inoculation on plant and soil community composition was most pronounced when the topsoil layer was removed whereas effects were weaker, but still significant, when soil inocula were introduced into intact topsoil.

The inoculation of early successional land with later successional soil organisms or soil to accelerate succession has received some attention. Inoculation with late-successional soil communities into grassland plant communities can increase the performance of late-

successional target plant species (Carbajo et al., 2011; De Deyn et al., 2003). The largest total plant biomass of late successional plant species was produced with soil from late successional grassland and the most even plant community composition with soil from semi-natural grassland (Carbajo et al., 2011). In contrast, Kardol et al. (2009a) found that the introduction of later successional soil organisms into a topsoil-removed soil did not facilitate the establishment of later successional plants. Overall, much work needs to be done to establish to what extent and under what conditions, soil communities may steer the direction of plant community development towards different target states.

Table 2. Current use of biota to aid in ecosystem restoration.

Country	Restoration community	Type / mode of introduction/elimination	Macro	Meso	Micro	Reference Author
Canada	forest	soil inoculum			X	St-Denis et al. (2017)
Netherlands	grassland	nematodes, micro-arthropods, beetle larvae	X	X		De Deyn et al. (2003)
Netherlands	grassland	soil inoculum		X	X	Carbajo et al. (2011)
Netherlands	grassland	soil inoculum			X	Kardol et al. (2006)
Netherlands	grassland	soil inoculum			X	Kardol et al. (2009a)
Netherlands	grassland heathland	soil inoculum	X	X	X	Wubs et al. (2016)
Worldwide	grasslands, shrublands, woodlands	soil inoculum			X	Neuenkamp et al. (2018)
Spain	native legume	mycorrhizae, rhizobia			X	Herrera et al. (1993)
USA	prairies	soil inoculum			X	Herzberger et al. (2015)
USA	shrubland	inoculation with fungi/algae			X	Hamman and Hawkes (2013)
Spain	shrubland	mycorrhizae, rhizobia			X	Requena et al. (2001)
worldwide	various – review	earthworms, millipedes and isopods	X			Snyder and Hendrix (2008)
worldwide	various – review	various	X	X	X	Harris (2009)
Australia	woodland	soil sterilization			X	Bourne et al. (2008)

Implications for restoration of degraded sites: Where to now?

There is a large body of theoretical research on soil biota and ecological restoration (Callaham et al., 2008; Decaëns et al., 2006; Heneghan et al., 2008; Kardol and Wardle, 2010). This knowledge, in combination with quantitative evidence, is providing a strong base for continued research. The number of studies on soil microbes exceeds the number of studies on macrofauna and mesofauna (Fig. 3). This highlights the complexity associated with soil microbes. Given recent advances in molecular approaches and the growing awareness of linkages between aboveground and belowground communities, there is significant scope for enhancing our current knowledge of these interactions in restored agricultural systems. Through this literature review, I have identified three main areas for future development: 1) use of recent molecular advances to further understand microbial communities in restored ecosystems; 2) developing our understanding of how variation in soil community structure and diversity alters ecosystem functions; and 3) strengthening knowledge of the factors that influence trajectories of change in restored systems. These areas of further research are particularly important for native woody systems which have received only limited research attention to date.

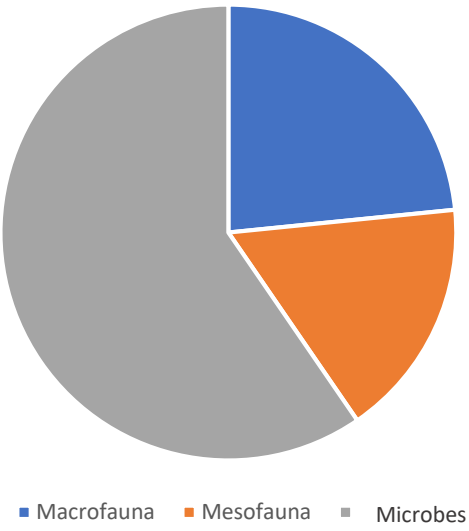


Figure 3. Summary of published research on the effects of both active and passive restoration on soil biology in agricultural landscapes from 2000-2019, broken down by the three broad groups, macrofauna, mesofauna and microbes (n=47).

Taking advantage of molecular advances

Quantification of the relationships between soil community structure, diversity and function in ecological processes has been limited due in part to the difficulties associated with studying soil biota. Much of the microbial community cannot be cultured in the laboratory, thus

representing a ‘black box’ within the soil (Tiedje et al., 1999). With rapid developments in molecular techniques, there is increasingly greater research emphasis on microbial community composition in revegetated landscapes. Such efforts are providing insights into the full microbial composition of soils and increase our ability to explore links between community structure and function, and the resilience or stability of soil ecosystems (Emmerling et al., 2002). The use of molecular fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis and terminal restriction-fragment length polymorphism (T-RFLP) and high-throughput sequencing that measures community structure can help inform management strategies for restoring agricultural land. One drawback of these methods is the inability to measure directly the functions and traits that microorganisms exhibit within their environment. However, further exploration of soil microbial communities using these techniques can offer high quality data on soil microbial communities and provide an avenue to understand the link between diversity, community structure and function (Tiedje et al., 1999).

Restoring function

Our understanding of how variability in soil community composition alters ecosystem function remains limited (Thrall et al., 2011). We need further research on how the restoration process affects soil biotic communities and how these changes in turn affect processes such as decomposition and carbon dynamics. At a community level, little is known about restoration effects given that most studies focus on only one or two taxa (see Table 1). While characterisation of community interactions can provide insight to functions that the whole community is undertaking, interactions between different soil taxa have been largely neglected within restoration. Integrated studies are needed that look at how different components of the biological community (e.g. macrofauna) are affected by restoration as well as how their interactions affect functional processes such as nutrient cycling. There have been limited rigorous experimental manipulations of soil factors which incorporate the real complexity of soil systems. Within restoration ecology, a greater understanding of soil ecological processes in revegetated sites can advance restoration success (Callaham et al., 2008).

Time constraints

Much research on succession has been conducted to assess the trajectories of change in agricultural restoration. In this context, chronsequences have proven to be a useful tool for quantifying soil change over decadal time scales and predicting recovery to undisturbed

conditions. In combination to chronosequence a Before-After-Controlled-Impact (BACI) design (for) can determine changes through time induced by the treatment, relative to the background changes through time. The nature of many altered ecosystems is that in some cases finding analog sites may be problematic. This, in combination with the need to obtain baseline data prior to the restoration of agricultural land (e.g. including reference communities which can be used to help measure the success of restoration efforts) can hinder research progress. The lack of analogue sites to compare against restored sites limits our ability to determine if soil communities in the latter are approaching a natural state. However, when analog sites are not present, using measures of soil function (i.e. microbial biomass, and enzyme activities) as indicators of the effectiveness of restoration may be valuable.

Conclusion

The relationships between soil biota, soil chemistry, and plant community structure during the transition from agricultural land to that of a restored system are critical to improving the success and cost-effectiveness of restoration efforts. Through a literature review, I have identified three main areas for future development: 1) use of recent molecular advances to further understand microbial communities in restored ecosystems; 2) increasing our understanding on how variation in soil community structure and diversity influences ecosystem function; and 3) strengthening knowledge regarding trajectories of change in restored systems; particularly how different restoration approaches might affect the rate and direction of change. These areas of further research are particularly important for native woody systems which have received limited research to date. The ability to determine the role that soil biota play in facilitating the establishment of woody vegetation and the rate at which a restoration stabilises on former agricultural lands will enhance the degree and rate at which a system recovers.

References

Adl, S.M., Coleman, D.C., Read, F., 2006. Slow recovery of soil biodiversity in sandy loam soils of Georgia after 25 years of no-tillage management. Agriculture, Ecosystems and Environment 114, 323-334.

Allison, S.D., Jastrow, J.D., 2006. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biology and Biochemistry 38, 3245-3256.

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3, 336-340.

Allison, V.J., Miller, R.M., Jastrow, J.D., Matamala, R., Zak, D.R., 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Science Society of America Journal 69, 1412-1421.

Andersen, A.N., Hoffmann, B.D., Müller, W.J., Griffiths, A.D., 2002. Using ants as bioindicators in land management: simplifying assessment of ant community responses. Journal of Applied Ecology 39, 8-17.

Andersen, A.N., Majer, J.D., 2004. Ants show the way Down Under: invertebrates as bioindicators in land management. Frontiers in Ecology and the Environment 2, 291-298.

Andersen, A.N., Sparling, G.P., 1997. Ants as indicators of restoration success: relationship with soil microbial biomass in the Australian seasonal tropics. Restoration Ecology 5, 109-114.

Anderson, J.D., Ingram, L.J., Stahl, P.D., 2008. Influence of reclamation management practices on microbial biomass carbon and soil organic carbon accumulation in semiarid mined lands of Wyoming. Applied Soil Ecology 40, 387-397.

Anderson, T.-H., Domsch, K.H., 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biology and Biochemistry 21, 471-479.

Andrén, O., Brussaard, L., Clarholm, M., 1999. Soil organism influence on ecosystem-level processes - Bypassing the ecological hierarchy? Applied Soil Ecology 11, 177-188.

Andrés, P., Mateos, E., 2006. Soil mesofaunal responses to post-mining restoration treatments. Applied Soil Ecology 33, 67-78.

Aronson, J., Floret, C., Le Floc'h, E., Ovalle, C., Pontanier, R., 1993. Restoration and rehabilitation of degraded ecosystems in arid and semi-arid Lands. I. A View from the South. Restoration Ecology 1, 8-17.

Bach, L.H., Grytnes, J.-A., Halvorsen, R., Ohlson, M., 2010. Tree influence on soil microbial community structure. Soil Biology and Biochemistry 42, 1934-1943.

Baker, G.H., 1998. Recognising and responding to the influences of agriculture and other land-use practices on soil fauna in Australia. Applied Soil Ecology 9, 303-310.

Baker, G.H., 2004. Managing earthworms as a resource in Australian pastures, Earthworm Ecology. CRC Press, 263-286.

Baker, G.H., Amato, M., Ladd, J., 2003. Influences of *Aporrectodea trapezoides* and *A. rosea* (Lumbricidae) on the uptake of nitrogen and yield of oats (*Avena fatua*) and lupins (*Lupinus angustifolius*): The 7th international symposium on earthworm ecology Cardiff Wales 2002. Pedobiologia 47, 857-862.

Balota, E.L., Yada, I.F., Amaral, H., Nakatani, A.S., Dick, R.P., Coyne, M.S., 2014. Long-term land use influences soil microbial biomass P and S, phosphatase and arylsulfatase activities, and S mineralization in a Brazilian oxisol. Land Degradation and Development 25, 397-406.

Banerjee, S., Kirkby, C.A., Schmutter, D., Bissett, A., Kirkegaard, J.A., Richardson, A.E., 2016. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biology and Biochemistry 97, 188-198.

Bardgett, R., 2005. The Biology of Soil: A community and ecosystem approach Oxford University Press, United States.

Bardgett, R.D., Bowman, W.D., Kaufmann, R., Schmidt, S.K., 2005. A temporal approach to linking aboveground and belowground ecology. Trends in Ecology and Evolution 20, 634-641.

Beare, M.H., Reddy, M.V., Tian, G., Srivastava, S.C., 1997. Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of decomposer biota. Applied Soil Ecology 6, 87-108.

Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology 68, 1-13.

Berg, M., Hemerik, L., 2004. Secondary succession of terrestrial isopod, centipede, and millipede communities in grasslands under restoration. Biology and Fertility of Soils 40, 163-170.

Biederman, L.A., Boutton, T.W., Whisenant, S.G., 2008. Nematode community development early in ecological restoration: The role of organic amendments. Soil Biology and Biochemistry 40, 2366-2374.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.J., Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.J., 2013. A review of earthworm impact on soil function and ecosystem services. European Journal of Soil Science 64, 161-182.

Bourne, M., Nicotra, A., Colloff, M., Cunningham, S., 2008. Effect of soil biota on growth and allocation by *Eucalyptus microcarpa*. Plant and Soil 305, 145-156.

Bowles, T.M., Acosta-Martínez, V., Calderón, F., Jackson, L.E., 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biology and Biochemistry 68, 252-262.

Bradford, M.A., Tordoff, G.M., Eggers, T., Jones, T.H., Newington, J.E., 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. Oikos 99, 317-323.

Brussaard, L., 1998. Soil fauna, guilds, functional groups and ecosystem processes. Applied Soil Ecology 9, 123-135.

Callaham, M.A., Rhoades, C.C., Heneghan, L., 2008. A striking profile: soil ecological knowledge in restoration management and science. Restoration Ecology 16, 604-607.

Carbajo, V., den Braber, B., van der Putten, W.H., De Deyn, G.B., 2011. Enhancement of late successional plants on ex-arable land by soil inoculations. PLoS One 6, e21943.

Carlile, M.J., Watkinson, S.C., Gooday, G.W., 2001. The fungi. Gulf Professional Publishing.

Carnovale, D., Baker, G., Bissett, A., Thrall, P., 2015. Earthworm composition, diversity and biomass under three land use systems in south-eastern Australia. Applied Soil Ecology 88, 32-40.

Carter, M.R., 1986. Microbial biomass and mineralizable nitrogen in solonetzic soils: Influence of gypsum and lime amendments. Soil Biology and Biochemistry 18, 531-537.

Cavagnaro, T.R., 2016. Life at the interface: above- and below-ground responses of a grazed pasture soil to reforestation. Applied Soil Ecology 100, 27-37.

Cavani, L., Manici, L.M., Caputo, F., Peruzzi, E., Ciavatta, C., 2016. Ecological restoration of a copper polluted vineyard: Long-term impact of farmland abandonment on soil bio-chemical properties and microbial communities. Journal of Environmental Management 182, 37-47.

Chan, K.Y., 2001. An overview of some tillage impacts on earthworm population abundance and diversity — implications for functioning in soils. Soil and Tillage Research 57, 179-191.

Chan, K.Y., Barchia, I., 2007. Soil compaction controls the abundance, biomass and distribution of earthworms in a single dairy farm in south-eastern Australia. Soil and Tillage Research 94, 75-82.

Clewell, A., Aronson, J., Winterhalder, K., 2004. The SER international primer on ecological restoration.

Cookson, W.R., Murphy, D.V., Roper, M.M., 2008. Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient. Soil Biology and Biochemistry 40, 763-777.

Courtney, R., Keith, A.M., Harrington, T., 2010. Nematode assemblages in bauxite residue with different restoration histories. Restoration Ecology, 19: 758-764.

Crittenden, S., Eswaramurthy, T., De Goede, R., Brussaard, L., Pulleman, M., 2014. Effect of tillage on earthworms over short-and medium-term in conventional and organic farming. Applied Soil Ecology 83, 140-148.

De Deyn, G.B., Raaijmakers, C.E., Van Der Putten, W.H., 2004. Plant community development is affected by nutrients and soil biota. Journal of Ecology 92, 824-834.

De Deyn, G.B., Raaijmakers, C.E., Zoomer, H.R., Berg, M.P., de Ruiter, P.C., Verhoef, H.A., Bezemer, T.M., van der Putten, W.H., 2003. Soil invertebrate fauna enhances grassland succession and diversity. Nature 422, 711-713.

Decaëns, T., Jiménez, J.J., Gioia, C., Measey, G.J., Lavelle, P., 2006. The values of soil animals for conservation biology. European Journal of Soil Biology 42, Supplement 1, S23-S38.

Dick, R., Sandor, J., Eash, N., 1994. Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru. Agriculture, Ecosystems and Environment 50, 123-131.

Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: managing the biotic component of soil quality. Applied Soil Ecology 15, 3-11.

Dunger, W., Voigtländer, K., 2005. Assessment of biological soil quality in wooded reclaimed mine sites. Geoderma 129, 32-44.

Dunger, W., Wanner, M., Hauser, H., Hohberg, K., Schulz, H.J., Schwalbe, T., Seifert, B., Vogel, J., Voigtländer, K., Zimdars, B., Zulka, K.P., 2001. Development of soil fauna at mine sites during 46 years after afforestation. Pedobiologia 45, 243-271.

Ekschmitt, K., Griffiths, B., 1998. Soil biodiversity and its implications for ecosystem functioning in a heterogeneous and variable environment. Applied Soil Ecology 10, 201-215.

Emmerling, C., Schloter, M., Hartmann, A., Kandeler, E., 2002. Functional diversity of soil organisms—a review of recent research activities in Germany. Journal of Plant Nutrition and Soil Science 165, 408-420.

Emmerling, C., Udelhoven, T., Schröder, D., 2001. Response of soil microbial biomass and activity to agricultural de-intensification over a 10 year period. Soil Biology and Biochemistry 33, 2105-2114.

Eviner, V.T., Hawkes, C.V., 2008. Embracing variability in the application of plant–soil interactions to the restoration of communities and ecosystems. Restoration Ecology 16, 713-729.

Fagan, K.C., Pywell, R.F., Bullock, J.M., Marrs, R.H., 2010. Are ants useful indicators of restoration success in temperate grasslands? Restoration Ecology 18, 373-379.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103, 626-631.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecology Letters 12, 1238-1249.

Filser, J., Mebes, K.H., Winter, K., Lang, A., Kampichler, C., 2002. Long-term dynamics and interrelationships of soil Collembola and microorganisms in an arable landscape following land use change. Geoderma 105, 201-221.

Fischer, C., Roscher, C., Jensen, B., Eisenhauer, N., Baade, J., Attinger, S., Scheu, S., Weisser, W.W., Schumacher, J., Hildebrandt, A., 2014. How do earthworms, soil texture and plant composition affect infiltration along an experimental plant diversity gradient in grassland? PLoS One 9, e98987.

Fraser, P.M., Beare, M.H., Butler, R.C., Harrison-Kirk, T., Piercy, J.E., 2003. Interactions between earthworms (*Aporrectodea caliginosa*), plants and crop residues for restoring properties of a degraded arable soil: The 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. Pedobiologia 47, 870-876.

Freckman, D.W., Ettema, C.H., 1993. Assessing nematode communities in agroecosystems of varying human intervention. Agriculture, Ecosystems and Environment 45, 239-261.

García-Ruiz, R., Ochoa, V., Hinojosa, M.B., Carreira, J.A., 2008. Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biology and Biochemistry 40, 2137-2145.

Gellie, N.J.C., Mills, J.G., Breed, M.F., Lowe, A.J., 2017. Revegetation rewilds the soil bacterial microbiome of an old field. Molecular Ecology 26, 2895-2904.

Gianfreda, L., Rao, M.A., Piotrowska, A., Palumbo, G., Colombo, C., 2005. Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Science of the Total Environment 341, 265-279.

Gibb, H., Cunningham, S.A., 2010. Revegetation of farmland restores function and composition of epigaeic beetle assemblages. Biological Conservation 143, 677-687.

Gibb, H., Pettersson, R.B., Hjältén, J., Hilszczanski, J., Ball, J.P., Johansson, T., Atlegrim, O., Danell, K., 2006. Conservation-oriented forestry and early successional saproxylic beetles: Responses of functional groups to manipulated dead wood substrates. Biological Conservation 129, 437-450.

Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M., Seoane, S., 2005. Different approaches to evaluating soil quality using biochemical properties. Soil Biology and Biochemistry 37, 877-887.

Giller, K.E., Beare, M.H., Lavelle, P., Izac, A.M.N., Swift, M.J., 1997. Agricultural intensification, soil biodiversity and agroecosystem function. Applied Soil Ecology 6, 3-16.

Gonzalez-Quiñones, V., Stockdale, E., Banning, N., Hoyle, F., Sawada, Y., Wherrett, A., Jones, D., Murphy, D., 2011. Soil microbial biomass—Interpretation and consideration for soil monitoring. Soil Research 49, 287-304.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30, 369-378.

Greenslade, P., 2007. The potential of Collembola to act as indicators of landscape stress in Australia. Australian Journal of Experimental Agriculture 47, 424-434.

Haines, P., Uren, N., 1990. Effects of conservation tillage farming on soil microbial biomass, organic matter and earthworm populations, in north-eastern Victoria. Australian Journal of Experimental Agriculture 30, 365-371.

Hamman, S.T., Hawkes, C.V., 2013. Biogeochemical and microbial legacies of non-native grasses can affect restoration success. Restoration Ecology 21, 58-66.

Hamonts, K., Bissett, A., Macdonald, B.C., Barton, P.S., Manning, A.D., Young, A., 2017. Effects of ecological restoration on soil microbial diversity in a temperate grassy woodland. Applied Soil Ecology 117, 117-128.

Harris, J., 2009. Soil microbial communities and restoration ecology: facilitators or followers? Science 325, 573-574.

Harris, J.A., 2003. Measurements of the soil microbial community for estimating the success of restoration. European Journal of Soil Science 54, 801-808.

Hartmann, A., Schmid, M., Tuinen, D., Berg, G., 2009. Plant-driven selection of microbes. Plant and Soil 321, 235-257.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. The ISME Journal 9, 1177.

Hedlund, K., 2002. Soil microbial community structure in relation to vegetation management on former agricultural land. Soil Biology and Biochemistry 34, 1299-1307.

Helgason, B.L., Walley, F.L., Germida, J.J., 2010. Long-term no-till management affects microbial biomass but not community composition in Canadian prairie agroecosytems. Soil Biology and Biochemistry 42, 2192-2202.

Hellawell, J.M., 1978. Biological surveillance of rivers; a biological monitoring handbook.

Hemerik, L., Brussaard, L., 2002. Diversity of soil macro-invertebrates in grasslands under restoration succession. European Journal of Soil Biology 38, 145-150.

Heneghan, L., Miller, S.P., Baer, S., Callaham, M.A., Montgomery, J., Pavao-Zuckerman, M., Rhoades, C.C., Richardson, S., 2008. Integrating soil ecological knowledge into restoration management. Restoration Ecology 16, 608-617.

Hernández-Hernández, R.M., López-Hernández, D., 2002. Microbial biomass, mineral nitrogen and carbon content in savanna soil aggregates under conventional and no-tillage. Soil Biology and Biochemistry 34, 1563-1570.

Herrera, M., Salamanca, C., Barea, J., 1993. Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified Mediterranean ecosystems. Applied and Environmental Microbiology 59, 129-133.

Herzberger, A.J., Meiners, S.J., Towey, J.B., Butts, P.A., Armstrong, D.L., 2015. Plant–microbe interactions change along a tallgrass prairie restoration chronosequence. Restoration Ecology 23, 220-227.

Hobbie, S.E., 1992. Effects of plant species on nutrient cycling. Trends in Ecology and Evolution 7, 336-339.

Hobbs, R.J., 1993. Can revegetation assist in the conservation of biodiversity in agricultural areas? Pacific Conservation Biology 1, 29.

Hobbs, R.J., 2005. Landscapes, ecology and wildlife management in highly modified environments–an Australian perspective. Wildlife Research 32, 389-398.

Hobbs, R.J., Harris, J.A., 2001. Restoration ecology: repairing the earth's ecosystems in the new millennium. Restoration Ecology 9, 239-246.

Hobbs, R.J., Norton, D.A., 1996. Towards a conceptual framework for restoration ecology. Restoration Ecology 4, 93-110.

Högberg, M.N., Högberg, P., Myrold, D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590-601.

Holtkamp, R., Kardol, P., van der Wal, A., Dekker, S.C., van der Putten, W.H., de Ruiter, P.C., 2008. Soil food web structure during ecosystem development after land abandonment. Applied Soil Ecology 39, 23-34.

Holtkamp, R., van der Wal, A., Kardol, P., van der Putten, W.H., de Ruiter, P.C., Dekker, S.C., 2011. Modelling C and N mineralisation in soil food webs during secondary succession on ex-arable land. Soil Biology and Biochemistry 43, 251-260.

Huhe, Borjigin, S., Cheng, Y., Nomura, N., Nakajima, T., Nakamura, T., Uchiyama, H., 2014. Effect of abandonment on diversity and abundance of free-living nitrogen-fixing bacteria and total bacteria in the cropland soils of Hulun Buir, Inner Mongolia. PLOS ONE 9, e106714.

James, A., Evison, L., 1979. Biological indicators of water quality. John Wiley.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Blair, J.M., Coleman, D.C., Whitman, W.B., 2010. Development of soil microbial communities during tallgrass prairie restoration. Soil Biology and Biochemistry 42, 302-312.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Schmidt, T.M., Coleman, D.C., Whitman, W.B., 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology and Biochemistry 43, 2184-2193.

Jia, G.-M., Pei-Dong, Z., Gang, W., Jing, C., Jing-Cheng, H., Ying-Ping, H., 2010. Relationship between microbial community and soil properties during natural succession of abandoned agricultural land. Pedosphere 20, 352-360.

Kardol, P., Bezemer, T.M., Van Der Putten, W.H., 2009a. Soil organism and plant introductions in restoration of species-rich grassland communities. Restoration Ecology 17, 258-269.

Kardol, P., Martijn Bezemer, T., Van Der Putten, W.H., 2006. Temporal variation in plant–soil feedback controls succession. Ecology Letters 9, 1080-1088.

Kardol, P., Newton, J.S., Bezemer, T.M., Maraun, M., van der Putten, W.H., 2009b. Contrasting diversity patterns of soil mites and nematodes in secondary succession. Acta Oecologica 35, 603-609.

Kardol, P., Wardle, D.A., 2010. How understanding aboveground–belowground linkages can assist restoration ecology. Trends in ecology and evolution 25, 670-679.

Kasel, S., Bennett, L.T., Tibbits, J., 2008. Land use influences soil fungal community composition across central Victoria, south-eastern Australia. Soil Biology and Biochemistry 40, 1724-1732.

Kemmitt, S.J., Lanyon, C.V., Waite, I.S., Wen, Q., Addiscott, T.M., Bird, N.R.A., O'Donnell, A.G., Brookes, P.C., 2008. Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass—a new perspective. Soil Biology and Biochemistry 40, 61-73.

Kluber, L.A., Miller, J.O., Ducey, T.F., Hunt, P.G., Lang, M., Ro, K.S., 2014. Multistate assessment of wetland restoration on CO_2 and N_2O emissions and soil bacterial communities. Applied Soil Ecology 76, 87-94.

Koehler, H.H., 2000. Natural regeneration and succession -- results from a 13 years study with reference to mesofauna and vegetation, and implications for management. Landscape and Urban Planning 51, 123-130.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75, 5111-5120.

Li, Y., Chang, S.X., Tian, L., Zhang, Q., 2018. Conservation agriculture practices increase soil microbial biomass carbon and nitrogen in agricultural soils: A global meta-analysis. Soil Biology and Biochemistry 121, 50-58.

Liu, Y., Axmacher, J.C., Wang, C., Li, L., Yu, Z., 2012. Ground Beetle (Coleoptera: Carabidae) assemblages of restored semi-natural habitats and intensively cultivated fields in Northern China. Restoration Ecology 20, 234-239.

Maharning, A.R., Mills, A.A., Adl, S.M., 2009. Soil community changes during secondary succession to naturalized grasslands. Applied Soil Ecology 41, 137-147.

Maraun, M., Salamon, J.-A., Schneider, K., Schaefer, M., Scheu, S., 2003. Oribatid mite and collembolan diversity, density and community structure in a model beech forest (*Fagus sylvatica*): effects of mechanical perturbations. Soil Biology and Biochemistry 35, 1387-1394.

Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural intensification and ecosystem properties. Science 277, 504-509.

McKinley, V., Peacock, A., White, D., 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biology and Biochemistry 37, 1946-1958.

Mendham, D.S., O'connell, A., Grove, T., 2002a. Organic matter characteristics under native forest, long-term pasture, and recent conversion to *Eucalyptus* plantations in Western Australia: microbial biomass, soil respiration, and permanganate oxidation. Soil Research 40, 859-872.

Mendham, D.S., Sankaran, K.V., O'Connell, A.M., Grove, T.S., 2002b. Eucalyptus globulus harvest residue management effects on soil carbon and microbial biomass at 1 and 5 years after plantation establishment. Soil Biology and Biochemistry 34, 1903-1912.

Minor, M., Cianciolo, J., 2007. Diversity of soil mites (Acari: Oribatida, Mesostigmata) along a gradient of land use types in New York. Applied Soil Ecology 35, 140-153.

Mukhopadhyay, S., Joy, V.C., 2010. Influence of leaf litter types on microbial functions and nutrient status of soil: Ecological suitability of forest trees for afforestation in tropical laterite wastelands. Soil Biology and Biochemistry 42, 2306-2315.

Nakamura, A., Proctor, H., Catterall, C.P., 2003. Using soil and litter arthropods to assess the state of rainforest restoration. Ecological Management and Restoration 4, S20-S28.

Neher, D.A., 2001. Role of nematodes in soil health and their use as indicators. Journal of Nematology 33, 161.

Neuenkamp, L., Prober, S.M., Price, J.N., Zobel, M., Standish, R.J., 2018. Benefits of mycorrhizal inoculation to ecological restoration depend on plant functional type, restoration context and time. Fungal Ecology.

Nielsen, M.N., Winding, A., Binnerup, S., 2002. Microorganisms as indicators of soil health. NERI Technical Report No. 388

Nielsen, U.N., Osler, G.H., Campbell, C.D., Burslem, D.F., van der Wal, R., 2010. The influence of vegetation type, soil properties and precipitation on the composition of soil mite and microbial communities at the landscape scale. Journal of Biogeography 37, 1317-1328.

Nunes, J.S., Araujo, A.S.F., Nunes, L.A.P.L., Lima, L.M., Carneiro, R.F.V., Salviano, A.A.C., Tsai, S.M., 2012. Impact of land degradation on soil microbial biomass and activity in Northeast Brazil. Pedosphere 22, 88-95.

Oehl, F., Sieverding, E., Mäder, P., Dubois, D., Ineichen, K., Boller, T., Wiemken, A., 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138, 574-583.

Osborne, C.A., Zwart, A.B., Broadhurst, L.M., Young, A.G., Richardson, A.E., 2011. The influence of sampling strategies and spatial variation on the detected soil bacterial communities under three different land-use types. FEMS Microbiology Ecology 78, 70-79.

Pajares, S., Gallardo, J.F., Masciandaro, G., Ceccanti, B., Etchevers, J.D., 2011. Enzyme activity as an indicator of soil quality changes in degraded cultivated Acrisols in the Mexican Trans-volcanic Belt. Land Degradation and Development 22, 373-381.

Pankhurst, Kirkby, Hawke, Harch, 2002. Impact of a change in tillage and crop residue management practice on soil chemical and microbiological properties in a cereal-producing red duplex soil in NSW, Australia. Biology and Fertility of Soils 35, 189-196.

Pankhurst, C.E., Hawke, B.G., McDonald, H.J., Kirkby, C.A., Buckerfield, J.C., Michelsen, P., O'Brien, K.A., Gupta, V.V.S.R., Doube, B.M., 1995. Evaluation of soil biological properties as potential bioindicators of soil health. Australian Journal of Experimental Agriculture 35, 1015-1028.

Pánková, H., Lepinay, C., Rydlová, J., Voříšková, A., Janoušková, M., Dostálek, T., Münzbergová, Z., 2018. Arbuscular mycorrhizal fungi and associated microbial communities from dry grassland do not improve plant growth on abandoned field soil. Oecologia 186, 677-689.

Patra, A.K., Le Roux, X., Grayston, S.J., Loiseau, P., Louault, F., 2008. Unraveling the effects of management regime and plant species on soil organic carbon and microbial phospholipid fatty acid profiles in grassland soils. Bioresource Technology 99, 3545-3551.

Paul, M., Catterall, C.P., Pollard, P.C., Kanowski, J., 2010. Recovery of soil properties and functions in different rainforest restoration pathways. Forest Ecology and Management 259, 2083-2092.

Pavao-Zuckerman, M.A., 2008. The nature of urban soils and their role in ecological restoration in cities. Restoration Ecology 16, 642-649.

Plassart, P., Akpa Vinceslas, M., Gangneux, C., Mercier, A., Barray, S., Laval, K., 2008. Molecular and functional responses of soil microbial communities under grassland restoration. Agriculture, Ecosystems and Environment 127, 286-293.

Porazinska, D.L., Bardgett, R.D., Blaauw, M.B., Hunt, H.W., Parsons, A.N., Seastedt, T.R., Wall, D.H., 2003. Relationships at the aboveground–belowground interface: plants, soil biota, and soil processes. Ecological Monographs 73, 377-395.

Post, W.M., Kwon, K.C., 2000. Soil carbon sequestration and land-use change: processes and potential. Global Change Biology 6, 317-327.

Postma-Blaauw, M.B., de Goede, R.G.M., Bloem, J., Faber, J.H., Brussaard, L., 2012. Agricultural intensification and de-intensification differentially affect taxonomic diversity of predatory mites, earthworms, enchytraeids, nematodes and bacteria. Applied Soil Ecology 57, 39-49.

Potthoff, M., Jackson, L.E., Steenwerth, K.L., Ramirez, I., Stromberg, M.R., Rolston, D.E., 2005. Soil biological and chemical properties in restored perennial grassland in California. Restoration Ecology 13, 61-73.

Potthoff, M., Steenwerth, K.L., Jackson, L.E., Drenovsky, R.E., Scow, K.M., Joergensen, R.G., 2006. Soil microbial community composition as affected by restoration practices in California grassland. Soil Biology and Biochemistry 38, 1851-1860.

Prober, S.M., Bissett, A., Walker, C., Wiehl, G., McIntyre, S., Tibbett, M., 2015. Spatial structuring of arbuscular mycorrhizal communities in benchmark and modified temperate eucalypt woodlands. Mycorrhiza 25, 41-54.

Proctor, H., Kanowski, J., Catterall, C.P., Wardell, johnson, G., Reis, T., 2011. Rainforest-restoration success as judged by assemblages of soil- and litter-dwelling mites (Arachnida: Acari). Zoosymposia 6, 234-254.

Raiesi, F., Salek-Gilani, S., 2018. The potential activity of soil extracellular enzymes as an indicator for ecological restoration of rangeland soils after agricultural abandonment. Applied Soil Ecology 126, 140-147.

Redi, B.H., Van Aarde, R.J., Wassenaar, T.D., 2005. Coastal dune forest development and the regeneration of millipede communities. Restoration Ecology 13, 284-291.

Requena, N., Perez-Solis, E., Azcón-Aguilar, C., Jeffries, P., Barea, J.-M., 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Applied and Environmental Microbiology 67, 495-498.

Reynolds, H.L., Packer, A., Bever, J.D., Clay, K., 2003. Grassroots ecology: plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology 84, 2281-2291.

Riggins, J.J., Davis, C.A., Hoback, W.W., 2009. Biodiversity of belowground invertebrates as an indicator of wet meadow restoration success (Platte River, Nebraska). Restoration Ecology 17, 495-505.

Ritz, K., Black, H.I.J., Campbell, C.D., Harris, J.A., Wood, C., 2009. Selecting biological indicators for monitoring soils: A framework for balancing scientific and technical opinion to assist policy development. Ecological Indicators 9, 1212-1221.

Roper, M., Gupta, V., 1995. Management-practices and soil biota. Soil Research 33, 321-339.

Rosenzweig, S.T., Carson, M.A., Baer, S.G., Blair, J.M., 2016. Changes in soil properties, microbial biomass, and fluxes of C and N in soil following post-agricultural grassland restoration. Applied Soil Ecology 100, 186-194.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4, 1340-1351.

Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology 75, 1589-1596.

Ruiz-Jaen, M.C., Aide, T.M., 2005. Restoration success: how is it being measured? Restoration Ecology 13, 569-577.

Ryan, M., Chilvers, G., Dumaresq, D., 1994. Colonisation of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbour. Plant and Soil 160, 33-40.

Sanchez-Maranon, M., Soriano, M., Delgado, G., Delgado, R., 2002. Soil quality in Mediterranean mountain environments. Soil Science Society of America Journal 66, 948-958.

Saviozzi, A., Levi-Minzi, R., Cardelli, R., Riffaldi, R., 2001. A comparison of soil quality in adjacent cultivated, forest and native grassland soils. Plant and soil 233, 251-259.

Scheu, S., 1992. Changes in the *lumbricid coenosis* during secondary succession from a wheat field to a beechwood on limestone. Soil Biology and Biochemistry 24, 1641-1646.

Scheu, S., Schulz, E., 1996. Secondary succession, soil formation and development of a diverse community of oribatids and saprophagous soil macro-invertebrates. Biodiversity and Conservation 5, 235-250.

Singh, B.K., Munro, S., Potts, J.M., Millard, P., 2007. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Applied Soil Ecology 36, 147-155.

Šnajdr, J., Dobiášová, P., Urbanová, M., Petránková, M., Cajthaml, T., Frouz, J., Baldrian, P., 2013. Dominant trees affect microbial community composition and activity in post-mining afforested soils. Soil Biology and Biochemistry 56, 105-115.

Snyder, B.A., Hendrix, P.F., 2008. Current and potential roles of soil macroinvertebrates (earthworms, millipedes, and isopods) in ecological restoration. Restoration Ecology 16, 629-636.

St-Denis, A., Kneeshaw, D., Bélanger, N., Simard, S., Laforest-Lapointe, I., Messier, C., 2017. Species-specific responses to forest soil inoculum in planted trees in an abandoned agricultural field. Applied Soil Ecology 112, 1-10.

Strickland, M.S., Lauber, C., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. Ecology 90, 441-451.

Swift, M.J., Izac, A.M.N., van Noordwijk, M., 2004. Biodiversity and ecosystem services in agricultural landscapes--are we asking the right questions? Agriculture, Ecosystems and Environment 104, 113-134.

Szlávecz, K., Csuzdi, C., 2007. Land use change affects earthworm communities in Eastern Maryland, USA. European Journal of Soil Biology 43, Supplement 1, S79-S85.

Thomas, F., Folgarait, P., Lavelle, P., Rossi, J.P., 2004. Soil macrofaunal communities along an abandoned rice field chronosequence in Northern Argentina. Applied Soil Ecology 27, 23-29.

Thrall, P.H., Millsom, D.A., Jeavons, A.C., Waayers, M., Harvey, G.R., Bagnall, D.J., Brockwell, J., 2005. Seed inoculation with effective root-nodule bacteria enhances revegetation success. Journal of Applied Ecology 42, 740-751.

Thrall, P.H., Oakeshott, J.G., Fitt, G., Southerton, S., Burdon, J.J., Sheppard, A., Russell, R.J., Zalucki, M., Heino, M., Ford Denison, R., 2011. Evolution in agriculture: the application of evolutionary approaches to the management of biotic interactions in agro-ecosystems. Evolutionary Applications 4, 200-215.

Tian, G., Vose, J.M., Coleman, D.C., Geron, C.D., Walker, J.T., 2004. Evaluation of the effectiveness of riparian zone restoration in the southern Appalachians by assessing soil microbial populations. Applied Soil Ecology 26, 63-68.

Tiedje, J.M., Asuming-Brempong, S., Nüsslein, K., Marsh, T.L., Flynn, S.J., 1999. Opening the black box of soil microbial diversity. Applied Soil Ecology 13, 109-122.

Trinder, C.J., Johnson, D., Artz, R.R.E., 2009. Litter type, but not plant cover, regulates initial litter decomposition and fungal community structure in a recolonising cutover peatland. Soil Biology and Biochemistry 41, 651-655.

van Groenigen, J.W., Lubbers, I.M., Vos, H.M.J., Brown, G.G., De Deyn, G.B., van Groenigen, K.J., 2014. Earthworms increase plant production: a meta-analysis. Scientific Reports 4, 6365-6365.

Vitousek, P., Hooper, D., 1994. Biological diversity and terrestrial ecosystem biogeochemistry, Biodiversity and ecosystem function. Springer, 3-14.

Wade, M.R., Gurr, G.M., Wratten, S.D., 2008. Ecological restoration of farmland: progress and prospects. Philosophical transactions of the Royal Society of London. Series B, Biological Sciences 363, 831-847.

Wang, H., Deng, N., Wu, D., Hu, S., 2017. Quantitative response relationships between net nitrogen transformation rates and nitrogen functional genes during artificial vegetation restoration following agricultural abandonment. Scientific Reports 7, 7752.

Wardle, D., Bonner, K., Barker, G., 2002. Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. Functional Ecology 16, 585-595.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629-1633.

Wardle, D.A., Giller, K.E., 1996. The quest for a contemporary ecological dimension to soil biology. Soil Biology and Biochemistry 28, 1549-1554.

White, P.S., Walker, J.L., 1997. Approximating nature's variation: selecting and using reference information in restoration ecology. Restoration Ecology 5, 338-349.

Wu, J., Joergensen, R., Pommerening, B., Chaussod, R., Brookes, P., 1990. Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. Soil Biology and Biochemistry 22, 1167-1169.

Wubs, E.J., van der Putten, W.H., Bosch, M., Bezemer, T.M., 2016. Soil inoculation steers restoration of terrestrial ecosystems. Nature Plants 2, 16107.

Yan, D., Mills, J.G., Gellie, N.J.C., Bissett, A., Lowe, A.J., Breed, M.F., 2018. High-throughput eDNA monitoring of fungi to track functional recovery in ecological restoration. Biological Conservation 217, 113-120.

Zaitsev, A.S., Wolters, V., Waldhardt, R., Dauber, J., 2006. Long-term succession of oribatid mites after conversion of croplands to grasslands. Applied Soil Ecology 34, 230-239.

Zeller, V., Bardgett, R.D., Tappeiner, U., 2001. Site and management effects on soil microbial properties of subalpine meadows: a study of land abandonment along a north–south gradient in the European Alps. Soil Biology and Biochemistry 33, 639-649.

Zhang, H., Li, G., Song, X., Yang, D., Li, Y., Qiao, J., Zhang, J., Zhao, S., 2013. Changes in soil microbial functional diversity under different vegetation restoration patterns for Hulunbeier Sandy Land. Acta Ecologica Sinica 33, 38-44.

Zornoza, R., Guerrero, C., Mataix-Solera, J., Arcenegui, V., García-Orenes, F., Mataix-Beneyto, J., 2009a. Assessing the effects of air-drying and rewetting pre-treatment on soil microbial biomass, basal respiration, metabolic quotient and soluble carbon under Mediterranean conditions. European Journal of Soil Biology 43, 120-129.

Zornoza, R., Guerrero, C., Mataix-Solera, J., Scow, K., Arcenegui, V., Mataix-Beneyto, J., 2009b. Changes in soil microbial community structure following the abandonment of agricultural terraces in mountainous areas of Eastern Spain. Applied Soil Ecology 42, 315-323.

Paper I: Earthworm composition, diversity and biomass under three land use systems in south-eastern Australia

In **Paper I** I examined earthworm composition, diversity and biomass in three land use systems: native shelterbelts dominated by *Acacia* and *Eucalyptus* species, agricultural pastures and native remnant woodland fragments as to further develop current knowledge on trajectories of change in earthworm communities in restored systems.

Carnovale, D., Baker, G., Bissett, A., Thrall, P., 2015. Earthworm composition, diversity and biomass under three land use systems in south-eastern Australia. Applied Soil Ecology 88, 32-40.

Abstract

In south-eastern Australia, strips of planted native trees and shrubs (shelterbelts) are frequently established to restore ecosystem services altered by agriculture. Despite their widespread use, little is known about the effects of establishing shelterbelts on soil macroinvertebrates, especially earthworms, which are of major importance in soil processes. We assessed earthworm composition, diversity and biomass in three land use systems: native shelterbelts dominated by *Acacia* and *Eucalyptus* species, agricultural pastures and native remnant woodland fragments dominated by *Eucalyptus blakelyi* and/or *Eucalyptus melliodora*. Earthworm communities differed significantly among systems, with abundance, biomass and diversity greatest under pasture. Within shelterbelts we saw a shift from high earthworm biomass and density to low with increasing time after establishment. Soil edaphic variables did not correlate strongly with earthworm biomass or density but were correlated with earthworm community composition. Overall, the introduction of native woody vegetation was associated with a decline in density and biomass of earthworms, including a decrease in the relative abundance of exotic species. As such, shelterbelts can be used to promote native earthworm relative abundance which may be important for local diversity, soil function and landscape connectivity.

Key words: *earthworm, shelterbelts, soil edaphic properties, agricultural restoration, exotic species*

Introduction

In response to a growing awareness of the need to conserve local biodiversity and reduce land degradation, the use of native plant species for ecological restoration has been widely implemented in Australian agricultural landscapes (Cleugh et al., 2002; Hobbs, 1993). One method of re-introducing woody species into agricultural land is through the planting of shelterbelts, which are strips of planted native trees and shrubs (Cleugh et al., 2002). The restoration and addition of shelterbelts to agricultural landscapes provides various ecosystem services including increased landscape connectivity, increased productivity and reduced erosion (Bird, 1998; Bird et al., 1993; Cleugh et al., 2002). Nevertheless, little information is available on the effects of restoration on local earthworm communities, despite the known role of earthworms as regulators of nutrient cycling, water infiltration and cycling of organic matter (Edwards, 2004; Lee, 1985).

The impact of agricultural practices on the abundance and diversity of earthworms, and in turn their influences on soil properties and plant production, is well studied worldwide (Edwards, 2004; Lee, 1985). This includes Australia, where exotic earthworms, notably Lumbricidae, have been studied in detail (Baker, 1998a; Baker, 2004). In southern Australia, the documented effects of exotic earthworm species on agricultural soils include influences on soil structure (Chan and Barchia, 2007), nutrient availability (Baker, 2007; Baker et al., 2003a), soil organic matter and lime burial (Baker et al., 1999; Baker et al., 1998; Chan et al., 2004), and beneficial microorganisms (Doube et al., 1994; Stephens and Davoren, 1997; Stephens et al., 1993).

Disturbance associated with agricultural practices in southern Australia is allied with reduced native earthworm abundance and invasion by exotic earthworm species, in particular European *Aporrectodea* species (Chan and Barchia, 2007; Chan and Heenan, 2006). However, our understanding of the ecological role of native species, especially the most common family of Megascolecidae remains poor (Baker et al., 2003b; Baker et al., 1997; Chan, 2004), although, it has been shown that native species of *Spenceriella* and *Gemascolex* (Megascolecidae) are inferior in improving soil structure, water infiltration, burial of surface dung and plant production compared with exotic Lumbricidae such as *Aporrectodea calignosa*, *Aporrectodea trapezoides* and *Aporrectodea longa* (Baker, 1998a; Baker et al., 2003b; Blakemore, 1997). Common exotics, *Aporrectodea calignosa*, *Aporrectodea trapezoids and Aporrectodea rosea* in southern Australia are regarded as endogeic species (Lee, 1985). They feed on the organic matter in the mineral horizons.

Ecological relationships between native and exotic species in an Australian context are not well understood. In southern New South Wales, Baker (2004) found no correlation between the abundance of native and exotic earthworms. Contrary to this, in Western Victoria native earthworms were generally absent where exotic species exceeded 400 individuals m^{-2}. In this case it is possible that competitive interactions were occurring between exotic and native species similar to that shown experimentally by Dalby et al. (1998a) for two exotic species, where removal of food, destruction of habitat and consumption of cocoons by *A. longa* reduced the abundance and biomass of *Microscolex dubius*. Similarly, Didham et al. (2007) argued that habitat modification alters ecological interactions (including competition) between invasive and native species. Changes in land use can alter preferential feeding on different quality litter by earthworms, and this can in turn influence community composition and altered soil edaphic

properties (Rajapaksha et al., 2013b). For example, the invasion of *A. trapezoides* and displacement of native *Argilophilus* species in areas converted to livestock grazing in California was facilitated by the rapid growth and reproduction rate of *A. trapezoides* in these altered systems (Winsome et al., 2006). However, in less productive natural grasslands, *A. trapezoides* failed to acquire enough resources to maintain its rapid growth rates or reach reproductive maturity. Further to this, in South Australia, Dalby et al (1998b) concluded that the European lumbricid, *A. longa,* was able to survive and grow in woodland soil, but did not reproduce.

The focus of this study was to improve understanding of how earthworm communities are altered by the restoration of agricultural land via planting native shelterbelts. We hypothesised that: 1) earthworm community composition and diversity were different under pastures, shelterbelts and native remnants; and 2) earthworm communities within shelterbelt habitats by chronosequence would show trajectories that converge towards those observed in local native remnants.

Methods

Study site and design

The study sites were located near Murrumbateman in New South Wales, Australia (34°57′0″S, 149°01′0″E). The region is predominantly agricultural land dominated by grazing. The climate is temperate, with warm to hot summers and cool winters. The average annual rainfall is 927 mm (Australian Bureau of Meterology, 2014). Rainfall is higher in spring to summer with October and November being slightly wetter than other times. Rainfall is lowest within the winter months of June and July.

Nine sites in total were selected. Six sites consisted of 2 parallel transects per plot, one within a shelterbelt established on old pastoral land and the other within an adjacent grazed pasture (Fig.1). Another three sites were selected within three native remnant woodlands. At each native remnant site, a single transect was sampled. These native remnants were small (approximately 1 hectare) fragments which had various levels of disturbance. The native remnants all had an overstory of native tree species, predominantly *E. blakelyi* and/or *E. melliodora*. The level of understory disturbance, primarily due to grazing, varied within and between the native remnant patches, thus exotic pasture species were typically present to varying degrees in native remnants.

The six shelterbelt transects were separated into three age classes: 0-5 years since establishment (young), 5-15 years (middle-aged), 15-20 years (old), with two (replicate) transects in each age class. This provided a chronosequence. At the time of establishment, each shelterbelt had been direct sown with a seed mix of tree and shrub species endemic to the Murrumbateman area. The primary trees used were a mix of *Acacia* and *Eucalyptus* species. The dominant *Eucalyptus* species within the seed mix consisted of *E. mannifera*, *E. blakelyi*, *E. viminalis* and *E. melliodora*, the dominant *Acacia* species were, *A. mearnsii*, *A. cardiophylla*, *A. decurrens* and *A. rubida*.

All transects were situated within a radius of 12 km, thus limiting environmental variability between sites. All sites were located on mid to lower slopes. Each transect was 90 m long. Shelterbelt width varied from 4-8 tree rows wide. Transects were centred through each shelterbelt to minimise any possible edge effects. In the pastures, transects were positioned parallel, approximately 15 metres away from the edge of the shelterbelt (Fig. 1). Transects within native remnants were positioned in the middle of the native remnant patch. Ten sample points were set along each transect at 10 m spacings. Paired transects (shelterbelt and pasture) within each site were sampled at the same time. Earthworms were collected at these sample points in late July in both 2012 and 2013. This timing ensured maximum surface activity of the earthworms and thus the most efficient collection (Baker et al., 1992a; Baker et al., 1992b)

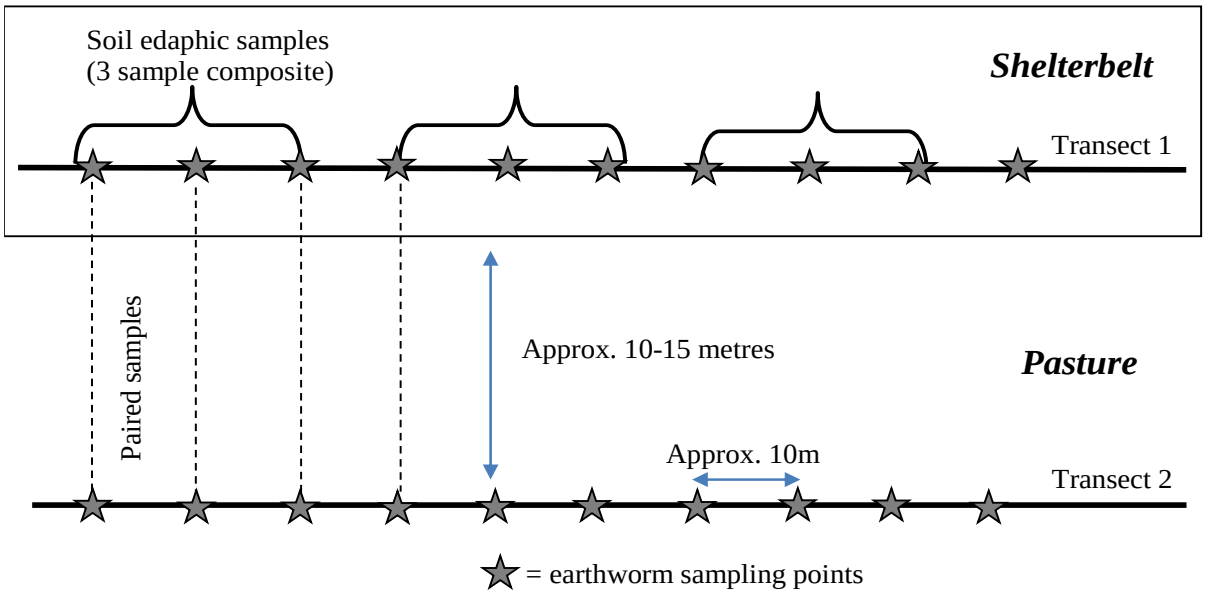


Figure 1. Experimental design for replicated field sites. Each site contained one transect in each of the shelterbelt and pasture. Ten sample points for earthworms and 3 (composite) sample points for soil edaphic properties were established along each transect. The design was replicated over 6 sites (shelterbelts=6 transects, pastures=6 transects). Sampling in native remnants was similar but consisted of 1 transect per site. The design was replicated over 3 native remnant sites (native remnants=3 transects).

Earthworm sampling

Soil sods including the plant root system and organic horizon, each 30 cm x 30 cm by 10 cm deep, were hand-sorted in the field (Baker et al., 1992b). Earthworms were placed in 70% ethanol and transferred to the lab for identification. Ethanol was replaced 48 hours after sampling to preserve the specimens for identification to species level where possible, using a dissecting microscope. Earthworms were washed clean, patted dry and weighed for biomass. Most of the earthworms were identified using previously published keys and descriptions (Baker and Barrett, 1994; Jamieson, 2001), and were grouped into adults or juveniles based on the presence or absence of a clitellum. A small number of earthworms, presumed to be native, could not be fully identified and were classed as morpho-species. Some juvenile earthworms could not be identified to species or morpho-species because of incomplete development of distinguishing features and were thus excluded from all further analysis.

Soil sampling and analysis

Soil cores (0-10cm deep) were collected in the mineral soil adjacent to the first nine soil sods hand-sorted for earthworms along each transect. Three consecutive soil samples along each transect were then bulked together resulting in three composite soil samples per transect. No sample was taken to correspond with the last earthworm sampling point. Soil moisture content was determined gravimetrically by oven drying 10 g subsamples at 105°C until weight became stable. Soil was air-dried and pH and electrical conductivity (EC) were analysed in a 1:5 water suspension which was shaken end over end for an hour, centrifuged down and read immediately with a Thermo Scientific Orion 3-Star portable meter. Total Carbon (C) and total Nitrogen (N) were determined on an oven dried basis on finely ground soil using the dry combustion method on an elemental analyser (*vario MAX CNS)*. The remaining parameters [particle size (sand, silt and clay), Organic C, Nitrate nitrogen (NO_3-N), Ammonium nitrogen (NH_4^+-N), Sulphur (S), Phosphorus (P), Potassium (K), Exchangeable cations (Ca, Mg, Na, K, Al) and trace elements (Cu, Fe, Mn Zn)] were determined by the CSBP Soil and Plant Analysis Laboratory (Perth, Australia). Organic C was approximated by the Walkley Black method (Walkley and Black, 1934), P and K measured according to Colwell (1963), S was extracted using the Blair/Lefroy method (Blair et al., 1991), and NO_3-N and NH_4^+-N were extracted with 1M potassium chloride and measured on a Lachat Flow Injection Analyser. DPTA trace elements were measured by atomic absorption spectroscopy (Rayment and Higginson, 1992).

Statistical analysis

Earthworm density (number of individuals per sample) and biomass were converted to a m^{-2} basis. Several different diversity and community composition indices were calculated for each sample point, including Shannon-Wiener index, Simpson's index, Margalef's richness index and Pielou's measure of species evenness. When necessary, data were log transformed to achieve homogeneity of variances and normality prior to analysis. Earthworm density m^{-2}, biomass (g/m^{-2}) and diversity indices were compared between pastures (n=60) and shelterbelts (n=60), and years (2012 and 2013) by two-way analysis of variance (ANOVA). Tukey HSD post hoc tests were used to test differences between pasture and shelterbelts across both sampling years. Native remnants were analysed separately as there were no comparable couplings of such sites with the other transects. Moreover, the native remnants comprised a very heterogeneous cluster with high variability within sites. Two-way ANOVA was used to test for significant differences between native sites and year, followed by Tukey HSD post hoc

tests for comparisons of means. A one-way ANOVA, blocked by site was used to assess differences between the 3 different age classes of shelterbelts, followed by Tukey HSD post hoc tests for comparisons of means.

For soil edaphic variables, native sites were combined due to limited replication within each native site. One-way ANOVA was then used to test for significant differences in soil edaphic properties between all three land uses [in this case, years were combined due to small sample numbers ($n=3yr^{-1}$ $transect^{-1}$)]. Linear regression models were used to assess correlations between soil edaphic properties and earthworm density, biomass diversity richness and evenness.

Multivariate analyses were used to assess changes in earthworm community composition. For diversity data, Bray–Curtis distance matrices were calculated on raw earthworm abundances. ANOSIM was used to test the significance of land use on earthworm assemblages and nMDS plots were used to visualise community composition. Earthworm species density data were averaged across replicate samples and compared to similarly averaged soil physico-chemical data, Distance Based Linear Models (DistLM) were used to test for differences in community composition and how these related to predictor environmental variables. For DistLM, log transformation was necessary for some of the environmental variables as indicated from a draftsman plot. Co-varying edaphic variables ($r^2>0.7$) were found and only one such variable was run in the model. Results were interpreted such that this single variable represented the behaviour of all variables with which it was found to co-vary. The best subset model selection procedure (Anderson et al., 2008) was used to identify variables that explained significant amounts of variation in earthworm community structure. The corrected Akaike information criterion (AICc) was used to select the predictor variables. Marginal tests were conducted to assess the statistical significance and percentage contribution of each soil edaphic variable alone (Anderson and Cribble, 1998; Legendre and Anderson, 1999; McArdle and Anderson, 2001). Distance-based redundancy *analysis* (*dbRDA*) was used for graphical visualization of the DistLM results. Univariate statistical analyses were conducted using Genstat software package (VSN International, 2011) and multivariate tests were conducted using PRIMER 6 (Clarke and Gorley, 2006; Clarke and Warwick, 2001) and PERMANOVA+ (Anderson et al., 2008).

Results

Density, biomass and species diversity

Earthworm density, biomass, diversity and richness were significantly related to land use (pasture, shelterbelt) ($p<0.05$) and year ($p<0.05$), with all variables higher under pasture (Table 1). Density was lower in 2013 compared with 2012 (Tables 1 and 2). However, the decline in overall earthworm density in 2013 did not alter land use effects ($p<0.05$). Diversity varied between native remnants with native site 3 having higher species diversity and richness than native sites 1 and 2 (Table 2). Earthworm density within shelterbelts was higher in young shelterbelts than in old shelterbelts ($p<0.001$); middle-aged shelterbelt earthworm density did not differ from either young or old shelterbelts (Fig. 2a and 2b).

Table 1. Earthworm abundance, biomass and diversity measures (Shannon-Wiener and Simpson index, species richness and evenness) in relation to land use (shelterbelt and pasture) and year (2012 and 2013). Values represent means (n=60). Different letters indicate significant differences, according to Tukey's test ($p<0.05$) between land uses across both years.

	2012		2013	
	Shelterbelt	Pasture	Shelterbelt	Pasture
Earthworm density (individuals m^{-2})	66.4 ab	196 c	36.9 a	114 b
Earthworm biomass (g m^{-2})	11.7 a	38.2 c	7.47 a	28.4b
Shannon-Weiner index (H')	0.36 a	0.78 b	0.22 a	0.64 bc
Simpson index (1/D)	0.33 b	0.51 c	0.18 a	0.44 b
Species richness	0.47 ab	0.77 c	0.27 a	0.60 bc
Pielou's species evenness (J')	0.46 b	0.75c	0.31a	0.69 c

Table 2. Earthworm abundance, biomass and diversity measures (Shannon-Wiener and Simpson index, species richness and evenness) in native remnants site 1, 2 and 3 by year (2012 and 2013). Means (n=30) followed by different letters indicate significant differences according to ANOVA and subsequent Tukey's test (p<0.05) across years.

	2012			2013		
	Native remnant 1	Native remnant 2	Native remnant 3	Native remnant 1	Native remnant 3	Native remnant 4
Earthworm density (individuals m^{-2})	61.6 b	137 bc	191 c	9.90 a	80.3 b	151 bc
Earthworm biomass (g/ m^2)	12.7b	30.2 bc	34.5 c	1.38 a	16.4 b	23.6 bc
Shannon- Weiner index (H')	0.39 abc	0.58 bc	1.13 d	0.13 ab	0.11 a	0.61 c
Simpson index (1/D)	0.27 ab	0.40 abc	0.68 c	0.20 ab	0.08 a	0.44 c
Species richness	0.48 ab	0.60 ab	1.01b	0.24 a	0.04 a	0.53 ab
Pielou's species evenness (J')	0.38 a	0.67 ab	0.89 b	0.48 a	0.20a	0.68 a

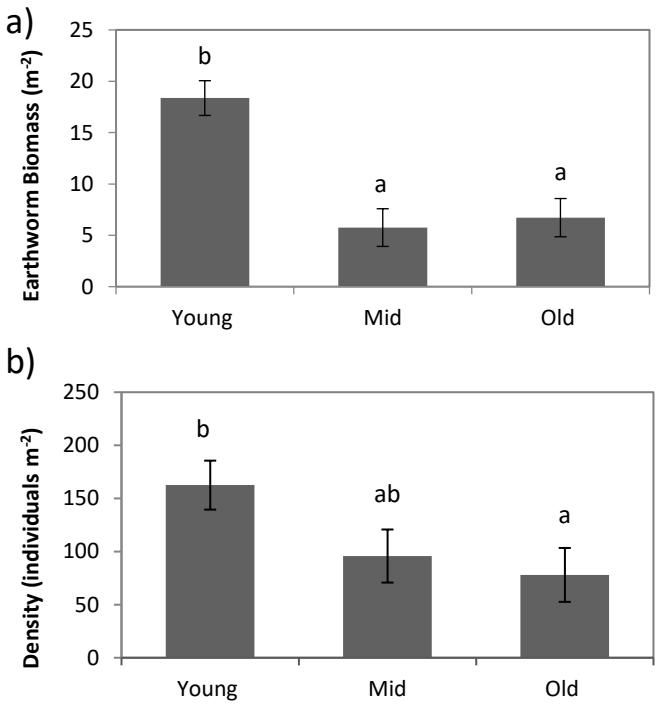


Figure 2. Mean (n=20) earthworm biomass (a) and density (b) in shelterbelts of different ages, young belts (<5years), middle-aged (5-10yrs) and old belts (>15 years). Error bars represent ±SE. Different letters indicate significant differences according to Tukey test (p<0.05).

Earthworm species and community composition

A total of 2818 earthworms were extracted from the soil samples, comprising eleven species, six of which were exotic (Table 3). Within the shelterbelts (n=6 transects), the native *S. macleayi* was the most abundant species. Within pastures, the exotic *A. rosea* and the native *S. macleayi* were the most abundant (Table 3). The abundance of *S. macleayi* did not differ significantly between pasture and shelterbelts. Exotic species such as *A. rosea* and *A. trapezoides* were more abundant in pastures than in shelterbelts. Populations of other species were too low and variable to make valid statistical comparisons.

Within and between native sites there was high variation in earthworm species density (Table 4). Native site 1 was dominated by *S. macleayi* but overall had lower earthworm density than site 3 in 2012 and site 2 and 3 in 2013 (Table 4). Native site 2 was dominated by exotic species such as *A.rosea, A. trapezoides* and *A. caliginosa.* The distribution of *A. caliginosa* within this site was restricted to localised samples within the site. Native site 3 had a high density of native species including *S. macleayi* and *Spenceriella* sp. 2.

Within shelterbelts, overall community composition was significantly affected by time since establishment (Global R=0.109, $p<0.001$, ANOSIM). ANOSIM and subsequent pairwise comparisons of the different age classes showed significantly different earthworm communities (Fig. 3) between young to middle-aged ($p<0.05$), young to old ($p<0.05$) and middle to old shelterbelts ($p<0.05$). Young shelterbelts had a higher proportion of exotic species (Fig. 4). Earthworm communities in middle and old aged belts were dominated by native *S. macleayi*. However, the relative abundance of exotic species increased in old shelterbelts compared with middle-aged shelterbelts. Community composition did not significantly differ between middle-aged and old shelterbelts and native remnant sites ($p=0.06$ and $p>0.05$) (Fig. 3). Community composition of earthworms was different between different land-uses (Global R=0.185, $p<0.001$, two-way ANOSIM, land use by sample year) (Fig. 5a). Community composition in shelterbelts was significantly different from both pasture systems and native remnants ($p<0.05$ for both comparisons).

Table 3. Mean (n=60) density m^{-2} (±SE) of each earthworm species in shelterbelts compared to pasture. Different letters indicate significant differences (p<0.05) between land use, determined by two sample T test.

Species	Shelterbelt	Pasture
Octolasion cyaneum (Savigny, 1826) $_{E}$	0.00	1.41(0.81)
Aporrectodea rosea (Savigny, 1826) $_{E}$	7.30(2.00)a	51.2 (10.4)b
Aporrectodea trapezoides (Dugès, 1828) $_{E}$	6.99 (1.27)a	28.8 (2.75)b
*Aporrectodea caliginosa (*Savigny, 1826) $_{E}$	0.1 (0.10)	0.19 (0.13)
Microscolex dubius (Fletcher,1887) $_{E}$	0.72 (0.39)a	2.73 (0.77)b
Microscolex phosphoreus (Dugès1837) $_{E}$	0.41(0.32)	0.94 (0.45)
*Spenceriella macleayi (*Fletcher 1887) $_{N}$	38.6 (5.62)	42.7 (5.15)
Spenceriella sp 1 $_{N}$	0.31 (0.18)a	5.36 (1.48)b
Spenceriella sp 2 $_{N}$	2.78 (1.16)a	18.8 (5.18)b
Native species A $_{N}$	0.21 (0.14)	3.48 (1.91)
Native species B $_{N}$	0.31 (0.18)a	1.22 (0.42)b

$_{E}$ = exotic species, $_{N}$ = Native species

Table 4. Mean (n=30) density m^{-2} (±SE) of earthworm species across three native remnants, determined by ANOVA and subsequent Tukey test (p<0.05)

Species	Native 1	Native 2	Native 3
Octolasion cyaneum (Savigny, 1826)	3.14 (2.14)	5.50 (4.89)	10.40 (4.26)
Aporrectodea rosea (Savigny, 1826)	3.14 (1.20) a	33.61(12.84) b	4.95 (3.87)a
Aporrectodea trapezoides (Dugès, 1828)	6.29 (2.50)	22.00 (14.72)	4.95 (2.03)
*Aporrectodea caliginosa (*Savigny, 1826)	0.79 (0.79) a	48.89 (18.97)b	18.15 (4.81)ab
Microscolex dubius (Fletcher,1887)	0.00	0.00	0.55 (0.55)
Microscolex phosphoreus (Dugès1837)	0.00	0.00	0.55 (0.55)
*Spenceriella macleayi (*Fletcher 1887)	36.9 (8.20)ab	11.0 (3.44)a	82.0 (19.23)b
Spenceriella sp 1	0.00	0.00	0.00
Spenceriella sp 2	0.00 a	0.61 (0.61)a	48.95 (11.27)b
Native species A	0.00	0.00	0.00
Native species B	0.786 (0.786)	0.00	0.00

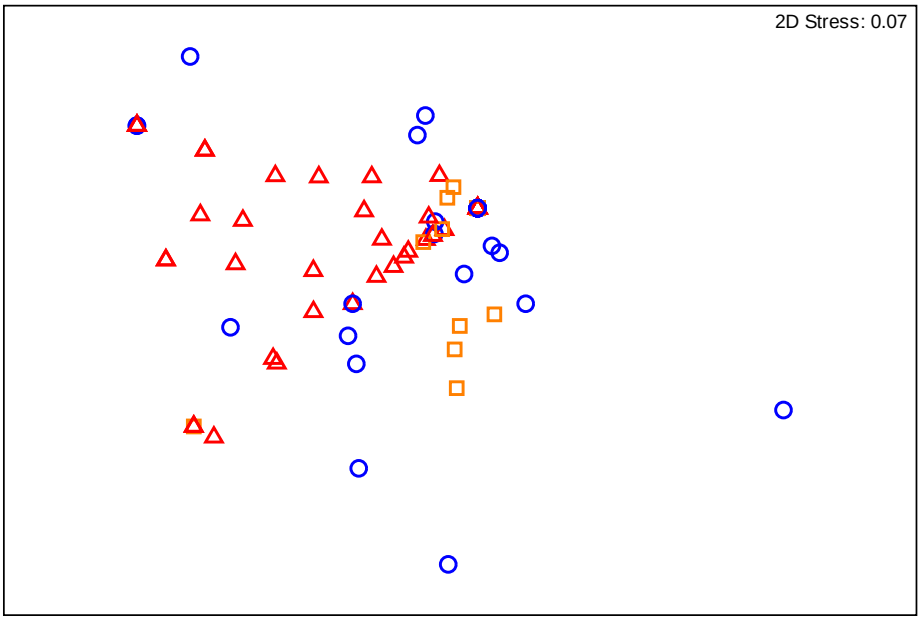


Figure 3. Non metric dimensional scaling (nMDS) analysis based on Bray Curtis similarity measure of earthworm community composition within shelterbelts of different ages, Δyoung shelterbelts (<5years), ○ old shelterbelts (>15 years) and □ middle-aged shelterbelts (5-10yrs). Points closest to each other in the nMDS plots indicate community compositions with a higher similarity.

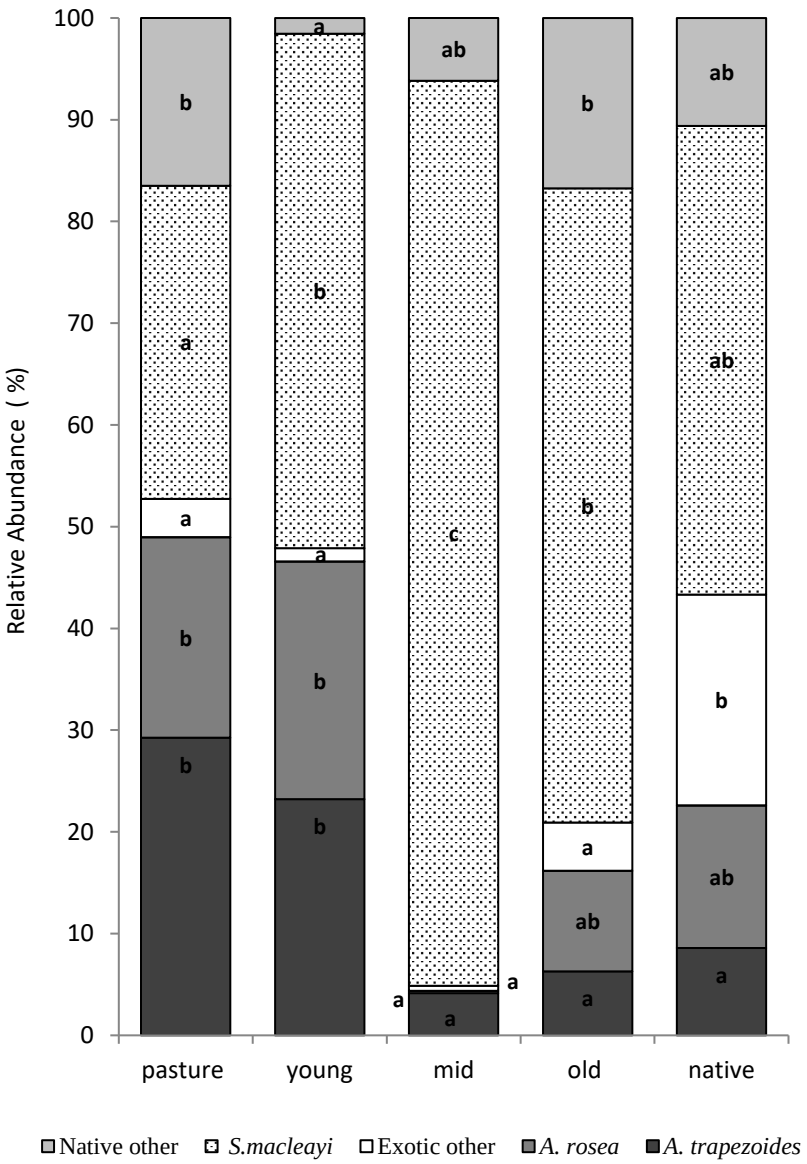


Figure 4. Relative abundance (%) of earthworm species with time since establishment and native and pastures. Young shelterbelts (<5years), old shelterbelts (>15 years) and middle-aged (5-10yrs). 'Native other' is a combination of the less dominant native species. 'Exotic other' is a combination of the less dominant exotic species. Different letters indicate significant differences according to ANOVA and subsequent Tukey's test (p<0.05) within each species or group of species.

Soil edaphic properties

Rainfall occurred approximately a week prior to sampling across all sampling plots with no rainfall events occurring during sampling. Soil moisture was significantly lower in shelterbelts than in native remnants and pasture sites (Table 5) at the time of sampling. Clay

content, pH, NO_3-N, K, Zn, Fe and exchangeable Mg, Cl, K, did not differ significantly between shelterbelts and pastures, but shelterbelts and pastures were significantly different from native remnant sites. Phosphorus levels were significantly lower in shelterbelts and native remnants compared to pastures. There were differences between all land uses for NH_4^+-N which was highest in shelterbelts and lowest in native remnants. Copper was highest in pastures and lowest in shelterbelts. Carbon:Nitrogen ratio and Aluminium in shelterbelts were intermediate between pastures and native remnants. For all other soil properties there was no difference between land use types (p>0.05) and they were subsequently excluded from further analyses.

Marginal tests indicate that moisture (17%, p=0.01), P (16%, p=0.03) and Cu (15%, p=0.04) most strongly correlated to earthworm community composition. Moisture, P and Cu had a slight positive correlation with earthworm density (Table 6). The combination of soil edaphic variables that most closely correlated with earthworm community was pH and P which correlated with S and Fe (r^2 >0.7) explained 36 % of the variation (Fig. 5b).

Table 5. Characteristics of the surface soil (0-10cm) under shelterbelts, native remnant and pasture systems combined for 2012 and 2013. Means ±SE followed by different letters indicate significant differences according to ANOVA and subsequent Tukey's test (p<0.05)

Soil parameters	Shelterbelt (n=36)	Native (n=18)	Pasture (n=36)
Soil moisture (%)	17.7 (0.57) a	29.7 (1.64) b	34.4 (0.68)b
pH	5.18 (0.07) a	5.66 (0.08) b	5.34 (0.06) a
EC (mS)	29.9 (2.51)	26.2 (2.86)	27.6 (1.91)
Total N (%)	0.168 (0.005)	0.153 (0.015)	0.174 (0.008)
Nitrate (mg kg^{-1})	5.00 (0.46) b	3.40 (0.58) a	4.00 (0.17) b
Ammonium (mg kg^{-1})	8.17 (0.18) c	5.6 (0.32) a	7.33 (0.23)b
Phosphorus (mg kg^{-1})	7.67 (0.21) b	6.20 (0.62) a	10.7 (0.81) c
Potassium (mg kg^{-1})	199 (12.27) b	152 (1.39) a	194 (4.54) b
Sulphur (mg kg^{-1})	6.43 (0.11)	6.88 (0.57)	7.03 (0.24)
Total C (%)	2.02 (0.07)	2.20 (0.20)	1.93 (0.07)
Organic C (%)	1.93 (0.07)	2.03 (0.17)	1.86 (0.06)
C: N ratio	12.1 (0.13) b	14.4 (0.36) c	11.2 (0.22) a
Copper (mg kg^{-1})	0.88 (0.02)a	1.02 (0.09) b	1.48 (0.03)c
Iron (mg kg^{-1})	250 (11.93) b	157 (10.26) a	287(13.53) b
Manganese (mg kg^{-1})	74.8 (3.46) ab	87.2 (2.66) b	70.08 (3.61) a
Zinc (mg kg^{-1})	1.26 (0.08) a	8.46 (3.60) b	1.403 (0.07) a
Aluminium ($cmol_c\ kg^{-1}$)	0.637 (0.02) c	0.122 (0.03) a	0.477 (0.02) b
calcium ($cmol_c\ kg^{-1}$)	1.42 (0.03) a	4.22 (00.45) c	1.72 (0.07)b
Magnesium ($cmol_c\ kg^{-1}$)	0.625 (0.03) a	1.25 (0.11) b	0.633 (0.03)a
Potassium ($cmol_c\ kg^{-1}$)	0.447 (0.03) b	0.306 (0.001)a	0.420 (0.010) b
Sodium ($cmol_c\ kg^{-1}$)	0.06 (0.002)a	0.064 (0.002) ab	0.068 (0.003) b
MBC	57.6 (6.16)	42.5 (5.00)	65.5 (7.86)
MBN	4.08 (0.36)	5.36 (0.76)	4.58 (0.39)
Sand (%)	68.6 (0.91)	65.3 (0.53)	67.34 (0.10)
Silt (%)	21.0 (0.76)	19.6 (0.88)	21.3 (0.73)
Clay (%)	10.4 (0.24) a	15.1 (0.68) b	11.3 (0.29) a

Table 6: Marginal test and best solution results from distance-based linear models (DISTLM) for soil edaphic variables predicting earthworm community composition. Significant results are in bold.

	Variable	***SS(trace)***	***Pseudo-F***	***p-Value***
Marginal test	P	4115.2	2.56	***0.021***
	Soil Moisture	4340	2.73	***0.024***
	Cu	3824	2.35	***0.044***
	pH	2599.2	1.51	0.184
	N	2132.3	1.21	0.309
	C:N	2304.3	1.32	0.280
	Mg	2534.7	1.47	0.183
	Al	2177.1	1.24	0.301
	Na	2895.5	1.70	0.115
	Silt %	2011.9	1.14	0.336
	Clay%	2503.8	1.45	0.201
	Ca	2035.9	1.15	0.338
Best solution[a]	AICc=112.72, r^2=0.36, RSS=15948, no of variables=2 (pH and P)			

[a] AICc= Corrected Akaike's information criterion, RSS=residual sum of squares.

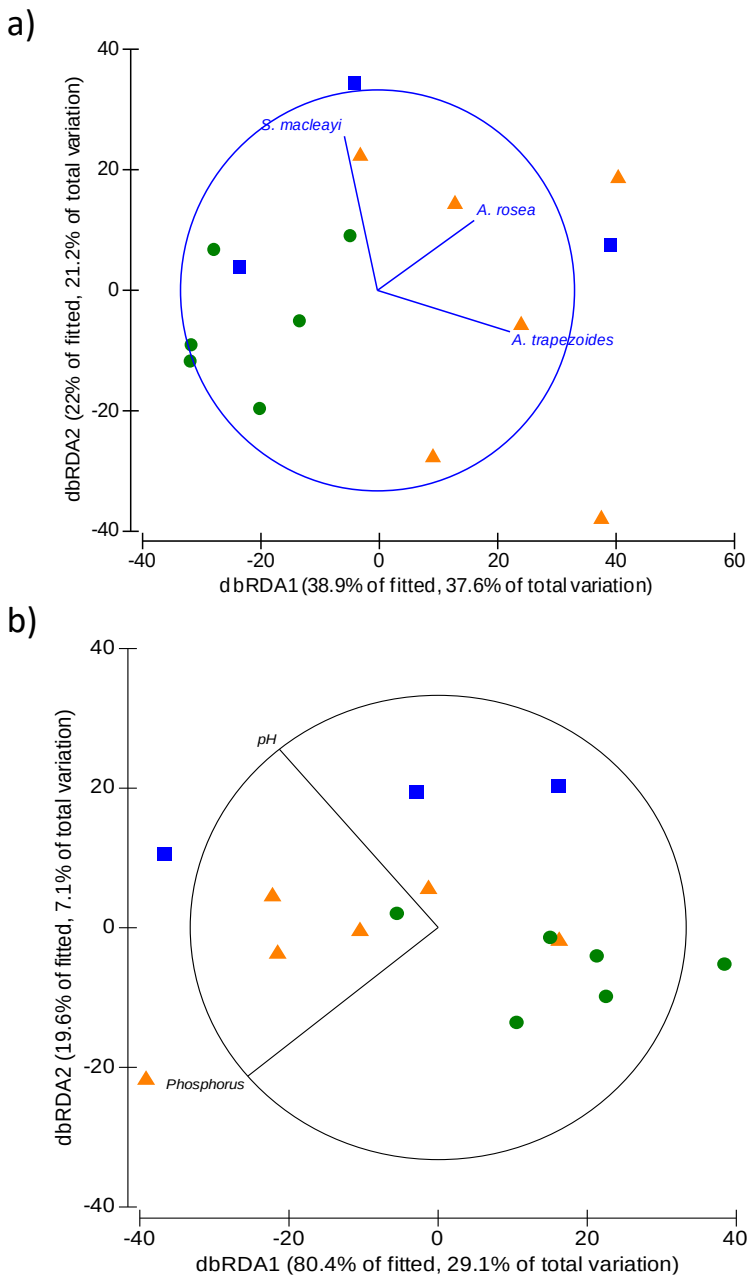


Figure 5. Plot distance-based redundancy analysis (dbRDA) ordination of earthworm community structure generated from a Bray-Curtis distance matrix. a) Relationship between the 3 dominant earthworm species and changes in earthworm community profiles within ■ native remnant woodland, ▲ pasture, ● shelterbelts b) Relationship between measured soil edaphic properties and changes in earthworm community profiles within native remnant woodland, pasture and shelterbelts. Vectors refer to relationships between dbRDA coordinate axes and predictors variables (soil properties). Only variables with correlations >0.4 are reported.

Discussion

Earthworm community composition, diversity, biomass and density were strongly influenced by the establishment of shelterbelts on agricultural land. This finding is contrary to that of Rajapaksha et al. (2013b), who found earthworm density and biomass in *Eucalyptus* short rotation (8-20 years) forestry (of similar age as shelterbelt age classes) established on pastoral land in the United Kingdom did not differ from the surrounding pasture. However, Smith et al. (2008) found that earthworm abundance after conifer afforestation was lower due to decreases in litter quality. Similarly, we demonstrated that conversion from pasture to native woody vegetation resulted in an overall decline in the density, biomass and species diversity, richness and evenness of both native and exotic earthworms. The decline in earthworm density within shelterbelts may be associated with the quality and quantity of litter produced by different plant communities (Muys et al., 1992; Rajapaksha et al., 2013a; Reich et al., 2005). *Eucalyptus* and *Acacia* litter contains higher lignin and phenol content, in particular tannins, which tend to have a greater C: N ratio, than pasture species (Bohlen et al., 1997; Crumsey et al., 2014; Hubbard et al., 1999). This is reflected in the higher soil C:N ratio observed in shelterbelts and native sites compared to pasture. Increased abundance of deep-rooted perennial vegetation (D. Carnovale, unpublished data) in shelterbelts is also associated with a decline in soil moisture content. It is thus quite likely that decreases in soil moisture within the shelterbelts at the time of sampling and the less degradable plant litter resulted in a decrease in the density and biomass of earthworms (Table 5). Due to low soil moisture levels in the shelterbelts, it is also possible that resident earthworms were forced to retreat to deeper soil horizons and as such may not have been captured by hand sorting (Edwards and Bohlen, 1992; Yeates, 1976). However, there was no evidence to suggest that the use of of a vermifuge would increase the efficiency of sampling as observations of soils to 30cm showed little to no evidence of burrowing indicative of anecic earthworms.

The overall landscape scale diversity (11 species) we observed is within the range of estimates for studies conducted at the similar spatial scales (Lavelle and Spain, 2001). However, earthworm diversity tended to be low within shelterbelt sites relative to other land-uses. Low earthworm diversity within local sites is not uncommon – other studies have recorded the presence of between one to five species at any one location (Lee, 1985; Smetak et al., 2007). The dominant exotic earthworm species found within pastures in the study area (*A. rosea* and *A. trapezoides*) were similar to those identified by Baker et al. (1992b) and (2004) in pasture

soils elsewhere in southern Australia. The native species, *S. macleayi*, dominated shelterbelts and native remnants (73.70% and 56.67% respectively). This is in agreement with several other authors (e.g. Wood 1974; Lawson 1993) who reported that native species are rarer in disturbed habitats compared with nearby natural habitats. Relatively few disturbances in recent years due to reduced tillage may partly explain the high density of *S. macleayi* we observed in pastures. Native earthworm species can occasionally comprise the majority of pasture communities in southern New South Wales (Baker, 2004).

The diversity, evenness and richness of earthworms within native sites were intermediate to those in pastures and shelterbelts. However, species composition was highly variable within native sites. What is surprising is the finding of the exotic species *O. cyaneum* and *A. caliginosa* within native remnant sites at greater density than in pastures and shelterbelts. Baker (1998b) showed that where rainfall is higher than 600 mm per year *A. caliginosa* is common, however, when lower than this, *A. caliginosa* becomes rarer. Moreover, as noted earlier, native remnant patches in this region are not pristine, having been subjected to significant modification since pastoral settlement as demonstrated by native site 2 which had a dense understory of the exotic perennial grass *Phalaris aquatica*. This increase in exotic ground cover may have contributed to more favourable litter quality and quantity, and thus further contributed to invasion by exotic earthworm species at this site.

Due to the lack of pristine native remnants within the study area we were unable to determine whether there is general convergence of earthworm communities in shelterbelts towards undisturbed native communities, as other factors (e.g. exotic ground cover) can influence the trajectory. Nevertheless, we were able to document a shift from exotic-dominated to native-dominated earthworm communities with increasing time after shelterbelt establishment. In addition, earthworm density in shelterbelts decreased with time after establishment. This is consistent with Zou and Bashkin (1998) and Szlávecz and Csuzdi (2007), whose studies showed declines in earthworm abundance in old forests, possibly due to a lack of high quality resources. In our study, earthworm biomass was also greater within young shelterbelts compared to old shelterbelts. However, with increasing time since establishment of shelterbelts there is a greater relative abundance of native species.

Following restoration of woody vegetation on agricultural land, one expectation is that soil properties such as soil carbon will increase due to higher organic matter inputs, whilst soil nutrients such as phosphorus and nitrogen may decrease in shelterbelts due to the removal of

fertiliser and manure inputs (Sauer et al., 2007). Such trends are likely to continue with time since establishment of shelterbelts. However, within our study, middle-aged shelterbelts had higher total C, N and P in the top 10 cm of soil than old and young shelterbelts which may reflect optimum resource availability from growth and organic matter production in middle-aged shelterbelts. Many other soil edaphic properties did not differ significantly between pasture and shelterbelts. Carbon density with the current study could not be assessed as bulk density measures were not recorded. However, within the same region no significant differences in soil carbon density in the top 10 cm was found in shelterbelts compared to pastures (D. Carnovale, unpublished data).

There were no strong correlations with earthworm density and biomass for any of the soil chemical properties measured. Similar to Siegrist (1998), we found no correlation between organic carbon and earthworm biomass or density. Earthworm density was not correlated with C:N ratio which is related to the digestibility of surface litter, indicating that litter quality alone may not be driving density. Measures of leaf litter inputs may have helped explain variation in earthworm density as demonstrated by Crumsey et al. (2014). This lack of strong correlation between soil chemical properties and earthworm abundance has also been shown in several other studies (Baker et al., 1992b; Hurisso et al., 2011; Morales et al., 2013; Siegrist et al., 1998).

Our sampling approach (e.g. bulking soil samples for physico-chemical analyses) could have obscured the level of heterogeneity within soil systems that earthworms would actually experience and respond to (Morales et al., 2013). However, despite the lack of strong correlations between individual soil chemical properties and earthworm density, variation in the earthworm community composition can be explained by a combination of pH, P, S, and Fe (Table 7). Higher pH and P in pastures compared to shelterbelts is associated with differing earthworm communities. The high pH in native remnants may confer a competitive advantage for particular species (Lobe et al., 2014). The lack of strong correlations between edaphic variables and earthworm density and biomass indicate that land use and times since establishment are better predictors.

Table 7. Results of a distance-based multivariate linear model (DistLM) for earthworm community composition showing the % variation explained by individual axes. The first two columns relate to the percentage explained in the fitted model (individuated with the DistLM analysis). The second two columns relate to the percentage explained out of the total variation in the resemblance matrix used to build up the DistLM model.

	% explained variation out of fitted model		% explained variation out of total variation	
Axis	Individual	Cumulative	Individual	Cumulative
1	80.4	80.34	29.1	29.1
2	19.6	100	7.12	36.3

Conclusions

Earthworm density and biomass was greatest in pastures independent of sampling years. We saw a shift from high earthworm biomass and density to low with increasing time since establishment. Overall the introduction of native woody vegetation was associated with a decline in density and biomass of earthworms, including a decrease in the relative abundance of exotic species possibly as a result of reduced soil moisture within shelterbelts. We cannot ascertain whether there is general convergence of earthworm communities in shelterbelts towards those in undisturbed native habitats due to the lack of pristine native remnants within the study area. Soil chemical variables were not strong predictors of earthworm biomass and density, but they can be used to explain overall community composition. The competitive nature between dominant exotic and native species remains unclear within these systems. Future studies addressing the consequences of changed community structure within shelterbelts and with time since establishment on ecological function are needed.

Acknowledgements

We are grateful to Linda Grevinga and Kathleen Anderson for their assistance with sampling and identification of earthworms. We are grateful to the farmers who allowed access to their property and Greening Australia for access to shelterbelts. We thank the three anonymous reviewers for their constructive comments and feedback. This research was supported by CSIRO and the Australian National University.

References

Anderson, M.J., Cribble, N.A., 1998. Partitioning the variation among spatial, temporal and environmental components in a multivariate data set. Australian Journal of Ecology. 23, 158–167.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth.

Australian Bureau of Meterology, 2014. Climate Statistics for Australian Locations. Australian Bureau of Meterology.

Baker, G.H., 1998a. The ecology, management, and benefits of earthworms in agricultural soils, with particular reference to southern Australia. In: Edwards, C.A. (Ed.), Earthworm Ecology 229–257.

Baker, G.H., 1998b. Recognising and responding to the influences of agriculture and other land-use practices on soil fauna in Australia. Applied Soil Ecology 9, 303–310.

Baker, G.H., 2004. Managing earthworms as a resource in Australian pastures. Earthworm Ecology. CRC Press, 263–286.

Baker, G.H., 2007. Differences in nitrogen release from surface and incorporated plant residues by two endogeic species of earthworms (Lumbricidae) in a red– brown earth soil in southern Australia. European Journal Soil Biology 1 (Suppl. 1), S165–S170.

Baker, G.H., Amato, M., Ladd, J., 2003a. Influences of *Aporrectodea trapezoides* and *A. rosea* (Lumbricidae) on the uptake of nitrogen and yield of oats (*Avena fatua*) and lupins (*Lupinus angustifolius*). Pedobiologia 47, 857–862.

Baker, G.H., Barrett, V., 1994. Earthworm Identifier. CSIRO Publishing.

Baker, G.H., Barrett, V.J., Grey-Gardner, R., Buckerfield, J.C., 1992a. The life history and abundance of the introduced earthworms *Aporrectodea trapezoides* and *A. caliginosa* (Annelida: Lumbricidae) in pasture soils in the Mount Lofty Ranges, South Australia. Australian Journal of Ecology 17, 177–188.

Baker, G.H., Buckerfield, J., Grey-Gardner, R., Merry, R., Doube, B., 1992b. The abundance and diversity of earthworms in pasture soils in the Fleurieu Peninsula, South Australia. Soil Biology and Biochemistry 24, 1389–1395.

Baker, G.H., Carter, P.J., Barrett, V.J., 1999. Influence of earthworms, *Aporrectodea* spp. (Lumbricidae), on lime burial in pasture soils in south-eastern Australia. Soil Restoration 37, 831–847.

Baker, G.H., Chan, K.Y., Munro, K., 2003b. Influences of native (Megascolecidae) and exotic (Lumbricidae) earthworms on ryegrass production in acidic pasture soils from south-eastern Australia. Pedobiologia 47, 830–834.

Baker, G.H., Obst, C.S., Barrett, V.J., 1998. Burial of lime by earthworms in acid soils in Western Australia. Proceedings of the 9th Australian Agronomy Conference 780–782.

Baker, G.H., Williams, P.M.L., Carter, P.J., Long, N.R., 1997. Influence of lumbricid earthworms on yield and quality of wheat and clover in glasshouse trials. Soil Biology and Biochemistry 29, 599–602.

Bird, P., 1998. Tree windbreaks and shelter benefits to pasture in temperate grazing systems. Agroforest. Syststems 41, 35–54.

Bird, P., Bicknell, D., Bulman, P., Burke, S., Leys, J., Parker, J., Van der Sommen, F., Voller, P., 1993. The role of shelter in Australia for protecting soils, plants and livestock. The Role of Trees in Sustainable Agriculture. Springer, 59–86.

Blair, G.J., Chinoim, N., Lefroy, R.D.E., Anderson, G.C., Crocker, G.J., 1991. A soil sulfur test for pastures and crops. Australian Journal of Soil Restoration 29, 619–626.

Blakemore, R.J., 1997. Agronomic potential of earthworms in brigalow soils of south- east Queensland. Soil Biology and Biochemistry 29, 603–608.

Bohlen, P.J., Parmelee, R.W., McCartney, D.A., Edwards, C.A., 1997. Earthworm effects on carbon and nitrogen dynamics of surface litter in corn agroecosystems. Applied Ecology 7, 1341–1349.

Chan, K.Y., 2004. Impact of tillage practices and burrows of a native Australian anecic earthworm on soil hydrology. Applied Soil Ecology 27, 89–96.

Chan, K.Y., Baker, G.H., Conyers, M.K., Scott, B., Munro, K., 2004. Complementary ability of three European earthworms (Lumbricidae) to bury lime and increase pasture production in acidic soils of south-eastern Australia. Applied Soil Ecology. 26, 257–271.

Chan, K.Y., Barchia, I., 2007. Soil compaction controls the abundance, biomass and distribution of earthworms in a single dairy farm in south-eastern Australia. Soil and Tillage Research 94, 75–82.

Chan, K.Y., Heenan, D.P., 2006. Earthworm population dynamics under conservation tillage systems in south-eastern Australia. Soil Research 44, 425–431.

Clarke, K., Gorley, R., 2006. PRIMER v6: user manual/tutorial. Plymouth Routines in Multivariate Ecological Research. Primer-E Ltd., Plymouth.

Clarke, K., Warwick, R., 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. PRIMER-E, Plymouth, UK.

Cleugh, H.A., Prinsley, R., Bird, P.R., Brooks, S.J., Carberry, P.S., Crawford, M.C.,Jackson, T.T., Meinke, H., Mylius, S.J., Nuberg, I.K., Sudmeyer, R.A., Wright, A.J., 2002. The Australian National Windbreaks Program: overview and summary of results. Australian Journal of Experimental Agriculture 42, 649–664.

Colwell, J., 1963. The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales by soil analysis. Australian Journal of Experimental Agriculture 3, 190–197.

Crumsey, J.M., Le Moine, J.M., Vogel, C.S., Nadelhoffer, K.J., 2014. Historical patterns of exotic earthworm distributions inform contemporary associations with soil physical and chemical factors across a northern temperate forest. Soil Biology and Biochemistry 68, 503–514.

Dalby, P.R., Baker, G.H., Smith, S.E., 1998a. Competition and cocoon consumption by the earthworm *Aporrectodea longa*. Applied Soil Ecology 10, 127–136.

Dalby, P.R., Baker, G.H., Smith, S.E., 1998b. Potential impact of an introduced lumbricid on a native woodland in South Australia. Applied Soil Ecology 9, 351–354.

Didham, R.K., Tylianakis, J.M., Gemmell, N.J., Rand, T.A., Ewers, R.M., 2007. Interactive effects of habitat modification and species invasion on native species decline. Trends in Ecology and Evolution 22, 489–496.

Doube, B.M., Stephens, P.M., Davoren, C.W., Ryder, M.H., 1994. Interactions between earthworms, beneficial soil microorganisms and root pathogens. Applied Soil Ecology. 1, 3–10. Earthworm Ecology. In: Edwards, C.A. (Ed.), CRC Press.

Edwards, C.A., Bohlen, P.J., 1992. The effects of toxic chemicals on earthworms. Reviews of Environmental Contamination and Toxicology. Springer, New York, pp. 23–99.

Hobbs, R.J., 1993. Can revegetation assist in the conservation of biodiversity in agricultural areas? Pac. Conservation Biology 1, 29.

Hubbard, V.C., Jordan, D., Stecker, J.A., 1999. Earthworm response to rotation and tillage in a Missouri claypan soil. Biology and Fertility of Soils 29, 343–347.

Hurisso, T.T., Davis, J.G., Brummer, J.E., Stromberger, M.E., Stonaker, F.H., Kondratieff, B.C., Booher, M.R., Goldhamer, D.A., 2011. Earthworm abundance and species composition in organic forage production systems of northern Colorado receiving different soil amendments. Applied Soil Ecology 48, 219–226.

Jamieson, B.G.M., 2001. Native Earthworms of Australia (Megascolecidae, Megascolecinae) [Electronic Resource] supplement/B.G.M. Jamieson. Dept. of Zoology and Entomology, University of Queensland, Brisbane.

Lavelle, P., Spain, A., 2001. Soil Ecology. Springer, Netherlands.

Lawson, L.M., 1993. The distribution and abundance of native and introduced earthworms in an area of pasture and native vegetation near Cape Jervis, South Australia. Flinders University, South Australia.

Lee, K.E., 1985. Earthworms: their ecology and relationships with soils and land use. Academic Press.

Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecological Monographs. 69, 1–24.

Lobe, J.W., Callaham Jr., M.A., Hendrix, P.F., Hanula, J.L., 2014. Removal of an invasive shrub (Chinese privet: *Ligustrum sinense* Lour) reduces exotic earthworm abundance and promotes recovery of native North American earthworms. Applied Soil Ecology 83, 133-139.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82, 290–297.

Morales, P.K., Yunusa, I.A.M., Lugg, G., Li, Z., Gribben, P., Eamus, D., 2013. Belowground eco-restoration of a suburban waste-storage landscape: earthworm dynamics in grassland and in a succession of woody vegetation covers. Landscape Urban Plan 120, 16–24.

Muys, B., Lust, N., Granval, P., 1992. Effects of grassland afforestation with different tree species on earthworm communities, litter decomposition and nutrient status. Soil Biology and Biochemistry 24, 1459–1466.

Rajapaksha, N.S.S., Butt, K.R., Vanguelova, E.I., Moffat, A.J., 2013a. Earthworm selection of short rotation forestry leaf litter assessed through preference testing and direct observation. Soil Biology and Biochemistry 67, 12–19.

Rajapaksha, N.S.S., Butt, K.R., Vanguelova, E.I., Moffat, A.J., 2013b. Effects of short rotation forestry on earthworm communities in the UK. Forest Ecology and Management 309, 96–104.

Rayment, G., Higginson, F.R., 1992. Australian laboratory handbook of soil and water chemical methods. Inkata Press Pty Ltd.

Reich, P.B., Oleksyn, J., Modrzynski, J., Mrozinski, P., Hobbie, S.E., Eissenstat, D.M., Chorover, J., Chadwick, O.A., Hale, C.M., Tjoelker, M.G., 2005. Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. Ecology Letters 8, 811–818.

Sauer, T., Cambardella, C., Brandle, J., 2007. Soil carbon and tree litter dynamics in a red cedar–scotch pine shelterbelt. Agroforest. Systems 71, 163–174.

Siegrist, S., Schaub, D., Pfiffner, L., Mäder, P., 1998. Does organic agriculture reduce soil erodibility? The results of a long-term field study on loess in Switzerland. Agriculture, Ecosystems and Environment 69, 253–264.

Smetak, K.M., Johnson-Maynard, J.L., Lloyd, J.E., 2007. Earthworm population density and diversity in different-aged urban systems. Applied Soil Ecology 37, 161– 168.

Smith, R.G., McSwiney, C.P., Grandy, A.S., Suwanwaree, P., Snider, R.M., Robertson, G. P., 2008. Diversity and abundance of earthworms across an agricultural land- use intensity gradient. Soil and Tillage Research 100, 83–88.

Stephens, P.M., Davoren, C.W., 1997. Influence of the earthworms *Aporrectodea trapezoides* and *A. rosea* on the disease severity of *Rhizoctonia solani* on subterranean clover and ryegrass. Soil Biology and Biochemistry 29, 511–516.

Stephens, P.M., Davoren, C.W., Ryder, M.H., Doube, B.M., 1993. Influence of the lumbricid earthworm *Aporrectodea trapezoides* on the colonization of wheat roots by *Pseudomonas corrugata* strain 2140R in soil. Soil Biology and Biochemistry 25, 1719–1724.

Szlávecz, K., Csuzdi, C., 2007. Land use change affects earthworm communities in Eastern Maryland, USA. Eur. J. Soil Biol. 1 (Suppl. 1), S79–S85.VSN International, 2011. GenStat for Windows, 14th ed. VSN International, Hemel Hempstead.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science 37, 29–38.

Winsome, T., Epstein, L., Hendrix, P.F., Horwath, W.R., 2006. Competitive interactions between native and exotic earthworm species as influenced by habitat quality in a California grassland. Applied Soil Ecology 32, 38–53.

Wood, T., 1974. The distribution of earthworms (Megascolecidae) in relation to soils, vegetation and altitude on the slopes of Mt. Kosciusko, Australia. J. Animal Ecology 87–106.

Yeates, G.W., 1976. Earthworm population of a pasture spray-irrigated with dairy shed effluent. N. Z. J. Agricultural Research 19, 387–391.

Zou, X., Bashkin, M., 1998. Soil carbon accretion and earthworm recovery following revegetation in abandoned sugarcane fields. Soil Biology and Biochemistry 30, 825–830.

Paper II: Shelterbelt influence on soil microbial community structure.

In **Paper II** I investigated how the presence of shelterbelts, and time since their establishment, affects soil microbial communities. This research complemented the research undertaken in **Paper I** by further characterising trajectories of change, this time focusing on the microbial community.

Carnovale, D., Thrall, P.H., Bissett, A., Baker, G., Shelterbelt influence on soil microbial community structure.

Abstract

Native trees and shrubs planted in rows (shelterbelts) have been established frequently on agricultural land to restore ecosystem services and conserve native biodiversity that has been altered by agricultural practices. Despite their wide use, our understanding of the effects of shelterbelts on soil biota remains limited. Changes in soil microbial community structure and abundance may have consequences for nutrient cycling, carbon sequestration and overall soil health. This study investigated how the presence of shelterbelts, and time since their establishment, affects soil microbial communities. We compared the abundance and structure of fungal and bacterial communities using quantitative PCR (qPCR) and terminal restriction fragment length polymorphism (T-RFLP) between shelterbelts, adjacent pastures and remnant native vegetation. There was no significant effect of site type on the fungal:bacterial (F:B) ratios (copy number), microbial biomass carbon, or on microbial biomass nitrogen. Fungal community composition in shelterbelts, regardless of age, was significantly different to pasture and native remnants. There was no significant difference in bacterial community composition between young shelterbelts (<5 years) and pastures, while middle-aged (5-15 years) and old (15-20 years) shelterbelts were significantly different to pasture communities. There was a significant difference in fungal and bacterial community composition between all shelterbelts and native remnants. Evaluation of the effect of time since shelterbelt establishment did not provide clear evidence that microbial communities in shelterbelts show trajectories that converge towards those observed in natural remnant patches, which may indicate that our native remnant sites may not represent true natural remnants. However, it can still be shown that the effects of agriculture are long-lasting and that the revegetated sites are different to remnant patches and may not be providing the same functions as remnant patches, even after 17 years post-establishment of a shelterbelt.

Key words: soil microbial communities, T-RFLP, chronosequence, shelterbelts

Introduction

Agricultural management practices may contribute to soil degradation. This can, in turn, result in changes in microbial community structure, with strong effects on soil microbial community biomass, abundance and community composition (Stark et al., 2008). In response to the growing awareness of the need to reduce land degradation, and conserve local biodiversity, the use of native plant species for ecological restoration has been widely implemented in Australian agricultural landscapes (Cleugh et al., 2002; Hobbs, 1993; Yunusa

et al., 2002). One method of re-introducing woody species into agricultural land is through the planting of native shelterbelts, which are strips, typically single or multiple rows of planted native trees and shrubs, or a strip of retained natural vegetation (Cleugh et al., 2002). The addition of shelterbelts to agricultural landscapes provides various ecosystem services including, but not limited to, increased landscape connectivity, increased productivity and reduced erosion (Bird, 1998; Bird et al., 1993; Cleugh et al., 2002; Yunusa et al., 2002). There is increasing evidence that shelterbelts conserve native faunal diversity (Lindenmayer and Hobbs, 2004), in particular bird diversity (Barrett et al., 2008; Cunningham et al., 2008; Munro et al., 2011). Nevertheless, little information is available on the effects of restoration on soil microbial communities.

Aboveground vegetation affects the size, activity and composition of soil microbial communities (da C Jesus et al., 2009; Lauber et al., 2008; Macdonald et al., 2009; Nüsslein and Tiedje, 1999; Singh et al., 2007b). For example, changes in aboveground plant community structure and composition alter the quality and diversity of plant litter (Bardgett and Shine, 1999; Six et al., 2006) which, in turn, may alter soil biotic community composition and function (Peršoh and Borken, 2017; Wardle et al., 2004). These changes can have consequences for critical ecosystem services such as nutrient cycling, carbon sequestration and longer-term sustainability (Singh et al., 2007a).

Microbial communities can respond more rapidly to changes in the environment than plant communities and thus may provide an early indication of the trajectory of recovery (Harris, 2009; Harris, 2003). The response of soil bacterial communities to land use change, both spatially and temporally, was addressed by Osborne et al. (2011) who found communities in a revegetated site were more similar to those in a remnant native woodland than the pasture from which they were rehabilitated, although time since establishment was not considered. More recently Cavagnaro (2016) found reforestation of agricultural land supported a greater microbial biomass, a higher fungal:bacterial ratio and a different microbial community (based on PLFA profiles) from that of the adjacent pasture. Others found a strong effect of vegetation type on microbial community structure in the rhizosphere (Costa et al., 2006; Grayston et al., 1998; Kuske et al., 2002). However, there is also evidence that both fungal and bacterial communities under distinct land-use types (hardwood and pine forests, cultivated and livestock pasture lands) did not necessarily result in distinct soil fungal or bacterial communities (Lauber et al., 2008). Rather, the composition of bacterial and fungal communities was most strongly

correlated with specific soil properties as shown by Lauber et al. (2008). Soil pH was the best predictor of bacterial community composition, whereas fungal community composition was most closely associated with changes in soil nutrient status. Further to this, many other studies have shown that soil physical and chemical properties are dominant factors in determining microbial community structure (Bossio et al., 1998; Buyer et al., 1999; Fierer and Jackson, 2006; Girvan et al., 2003; Singh et al., 2007b).

There have been limited studies on the effect of time since establishment of restoration of woody vegetation on soil microbial community and trajectories of recovery. Recently Yan et al. (2018) and Gellie et al. (2017), monitored fungi and bacteria following the restoration of woody vegetation on cleared land using high-throughput environmental DNA (eDNA). They found that after just 10 (fungi) and 8 years (bacteria) of active native plant revegetation, an observed shift in the respective microbial communities, towards that of the natural communities, could be seen. Further, Banning et al. (2011), using a chronosequence of jarrah forest systems (up to 18 years) on rehabilitated bauxite mining sites, demonstrated that bacterial communities in rehabilitated sites became more similar to those of the surrounding forest with rehabilitation age. Similar trends were, however, not evident with the fungal community. Overall, they found that shifts towards native systems in the soil bacterial community were only detected in sites that were more than 10 years post-revegetation. Jangid et al. (2011) found that land use history had a stronger impact on soil microbial community composition than either vegetation or soil properties and, over time (>50 years), microbial communities in disturbed soils returned toward their native state.

In this study we used shelterbelts that varied in time since establishment to investigate impacts on soil fungal and bacterial communities in a rural landscape in New South Wales (NSW), Australia. It was hypothesised that: 1) soil microbial communities would differ in restored vegetative shelterbelts compared to pasture sites, and that these differences would become greater with shelterbelt age; 2) microbial communities in shelterbelts would show trajectories that converge towards those observed in nearby natural remnant patches; and 3) soil physico-chemical properties commonly reported to change with revegetation under different vegetation types, such as soil carbon, nitrogen and phosphorus, would correlate with altered microbial communities.

Methods

Study site description and experimental design

Nine sites in total were selected as described in detail in Carnovale et al. (2015) and **Paper I** in this thesis. Study sites were located near Murrumbateman in New South Wales, Australia. Briefly, six sites consisted of two parallel transects per plot, one within a shelterbelt established on previous pastoral land and the other within an adjacent grazed pasture. An additional three sites were selected within three nearby native remnant woodlands, with one transect per site. The six shelterbelt transects were separated into three age classes: 0-5 years since establishment (young), 5-15 years (middle-aged), 15-20 years (old), with two (replicate) transects in each age class. Each shelterbelt had been direct sown with a seed mix of locally endemic tree and shrub species. The primary tree species used were a mix of *Acacia* and *Eucalyptus* species. The dominant *Eucalyptus* species within the seed mix consisted of *E. mannifera*, *E. blakelyi*, *E. viminalis* and *E. melliodora*, while the dominant *Acacia* species were *A. mearnsii*, *A. cardiophylla*, *A. decurrens* and *A. rubida*. Shelterbelts were fenced off and had not received any mulches, amendments or irrigation.

In 2011 two native remnant sites were sampled. At each native remnant site, a single transect was sampled. These native remnants were small (approximately 1 hectare) fragments which had various levels of disturbance. The native remnants all had an overstory of native tree species, predominantly *E. blakelyi* and/or *E. melliodora*. The level of understory disturbance varied within and between the native remnant patches, in particular due to occasional grazing, thus exotic pasture species were typically present to varying degrees in these sites and were also present in the shelterbelts. In these grazed landscapes (which have been highly modified) it is very difficult, if not impossible, to find pristine/undisturbed native patches.

All transects were situated within a radius of 12 km, thus limiting landscape scale environmental variability between sites. All sites were located on mid to lower slopes. Each transect was 90 m long. Shelterbelt width varied from 4-8 tree rows wide. Transects were centred through each shelterbelt, to minimise any possible edge effects. In the pastures, transects were positioned parallel to, and approximately 15 metres away from, the edge of the shelterbelt. Transects within native remnants were positioned in the middle of the native remnant patch. A total of 9 sample points were located along each transect at 10 m spacings irrespective of tree placement. Three composite soil samples (three consecutive sample points) per transect were collected. Each composite sample consisted of three core samples 5cm by

10cm deep. Paired transects (shelterbelt and pasture) within each site were sampled on the same day. Sampling occurred during one-week periods in October 2011 and April 2012. All samples were stored at 4°C in the field.

Physical and chemical Soil Analysis

Soil samples for chemical analysis were air-dried and sieved (2mm). Soil moisture content was determined gravimetrically by oven drying subsamples at 110 degrees for 72 hours. Soil pH and electrical conductivity (EC) were analysed in a 1:5 water suspension which was shaken end over end for 1 hour, centrifuged down for 1 hour and read immediately with a Thermo Scientific Orion 3-Star portable meter. Total Carbon (C) and total Nitrogen (N) were determined on an oven dried basis on finely ground soil using the dry combustion method on an elemental analyser (*vario MAX CNS)*. The remaining parameters [particle size (sand, silt and clay), Organic C, Nitrate N (NO_3-N), Ammonium N (NH_4^+-N), Sulphur (S), Phosphorus (P), Potassium (K), Exchangeable cations (Ca, Mg, Na, K, Al) and trace elements (Cu, Fe, Mn Zn)] were determined by the CSBP Soil and Plant Analysis Laboratory (Perth Australia). Organic C was determined by the Walkley Black method (Walkley and Black, 1934). P and K measured according to Colwell (1963). Sulphur was extracted using the Blair/Lefroy method (Blair et al., 1991), and NO_3-N and NH_4^+-N were extracted with 1M potassium chloride and measured on a Lachat Flow Injection Analyser. DPTA trace elements were measured by atomic absorption spectroscopy (Rayment and Higginson, 1992).

Microbial biomass

Microbial biomass C and N were estimated by the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987) using 10g of moist 2mm sieved soils extracted in 0.5 M K_2SO_4 within 24 hours after collection. Carbon and N in the extracts were measured using a Shimadzu Total Carbon and Nitrogen analyser (Shimadzu TOC-VCSH TNM). Microbial biomass C was calculated as *EC/k*EC, where *EC*=(organic C extracted from fumigated soil)–(organic C extracted from non-fumigated soil) and *k*EC=0.45 (Wu et al., 1990). Microbial biomass N was calculated as *E*N/*k*EN, where *E*N=(total N extracted from fumigated soil)–(total N extracted from non-fumigated soil) and *k*EN=0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996).

DNA extraction

The soil composite samples were frozen at -20° C until analysis (no longer than one year). Total soil DNA was extracted from 0.25 ± 0.01 g of each soil sample using the Power soil DNA

extraction Kit (MoBio Laboratories, Carlsbad, California, USA), with the following modification to manufacturer's instructions: samples were shaken in a Bio101 Savant bead beater on maximum speed for 2 min instead of vortexing. DNA quality was assessed by 1% agarose gel and DNA quantity was determined using PicoGreen dsDNA Quantitation Reagent (Invitrogen). DNA concentrations were standardised to 2 ng/µl prior to T-RFLP and qPCR.

Real time qPCR for Fungi and Bacteria Abundance

The abundances of bacterial 16S rRNA genes and fungal internal transcribed spacer (ITS) gene regions were determined using qPCR as described in Fierer et al. (2005). Primers targeting bacterial 16S rRNA gene were Eub338 (Lane, 1991) and Eub518 (Muyzer et al., 1993) and for fungal ITS primers were 5.8s (Vilgalys and Hester, 1990) and ITS1F (Gardes and Bruns, 1993). qPCR assays were performed in triplicate 10µl reactions containing 5 µl SsoAdvancedTM SYBR® green 246 Supermix (Bio-Rad, Hercules, CA), 0.4 µl of forward and reverse primers (10 µM), 2.2 µl H_2O and 2 µl template DNA. Bacterial 16S rRNA genes quantification was carried out under the following conditions, 95ºC for 2 min followed by 95ºC for 10s, 53ºC for 30s and 72ºC for 20s. Fungal quantification was as follows: 95ºC for 1 min followed by 95ºC for 5s, 53ºC for 20s and 72ºC for 20s. Forty cycles were run for bacteria and fungi, followed by a melting curve to confirm the amplified products were of the appropriate size. For each plate a standard curve was run with a 10-fold dilution series from 10^9 copies to 10^4 copies. Standards were prepared from PCR product generated using the primers above from *Pseudomonas fluorescens* strain 5 and *Fusarium oxysporum vasinfectum* and purified using the Agencourt PCR purification system. Standards were quantified using Picogreen (Invitrogen, Grand Island, NY, USA). Amplification efficiency was > 90% and r^2 was > 0.99 for all bacterial and fungal calibration curves.

T-RFLP

Bacterial and fungal soil communities were analysed using terminal-restriction fragment length polymorphism (T-RFLP) (Thies, 2007). Profiles of bacterial 16S rRNA genes present in each DNA extract were performed using the 6-carboxyfluorescein (FAM)-labelled FAM27f (5′-*AGTTTGATCMTGGCTCAG*-3′) and BAC519r (5′-*GWATTACCGCGGCKGCTG* -3′) primer. Fungal internal transcribed spacer (ITS) regions were amplified from soil DNA using fungal primers: FAMITS1-F (5′-*CTTGGTCATTTAGAGGAAGTAA*-3′) (Gardes and Bruns, 1993) and ITS4 (5- *TCCTCCGCTTATTGATATGC*-3′) (White et al., 1990). 50µL PCR reactions contained 1ng of extracted DNA, 1U of MyTaq DNA Polymerase (bioline) and 1x supplied

buffer, and 0.5µM of each of the oligonucleotide primers. Fungi PCR mix was as above with the addition of 0.5µL BSA (Promega). Bacterial PCRs were run at 94°C for 5 min, 40 cycles of 94°C for 30s, 58°C for 30s and 72°C for 45s followed by a final extension step at 72°C for 7 min. We confirmed amplification in a 1% agarose gel. The PCR product was then purified using Agencourt AMPure XP system, according to the manufacturer's instructions. Twenty five nanograms of the FAM-labeled PCR products were each added to restriction endonucleases *HinfI*, in a volume of 25µL that included 5 U of *HinfI* and the supplied buffer at 1X. Restriction digests were incubated at 37ºC for 4 h. Digested products were isopropanol precipitated, resuspended in 10µL of formamide containing LIZ600 size standard (Applied Biosystems, UK), then denatured at 95°C for 2 min. T-RFLP profiles were generated by separating fragments on an ABI 3130xl Genetic Analyser (Applied Biosystems, UK).

Terminal-restriction fragments (T-RFs) generated by the sequencer were exported and initially analysed for absolute peak areas using the size-calling software GeneMapper™ 4.0 (Applied Biosystems, UK). First T-RF profiles were checked for stable baselines, voltage and calibrations and profiles were trimmed to between 50 and 600bp to avoid T-RFs caused by primer-dimers and to obtain fragments within the linear range of the internal size standard. Data was then uploaded to the online T-RFLP analysis software package T-REX (http://trex.biohpc.org/; Culman et al., 2009). Noise filtering was carried out by removing peaks whose area was less than twice the standard deviation computed over all peaks (Abdo et al., 2006). T-RFs were aligned where peaks with values less than the threshold (1 bp) were identified and grouped into a T-RF (Smith et al., 2005). The relative abundance of a detected T-RF for a sample was calculated as signal area of each peak divided by the total peak area of all the peaks of the T-RFLP profile for that sample. For statistical analysis the relative abundance of a detected T-RF within a given T-RFLP profile was applied.

Statistical analysis

Statistical analysis was conducted with restricted maximum likelihood (REML) models using the following design [Fixed model=site type (pasture=P, young shelterbelt=YB, middle-aged shelterbelt=MB, old shelterbelt=OB and native remnants=N), Random model=Site/year) for all analysis where possible to account for uneven replication. When necessary (Total C, P, NO_3-N, Mn, Zn, Ca) data was log transformed to achieve homogeneity of variances and normality prior to analysis. Linear regression models were used to assess correlations between soil edaphic properties. All statistical tests were performed at p=0.05.

Multivariate analysis was used to assess changes in microbial community composition. Relative abundance of identified TRFs were used to produce Bray Curtis similarity matrices. Edaphic data, transformed as indicated above, were normalised and Euclidean distance used to calculate distance matrices. PERMANOVA (Anderson, 2001) was used to test significance of site type on microbial assemblages. General differences between soil microbial communities were visualised using non-metric multidimensional scaling (nMDS) and canonical analysis of principle coordinates (CAP) (Anderson and Willis, 2003). Distance Based Linear Models (DistLM) (Legendre and Anderson, 1999) were used to test for differences in community composition and how these related to predictor environmental variables. For DistLM, co-varying edaphic variables (r^2>0.70) were found and only one such variable was run in the model (Supplementary Table 1). Results were interpreted such that this single variable represented the behaviour of all variables with which it was found to co-vary. The stepwise model selection procedure (Anderson et al., 2008) was used to identify variables that explained significant amounts of variation in bacterial and fungal communities. A corrected Akaike information criterion (AICc) was used to select the predictor variables. Marginal tests were conducted to assess the statistical significance and percentage contribution of each soil edaphic variable alone (Anderson and Cribble, 1998; Legendre and Anderson, 1999; McArdle and Anderson, 2001). Distance-based redundancy *analysis* (*dbRDA*) was used for graphical visualization of the DistLM results. Statistical analysis was conducted using Genstat software 16th edition package (VSN International, 2011) and multivariate tests were conducted using PRIMER 6 (Clarke and Gorley, 2006; Clarke and Warwick, 2001) and PERMANOVA + (Anderson et al., 2008).

Results

Soil edaphic properties

REML models of soil edaphic properties (0-10cm) indicated significant differences (p<0.05) between site types (shelterbelts of differing age, pasture and native remnants) for most soil properties excluding total C and N, EC, S, Mn, Mg, and silt (Table 1). Soil moisture was significantly lower in shelterbelts than in native remnants and pasture sites at the time of sampling (p<0.05). Clay content, pH, NO_3-N, K, Zn, Fe and exchangeable Mg, Cl, K, did not differ significantly between shelterbelts of different ages and pastures, but shelterbelts and pastures were significantly different from native remnant sites (Table 1). Phosphorus levels were significantly lower in shelterbelts and native remnants compared to pastures. There were differences between all land uses for NH_4^+-N, which was highest in shelterbelts and lowest in

native remnants. Copper was highest in pastures and lowest in shelterbelts. C:N ratio and aluminium levels in shelterbelts were intermediate between pastures and native remnants.

The time since establishment of shelterbelts further influenced soil properties (Table 1). Within shelterbelts the C:N ratio, Total C and N, NO_3-N, phosphorus, sulphur, organic C, Fe, Zn, Ca, and Mg was highest within middle-aged shelterbelts. With increasing time since establishment there was a decrease in soil moisture recorded at time of sampling, and an increase in EC, NH_4^+-N, Cu, and Mn.

There was no significant effect of site type on the F:B ratio, MBC, or on MBN. There was a significant difference between site types (shelterbelts of differing age, pasture and native remnants) with respect to the microbial C:N ratio (Table 2). Microbial C:N was highest under pasture and lowest under native sites. Microbial C:N under young, middle-aged and old shelterbelts were more similar to pasture sites, relative to native remnants. No strong correlations between soil edaphic properties and MBC, MBN, the microbial biomass C:N ratio or the F:B ratio were evident.

Table 1. Characteristics of the surface soil (0-10cm) under shelterbelts of 3 age classes: 0-5 years since establishment (young), 5-15 years (middle-aged), 15-20 years (old); native remnant patches; and pasture systems. Values represent means ±SE. REML model (fixed model=site type, random model = site/year) results for effects of site type on soil properties. Only soil properties varying significantly among site types are shown. YS=Young shelterbelt, MS=middle-aged shelterbelt, OS=old shelterbelt.

Site type	**C: N ratio**	**pH**	**Soil moisture (%)**	**P (mg kg^{-1})**	**NO_3-N (mg kg^{-1})**	**NH_4^+-N (mg kg^{-1})**	**K (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Fe (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Al ($cmol_c$ kg^{-1})**	**Ca ($cmol_c$ kg^{-1})**	**Na ($cmol_c$ kg^{-1})**
Pasture	11.24 (0.22)	5.31 (0.05)	34.4 (0.68)	10.7 (0.81)	4.0 (0.17)	7.3 (0.23)	194 (4.54)	1.48 (0.03)	287 (13.5)	1.40 (0.07)	0.477 (0.02)	1.72 (0.07)	0.07 (0.003)
YS	12.14 (0.15)	5.30 (0.08)	10.8 (2.01)	7.5 (0.15)	2.5 (0.15)	7.5 (0.15)	168 (3.17)	0.84 (0.04)	232 (7.93)	1.04 (0.09)	0.709 (0.003)	1.20 (0.02)	0.06 (0.006)
MS	12.48 (0.11)	5.08 (0.13)	8.99 (0.88)	9 (0.30)	8.5 (0.45)	8.5 (0.45)	161 (15.4)	0.84 (0.003)	342 (2.13)	1.57 (0.20)	0.766 (0.01)	1.63 (0.03)	0.07 (0.002)
OS	11.53 (0.29)	5.14 (0.10)	7.55 (0.65)	6.5 (0.15)	4 (0)	8.5 (0.15)	270 (22.8)	0.97 (0.005)	177 (2.65)	1.18 (0.07)	0.437 (0.006)	1.44 (0.02)	0.06 (0.002)
Native	14.44 (0.36)	5.62 (0.07)	29.7 (1.64)	6.20 (0.62)	3.4 (0.58)	5.6 (0.32)	152 (1.39)	1.02 (0.09)	157 (10.3)	8.46 (3.60)	0.122 (0.03)	4.22 (0.45)	0.06 (0.002)
Wald statistic	20.45	21.58	18.21	27.75	55.38	25.82	13.9	22.94	19.23	39.85	429.91	66.09	28.08
n.d.f.	4	4	4	4	4	4	4	4	4	4	4	4	4
F statistic	4.97	5.25	4.41	6.63	13.35	6.17	3.34	5.54	4.58	9.67	102.63	15.9	6.7
d.d.f.	39.2	22.9	39.3	22.8	22.9	22.7	23.1	22.1	22.8	23.1	22.8	23	22.8
F pr	***0.002***	***0.004***	***0.005***	***0.001***	***<0.001***	***0.002***	***0.027***	***0.003***	***0.007***	***<0.001***	***<0.001***	***<0.001***	***0.001***
Transformed				y	y					y		Y	

Table 2. Microbial characteristics of the surface soil (0-10cm) under shelterbelts, native and pasture systems. Values shown are means ±SE and results from a REML model (fixed model =site type, random model=site/year) for effects of site type on soil properties.

	microbial C: N ratio	***MBC***	***MBN***	***F: B ratio***
Pasture	17.27 (1.9)	342.3 (39.6)	20.42 (1.7)	0.30 (0.03)
Young shelterbelt	15.35 (1.7)	358.6 (80.2)	22.23 (4.1)	0.24 (0.03)
Mid shelterbelt	15.8 (1.8)	304.4 (57.5)	17.69 (2.3)	0.23 (0.03)
Old shelterbelt	14.19 (0.9)	244.9 (42.6)	17.33 (2.8)	0.32 (0.03)
Native	9.89 (0.6)	230.4 (26.5)	24.27 (3.3)	0.19 (0.04)
Wald statistic	12.35	6.34	4.62	5.78
n.d.f.	4	4	4	4
F statistic	3.04	1.56	1.13	1.41
d.d.f.	49	51.8	35.6	22.2
F pr	***0.026***	0.2	0.357	0.263

Microbial community structure PERMANOVA analysis of T-RFLP community fingerprint patterns indicated that differences in microbial community structure could be attributed to site type for both bacterial and fungal communities (fungi p=0.001; bacteria p=0.001) (Table 3). PERMANOVA pairwise results and nMDS plots of T-RFLP fungal community composition indicated significantly different community profiles between shelterbelts, pasture and native sites (Table 3, Fig. 1a). Regardless of age, fungal community composition in shelterbelts was significantly different to pasture and native remnants. Differentiation between fungal communities in shelterbelts of differing ages was also evident, with the greatest differences between young versus middle-aged, and young versus old shelterbelts.

Bacterial T-RFLP community profiles were also altered by site type. PERMANOVA pairwise results and nMDS plots of T-RFLP bacterial community composition showed significantly different community profiles between shelterbelts and native sites (Table 3, Figure 1b). There was no significant difference in bacterial community composition between young shelterbelts (<5 years) and pastures (Table 3). Middle-aged and old shelterbelts were significantly different to pasture communities. However, there was no statistical difference in bacterial community composition between middle-aged and old shelterbelts.

Table 3. Pairwise tests from PERMANOVA testing microbial community structure in response to site type (YB: young belt, MB: middle-aged belt, OB: old belt, P: pasture and N: native remnant).

	Fungi			Bacteria		
Comparison	Psedo f	*p*(perm)	P(MC)	Psedo f	*p*(perm)	P(MC)
YB, MB	1.74	***0.002***	***0.02***	1.73	***0.007***	***0.036***
YB, OB	1.89	***0.002***	***0.013***	1.71	***0.01***	***0.03***
OB, MB	1.63	***0.017***	0.054	1.20	0.197	0.249
YB, N	1.48	***0.006***	***0.037***	1.84	***0.002***	***0.008***
MB, N	1.79	***0.001***	***0.003***	2.27	***0.002***	***0.001***
OB, N	1.69	***0.001***	***0.005***	1.99	***0.001***	***0.007***
YB, P	2.54	***0.001***	***0.001***	1.26	0.095	0.11
MB, P	3.64	***0.001***	***0.001***	2.01	***0.001***	***0.002***
OB, P	3.47	***0.001***	***0.001***	1.66	***0.013***	***0.007***
P, N	2.29	***0.001***	***0.001***	2.06	***0.001***	***0.002***

MC= monte carlo *p*

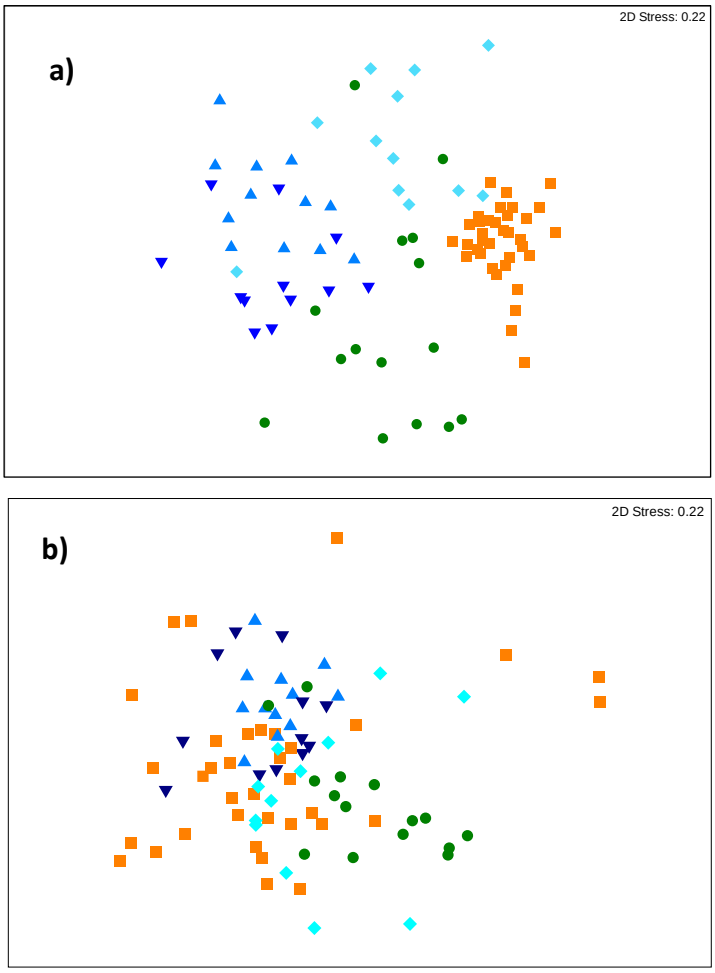


Figure 1. Non-metric dimensional scaling (nMDS) based on Bray Curtis similarity measure of a) fungi community composition by site type; and b) bacteria by site type. Symbols are as follows: ● native remnant, ■ pasture, ♦ young shelterbelts, ▲ middle-aged shelterbelts and ▼ old shelterbelts.

Soil edaphic Properties and soil biological community

The relationships between soil bacterial and fungal communities were assessed using DistLM and visualised with dbRDA (Fig. 2). The co-varying variables (r>0.70) that were removed included total N, S, Fe, Mg, Ca, silt, and clay (Supplementary Table 1). Soil edaphic properties were not strongly correlated to either fungal or bacterial community composition (24 and 28 % variation respectively). Marginal tests indicated that phosphorus (5.1%, p=0.001), Mn (4.4%, p=0.001) and NO_3-N (4.3%, p=0.001) most strongly correlated to fungal community composition (Table 4). The soil edaphic variables that explained the most variation in fungal communities (24% of the variation) (Table 4, Fig. 2) were P (which correlated with S and DTPA Fe), nitrate N, MBC, NH_4^+-N, Al (which correlated with Ca and clay % and C:N ratio).

Marginal tests indicated that Al (7.67%, p=0.001), NO3-N (6.2%, p=0.001), and Zn (6.2%, p=0.001) most strongly correlated to bacterial community composition (Table 5). The soil edaphic variables that most closely correlated with bacterial community composition were soil moisture, total N, C: N ratio, Al, Zn and Mn which explained 28% of the variation in bacterial community composition.

Table 4. Tests for relationships between the community structure of fungi and soil edaphic variables using non-parametric multivariate multiple regression analysis (DistLM). On the left are the marginal tests of individual sets. On the right are the partial (conditional) tests, where the amount explained by each variable added to the model is conditional on variables already in the model. *p*-values less than 0.05 are in bold (%Var: the percentage of the multivariate variance in the community structure explained by that variable; %Cumul: cumulative percentage of variance explained).

Marginal test				***Sequential tests***				
Variable	***Pseudo-F***	***p***	***% Var***	***Variable***	***Pseudo-F***	***p***	***% Var***	***% Cumul.***
pH	1.7	***0.045***	1.98	+ P	4.55	***0.001***	5.14	5.14
Ec	2.1	***0.013***	2.44	+ NO_3-N	5.22	***0.001***	5.61	10.75
Soil moisture	3.13	***0.001***	3.59	+MBC	3.12	***0.001***	3.27	14.02
MBC	3.1	***0.001***	3.56	+NH_4^+-N	3.16	***0.001***	3.22	17.24
MBN	2.49	***0.005***	2.88	+Al	3.15	***0.001***	3.13	20.38
C%	1.81	***0.029***	2.11	+C%	2.95	***0.001***	2.87	23.24
CNRatio	1.72	***0.04***	2.01	+moisture %	2.44	***0.002***	2.33	25.58
NH_4^+-N	2.06	***0.013***	2.4	- NO_3-N	1.66	***0.018***	1.58	24
NO_3-N	3.74	***0.001***	4.26					
P	4.55	***0.001***	5.14					
K	1.74	***0.037***	2.03					
Cu	2.09	***0.009***	2.43					
Mn	3.71	***0.001***	4.23					
Zn	2.18	***0.013***	2.53					
Al	3.36	***0.001***	3.84					
Exc. Sodium	2.73	***0.005***	3.15					
% Sand	1.4	0.119	1.64					
MBC:MBN	2.1101	0.011	2.4505E-2					
Best solution	AICc =656.89, R^2= 0.24, 6 variables (soil moisture, MBC, Total C, NH_4^+-N, P and Al)							

Table 5. Tests for relationships between bacterial community structure and soil edaphic variables using non-parametric multivariate multiple regression analysis (DistLM). On the left are the marginal tests of individual sets. On the right are the partial (conditional) tests, where the amount explained by each variable added to the model is conditional on variables already in the model. *p*-values less than 0.05 are in bold (%Var: the percentage of the multivariate variance in the community structure explained by that variable; %Cumul: cumulative percentage of variance explained).

Marginal test				***Sequential tests***				
Variable	***Pseudo-F***	***p***	***% Var***	***Variable***	***Pseudo-F***	***p***	***% Var***	***% Cumul.***
pH	4.19	***0.001***	4.75	+Al	6.98	***0.001***	7.67	7.67
Ec	3.19	***0.001***	3.65	+ NO_3-N	4.68	***0.001***	4.92	12.59
Soil moisture	4.2	***0.001***	4.76	+MBC	3.41	***0.001***	3.49	16.09
MBC	2.99	***0.003***	3.43	+Mn	2.54	***0.002***	2.55	18.64
MBN	3.24	***0.001***	3.71	+C%	2.73	***0.002***	2.68	21.32
C%	3.21	***0.001***	3.68	- NO_3-N	1.99	***0.023***	1.96	19.36
CNRatio	2.31	***0.007***	2.68	+moisture %	3.46	***0.001***	3.35	22.71
NH_4^+-N	3.04	***0.001***	3.49	+P	2.76	***0.001***	2.61	25.31
NO_3-N	5.56	***0.001***	6.21	+Na	2.71	***0.001***	2.51	27.83
P	1.89	***0.034***	2.19					
K	2.61	***0.004***	3.01					
Cu	3.66	***0.001***	4.18					
Mn	2.65	***0.002***	3.06					
Zn	5.58	***0.001***	6.22					
Al	6.98	***0.001***	7.67					
Na	1.45	0.133	1.69					
% Sand	1.28	0.201	1.5					
MBC:MBN	2.11	0.011	0.0					
Best solution	AICc =563.54, R^2= 0.278, 7 variables (Soil moisture, MBC, Total C, P, Mn, Al and Na)							

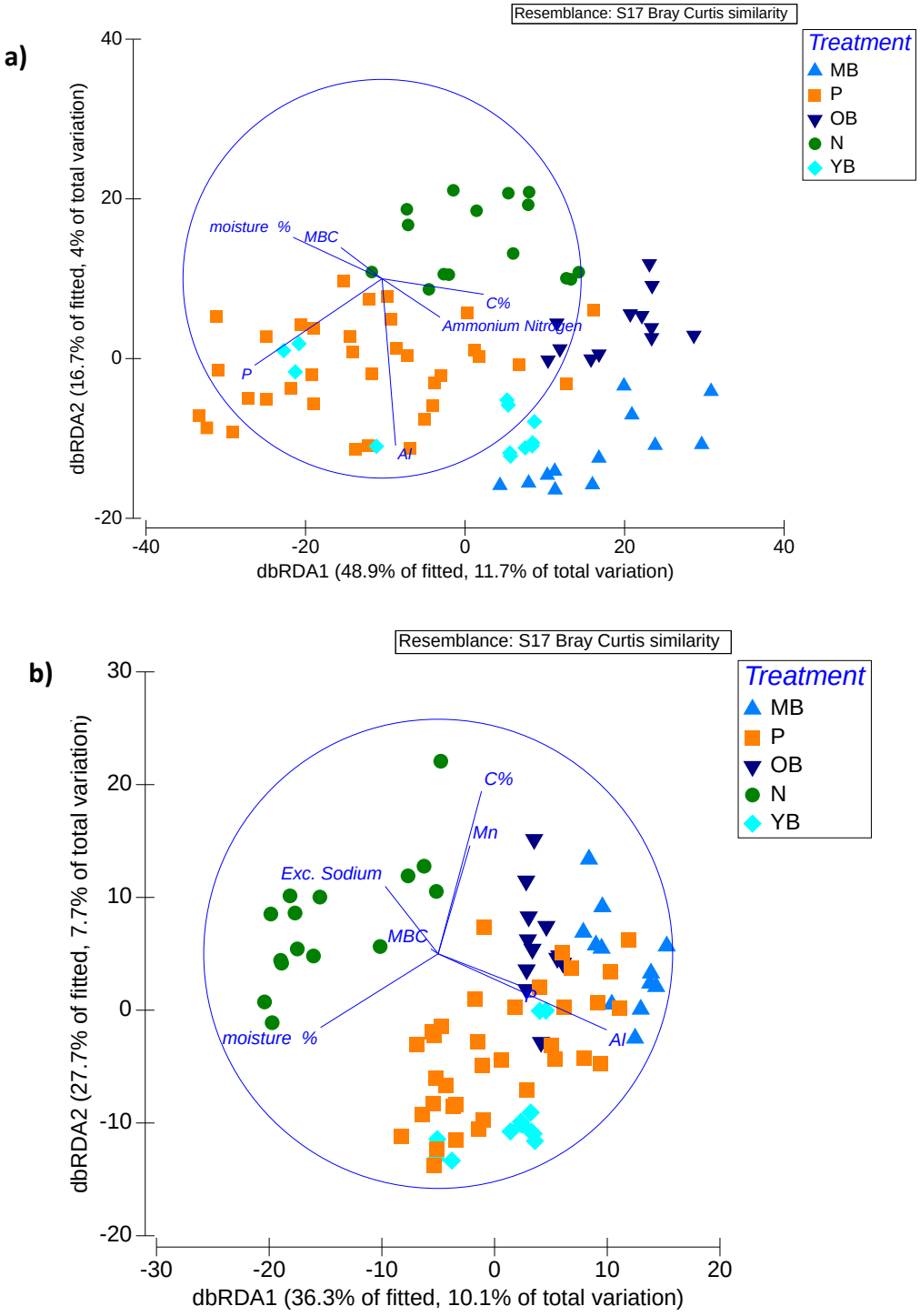


Figure 2. Plot distance-based redundancy analysis (dbRDA) ordination of microbial community structure in ●native remnants, ■ pasture, ♦ young shelterbelts, ▲middle-aged shelterbelts and▼ old shelterbelts. Results were generated from a Bray-Curtis distance matrix where (a) represents fungal community profiles and (b) represents relationships between measured soil edaphic properties and changes in bacterial community profiles. Vectors refer to relationships between dbRDA coordinate axes and predictor variables (soil properties). Only variables with Pearson correlations >0.4 are reported.

Discussion

Our results demonstrate both soil fungal and bacterial community structure differed in restored vegetative shelterbelts compared to pasture sites, and that these community changes increased with shelterbelt age. Despite the fact that time since establishment was between 15-20 years for the oldest shelterbelts in our study, we did not find evidence that microbial communities in older shelterbelts were more similar to those observed in natural remnant patches. Changes in soil edaphic properties under different vegetation types weakly correlated with altered microbial communities.

Soil edaphic properties such as C:N ratio and P significantly differed across land-use types, with C:N highest in the native remnants and lowest in the pasture with P showing opposite trends. Many of the other edaphic factors (Total C, N, EC, S, Mn, Mg, and silt) did not vary consistently with land-use (Table 1, Table 2). Lauber et al. (2008) also found that despite the clear distinctions between land uses (i.e. vegetation type and land-use history) there was a lack of a consistent land-use effect on soil edaphic factors such as C:N ratio, pH, moisture, and bulk density. They attributed this to variability in land management practices (e.g. fertilization and liming) within a given land-use or an inherent variability in soil types across the landscape. This may in part explain some of the variation observed within our study. However, variation due to soil types within our study is limited due to sites being in close proximity, thus the variation may be a result of land management. We were unable to find significant increases in soil carbon up to 15-20 years since establishment. This is consistent with Hoogmoed et al. (2012) who, through a meta-analysis of published data, found no evidence for substantial changes in soil C, N nor the C:N ratio over 30 years of afforestation. This is further supported by Cunningham et al. (2015a) who showed that soil C was slower to accumulate in native mixed species planting, with increases relative to adjacent pastures observed only after 45 years.

In our study we observed a significant difference in the soil C:N ratio between treatments, where C:N ratios were highest under native remnants, lowest under pasture, and shelterbelts had intermediate values. The increase in soil C:N ratio is likely due to larger C inputs (i.e. litter mass), and an increase in the C:N ratio of the litter produced by tree species compared with that of pasture species (Cavagnaro et al., 2016; De Deyn et al., 2008). Soil C:N ratio may directly influence the F:B due to stoichiometric constraints as bacteria are generally considered to require more nitrogen per unit biomass C accumulation than fungi (Bardgett and McAlister, 1999; De Deyn et al., 2008). We found a weak negative correlation between the F:B ratio and

the C:N ratio (F prob 0.04), indicating soil C:N ratio is not the main driver of the F:B ratio in shelterbelt systems.

The MBC:MBN ratio tended to decline with increasing time since establishment and was lowest in native remnant patches. Some studies have attributed a higher MBC:MBN ratio to a greater proportion of fungi rather than bacteria in the soil microbial community (Bolat, 2014; Chen et al., 2015). We were unable to find a correlation between the F:B ratio and MBC:MBN ratio. Fungal and bacterial communities differed in their response to land use change. As illustrated by nMDS (Fig. 1), there was a more distinct change in fungal community composition than bacterial communities within our study. This differentiation can partly be explained by the fact that fungi have been shown to be more susceptible to disturbance, such as land cover change than bacteria due to dispersal limitations (He et al., 2007; Hedlund et al., 2004). For example, Hedlund et al. (2004) noted that less efficient dispersers and colonisers such as mycorrhizal fungi are more susceptible to land use change involving habitat loss.

These changes in fungal and bacterial community composition were observed irrespective of local variation in soils. Site effects (i.e. differences between shelterbelt sites) were also found within our study. However, shelterbelt communities grouped together irrespective of site, which is in contrast to Cavagnaro et al. (2016) who attributed a lack of effect at larger scales to a stronger response of soil microbial PLFAs to local variation in soils. They found no consistent change in the soil microbial PLFA community with reforestation at the level of total biomass or fungal:bacterial ratio, although there were clear changes in microbial communities between reforestation-pasture pairs sampled on the same farm.

We found that differences in fungal communities between shelterbelts and pasture sites increased with time since establishment. Old shelterbelts differed significantly from both pastures and young shelterbelts and were most similar to middle-aged shelterbelts (Table 4). The fungal community in remnant sites appeared to be more variable which can be attributed to increased heterogeneity, both in terms of composition of plant species present and spatial distribution of litter within native sites compared to pastures (Cavagnaro et al., 2016). There was a significant difference in fungal community composition between old shelterbelts and native sites. However, without further detailed analysis of species/functional groups, it is unclear if fungal communities in old shelterbelts are showing trajectories that are converging towards those in native sites.

This slow transition of community structure supports the current understanding that establishment of a diverse, native soil assemblage may be slow following reforestation due to resource availability or abiotic factors (Cunningham et al., 2015b). These factors may differentially affect the dynamics of recovery for fungi and bacterial communities. In Australia, comparisons using DNA- and RNA-based molecular techniques between single sites of native forest and adjoining plantations (*Araucaria cunninghamii* or *Pinus elliotti*), indicated that plantation establishment significantly altered soil fungal community composition (Bastias et al., 2007; He et al., 2005). These changes occurred over time spans of 12-22 years (Bastias et al., 2007; Chapela et al., 2001; Yan et al., 2018) and 30 years (Behera and Sahani, 2003), which are roughly comparable with other indications of 25–30 years for fungal community recovery after disturbance (Hedlund et al., 2004; McLean and Huhta, 2002).

Similar results were observed for bacterial communities: stand age did have some impact on bacterial communities. There was no significant difference in bacterial community composition between young shelterbelts (<5 years) and pastures, however, middle-aged and old shelterbelts were significantly different to pasture communities. Osborne et al. (2011) found that soil bacterial community composition varied significantly in the conversion of woodland to pasture to revegetation within an Australian landscape. While their study only included a single site of each of these types of land use, the results showed that a revegetation plot was more similar in both community composition and soil chemical properties to the undisturbed woodland as compared with the pasture site, despite being returned to native vegetation only 20 years earlier. Results from our study across multiple sites indicate bacterial communities were neither more similar to pasture nor native remnant sites. This may indicate that the legacy of agriculture can have a lasting influence on soil bacterial community structure and that revegetated sites are different to remnant patches and may not provide the same functions as remnant patches, or it may indicate that the native remnant sites used in this study do not represent true natural remnants.

Soil edaphic properties were not strongly correlated to either fungal (24% variation) or bacterial (28% variation) community composition. This is consistent with other studies, which often also report large components of unexplained variation (Banning et al., 2011; Bissett et al., 2011). There was, however, a range of soil edaphic factors important for shaping microbial community structure in shelterbelts, native remnants and pastures (Figure 2, Table 5 and 6). Among the soil edaphic factors we investigated, multivariate analysis suggested that variation in bacterial communities was associated with soil moisture, MBC, total C, P, Mn, Al and Na.

These variables collectively accounted for 28% of the variability in bacterial community composition. The fungal community responded in a similar manner with the soil edaphic factors soil moisture, MBC, total C, NH_4^+-N, P and Al collectively accounting for 24% of the variability. This is contrary to Lauber et al. (2008) and van der Wal et al. (2006) who suggest that that the biogeography of each group would be controlled by separate edaphic factors which may vary among land-uses. It may be that the fungal and bacterial community composition may respond differentially to finer scale variations in edaphic properties such as shifts in carbon pools (de Moraes Sá et al., 2018; Six et al., 2006). Unlike other studies in which pH has been shown to be a dominant factor driving microbial community composition (Fierer and Jackson, 2006), we found no significant effect of pH on soil bacterial communities, probably because the range of soil pH across all sites was small (Macdonald et al., 2009).

Our results demonstrate that soil fungal and bacterial community structure varied significantly between three land cover types. Soil microbial communities differed in restored vegetative shelterbelts compared to pasture sites and these differences become greater with shelterbelt age. We were unable to demonstrate a clear trajectory of change in the microbial communities in shelterbelts towards those observed in nearby natural remnant patches. Changes in individual soil edaphic properties under different vegetation types only weakly correlated with changes in microbial communities. There was, however, a range of soil edaphic factors that were important in shaping microbial community. From the findings of this research it is clear that the legacy of agriculture on soil microbial community structure is still strong even after 15-20 years of establishment of woody vegetation. This further highlights the need to study changes in soil edaphic properties and microbial communities at sites that have been reforested for a longer time.

Acknowledgements

We are grateful to Shamsul Hoque for his assistance with laboratory work. We are thankful to the farmers who allowed access to their property and Greening Australia for access to shelterbelts. This research was supported by CSIRO and the Australian National University.

References

Abdo, Z., Schüette, U.M.E., Bent, S.J., Williams, C.J., Forney, L.J., Joyce, P., 2006. Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. Environmental Microbiology 8, 929-938.

Anderson, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences 58, 626-639.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA + for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth.

Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84, 511-525.

Ball, B.A., Bradford, M.A., Coleman, D.C., Hunter, M.D., 2009. Linkages between below and aboveground communities: Decomposer responses to simulated tree species loss are largely additive. Soil Biology and Biochemistry 41, 1155-1163.

Banning, N.C., Gleeson, D.B., Grigg, A.H., Grant, C.D., Andersen, G.L., Brodie, E.L., Murphy, D., 2011. Soil microbial community successional patterns during forest ecosystem restoration. Applied and Environmental Microbiology 77, 6158-6164.

Bardgett, R.D., McAlister, E., 1999. The measurement of soil fungal: bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils 29, 282-290.

Bardgett, R.D., Shine, A., 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biology and Biochemistry 31, 317-321.

Bardgett, R.D., Wardle, D.A., 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84, 2258-2268.

Barrett, G.W., Freudenberger, D., Drew, A., Stol, J., Nicholls, A., Cawsey, E., 2008. Colonisation of native tree and shrub plantings by woodland birds in an agricultural landscape. Wildlife Research 35, 19-32.

Bastias, B.A., Anderson, I.C., Xu, Z., Cairney, J.W.G., 2007. RNA- and DNA-based profiling of soil fungal communities in a native Australian eucalypt forest and adjacent *Pinus elliotti* plantation. Soil Biology and Biochemistry 39, 3108-3114.

Behera, N., Sahani, U., 2003. Soil microbial biomass and activity in response to Eucalyptus plantation and natural regeneration on tropical soil. Forest Ecology and Management 174, 1-11.

Bird, P., 1998. Tree windbreaks and shelter benefits to pasture in temperate grazing systems. Agroforest Systems 41, 35-54.

Bird, P., Bicknell, D., Bulman, P., Burke, S., Leys, J., Parker, J., Van der Sommen, F., Voller, P., 1993. The role of shelter in Australia for protecting soils, plants and livestock, The Role of Trees in Sustainable Agriculture. Springer, 59-86.

Bissett, A., Richardson, A.E., Baker, G., Thrall, P.H., 2011. Long-term land use effects on soil microbial community structure and function. Applied Soil Ecology 51, 66-78.

Blair, G.J., Chinoim, N., Lefroy, R.D.E., Anderson, G.C., Crocker, G.J., 1991. A soil sulfur test for pastures and crops. Australian Journal of Soil Research 29, 619-626.

Bolat, İ., 2014. The effect of thinning on microbial biomass C, N and basal respiration in black pine forest soils in Mudurnu, Turkey. European Journal of Forest Research 133, 131-139.

Bossio, D., Scow, K., Gunapala, N., Graham, K., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology 36, 1-12.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837-842.

Buyer, J.S., Roberts, D.P., Russek-Cohen, E., 1999. Microbial community structure and function in the spermosphere as affected by soil and seed type. Canadian Journal of Microbiology 45, 138-144.

Carnovale, D., Baker, G., Bissett, A., Thrall, P., 2015. Earthworm composition, diversity and biomass under three land use systems in south-eastern Australia. Applied Soil Ecology 88, 32-40.

Cavagnaro, T.R., 2016. Life at the interface: above- and below-ground responses of a grazed pasture soil to reforestation. Applied Soil Ecology 100, 27-37.

Cavagnaro, T.R., Cunningham, S.C., Fitzpatrick, S., 2016. Pastures to woodlands: changes in soil microbial communities and carbon following reforestation. Applied Soil Ecology 107, 24-32.

Chapela, I.H., Osher, L.J., Horton, T.R., Henn, M.R., 2001. Ectomycorrhizal fungi introduced with exotic pine plantations induce soil carbon depletion. Soil Biology and Biochemistry 33, 1733-1740.

Chen, X.-L., Wang, D., Chen, X., Wang, J., Diao, J.-J., Zhang, J.-y., Guan, Q.-W., 2015. Soil microbial functional diversity and biomass as affected by different thinning intensities in a Chinese fir plantation. Applied Soil Ecology 92, 35-44.

Clarke, K., Gorley, R., 2006. PRIMER v6: user manual/tutorial (Plymouth routines in multivariate ecological research). Plymouth: Primer-E Ltd.

Clarke, K., Warwick, R., 2001. Change in Marine Communities: An approach to statistical analysis and interpretation. PRIMER-E: Plymouth, UK.

Cleugh, H.A., Prinsley, R., Bird, P.R., Brooks, S.J., Carberry, P.S., Crawford, M.C., Jackson, T.T., Meinke, H., Mylius, S.J., Nuberg, I.K., Sudmeyer, R.A., Wright, A.J., 2002. The Australian National Windbreaks Program: overview and summary of results. Australian Journal of Experimental Agriculture 42, 649-664.

Clewell, A., Aronson, J., Winterhalder, K., 2004. The SER international primer on ecological restoration.

Coleman, D.C., 2008. From peds to paradoxes: Linkages between soil biota and their influences on ecological processes. Soil Biology and Biochemistry 40, 271-289.

Colwell, J., 1963. The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales by soil analysis. Australian Journal of Experimental Agriculture 3, 190-197.

Costa, R., Götz, M., Mrotzek, N., Lottmann, J., Berg, G., Smalla, K., 2006. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiology Ecology 56, 236-249.

Culman, S.W., Bukowski, R., Gauch, H.G., Cadillo-Quiroz, H., Buckley, D.H., 2009. T-REX: software for the processing and analysis of T-RFLP data. BMC Bioinformatics 10, 171.

Cunningham, R.B., Lindenmayer, D.B., Crane, M., Michael, D., Macgregor, C., Montague-Drake, R., Fischer, J., 2008. The combined effects of remnant vegetation and tree planting on farmland birds. Conservation Biology 22, 742-752.

Cunningham, S.C., Cavagnaro, T.R., Mac Nally, R., Paul, K.I., Baker, P.J., Beringer, J., Thomson, J.R., Thompson, R.M., 2015a. Reforestation with native mixed-species plantings in a temperate continental climate effectively sequesters and stabilizes carbon within decades. Global Change Biology 21, 1552-1566.

Cunningham, S.C., Mac Nally, R., Baker, P.J., Cavagnaro, T.R., Beringer, J., Thomson, J.R., Thompson, R.M., 2015b. Balancing the environmental benefits of reforestation in agricultural regions. Perspectives in Plant Ecology, Evolution and Systematics 17, 301-317.

da C Jesus, E., Marsh, T.L., Tiedje, J.M., de S Moreira, F.M., 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. The ISME journal 3, 1004-1011.

De Deyn, G.B., Cornelissen, J.H., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters 11, 516-531.

de Moraes Sá, J.C., Potma Gonçalves, D.R., Ferreira, L.A., Mishra, U., Inagaki, T.M., Ferreira Furlan, F.J., Moro, R.S., Floriani, N., Briedis, C., de Oliveira Ferreira, A., 2018. Soil carbon fractions and biological activity based indices can be used to study the impact of land management and ecological successions. Ecological Indicators 84, 96-105.

Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117-4120.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103, 626-631.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113-118.

Gellie, N.J.C., Mills, J.G., Breed, M.F., Lowe, A.J., 2017. Revegetation rewilds the soil bacterial microbiome of an old field. Molecular Ecology 26, 2895-2904.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Applied and Environmental Microbiology 69, 1800-1809.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30, 369-378.

Harris, J., 2009. Soil Microbial communities and restoration ecology: facilitators or followers? Science 325, 573-574.

Harris, J.A., 2003. Measurements of the soil microbial community for estimating the success of restoration. European Journal of Soil Science 54, 801-808.

He, J., Xu, Z., Hughes, J., 2005. Analyses of soil fungal communities in adjacent natural forest and hoop pine plantation ecosystems of subtropical Australia using molecular approaches based on 18S rRNA genes. FEMS Microbiology Letters 247, 91-100.

He, Y., Xu, J., Ma, Z., Wang, H., Wu, Y., 2007. Profiling of PLFA: Implications for nonlinear spatial gradient of PCP degradation in the vicinity of *Lolium perenne* L. roots. Soil Biology and Biochemistry 39, 1121-1129.

Hedlund, K., Griffiths, B., Christensen, S., Scheu, S., Setälä, H., Tscharntke, T., Verhoef, H., 2004. Trophic interactions in changing landscapes: responses of soil food webs. Basic and Applied Ecology 5, 495-503.

Hobbs, P.R., Sayre, K., Gupta, R., 2007. The role of conservation agriculture in sustainable agriculture. Philosophical Transactions of the Royal Society B: Biological Sciences 363, 543-555.

Hobbs, R.J., 1993. Can revegetation assist in the conservation of biodiversity in agricultural areas? Pacific Conservation Biology 1, 29.

Hoogmoed, M., Cunningham, S., Thomson, J., Baker, P., Beringer, J., Cavagnaro, T., 2012. Does afforestation of pastures increase sequestration of soil carbon in Mediterranean climates? Agriculture, Ecosystems and Environment 159, 176-183.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Schmidt, T.M., Coleman, D.C., Whitman, W.B., 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology and Biochemistry 43, 2184-2193.

Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the k EN value. Soil Biology and Biochemistry 28, 33-37.

Kavanagh, R., Law, B., Lemckert, F., Stanton, M., Chidel, M., Brassil, T., Towerton, A., Herring, M., 2005. Biodiversity in eucalypt plantings established to reduce salinity. A report for the RIRDC/Land and Water Australia FWPRDC/MDBC Joint Venture Agroforestry Program. (Rural Industries Research and Development Corporation: Canberra.).

Kuske, C.R., Ticknor, L.O., Miller, M.E., Dunbar, J.M., Davis, J.A., Barns, S.M., Belnap, J., 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Applied and Environmental Microbiology 68, 1854-1863.

Lane, D., 1991. 16S/23S rRNA sequencing. Nucleic acid techniques in bacterial systematics, 125-175.

Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40, 2407-2415.

Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecological Monographs 69, 1-24.

Li, D., Wen, L., Jiang, S., Song, T., Wang, K., 2018. Responses of soil nutrients and microbial communities to three restoration strategies in a karst area, southwest China. Journal of Environmental Management 207, 456-464.

Lindenmayer, D., Hobbs, R., 2004. Fauna conservation in Australian plantation forests–a review. Biological Conservation 119, 151-168.

Macdonald, C.A., Thomas, N., Robinson, L., Tate, K.R., Ross, D.J., Dando, J., Singh, B.K., 2009. Physiological, biochemical and molecular responses of the soil microbial community after afforestation of pastures with *Pinus radiata*. Soil Biology and Biochemistry 41, 1642-1651.

Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural intensification and ecosystem properties. Science 277, 504-509.

McLean, M., Huhta, V., 2002. Microfungal community structure in anthropogenic birch stands in central Finland. Biology and Fertility of Soils 35, 1-12.

Munro, N.T., Fischer, J., Barrett, G., Wood, J., Leavesley, A., Lindenmayer, D.B., 2011. Bird's response to revegetation of different structure and floristics—are “restoration plantings” restoring bird communities? Restoration Ecology 19, 223-235.

Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59, 695-700.

Nüsslein, K., Tiedje, J.M., 1999. Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. Applied and Environmental Microbiology 65, 3622-3626.

Oehl, F., Sieverding, E., Mäder, P., Dubois, D., Ineichen, K., Boller, T., Wiemken, A., 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138, 574-583.

Osborne, C.A., Zwart, A.B., Broadhurst, L.M., Young, A.G., Richardson, A.E., 2011. The influence of sampling strategies and spatial variation on the detected soil bacterial communities under three different land-use types. FEMS Microbiology Ecology 78, 70-79.

Peršoh, D., Borken, W., 2017. Impact of woody debris of different tree species on the microbial activity and community of an underlying organic horizon. Soil Biology and Biochemistry 115, 516-525.

Rayment, G., Higginson, F.R., 1992. Australian laboratory handbook of soil and water chemical methods. Inkata Press Pty Ltd.

Ryan, M., Chilvers, G., Dumaresq, D., 1994. Colonisation of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbour. Plant and Soil 160, 33-40.

Sauer, T., Cambardella, C., Brandle, J., 2007. Soil carbon and tree litter dynamics in a red cedar–scotch pine shelterbelt. Agroforest Syst 71, 163-174.

Singh, B.K., Munro, S., Potts, J.M., Millard, P., 2007a. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Applied Soil Ecology 36, 147-155.

Singh, B.K., Tate, K.R., Kolipaka, G., Hedley, C.B., Macdonald, C.A., Millard, P., Murrell, J.C., 2007b. Effect of afforestation and reforestation of pastures on the activity and population dynamics of methanotrophic bacteria. Applied and Environmental Microbiology 73, 5153-5161.

Six, J., Frey, S., Thiet, R., Batten, K., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555-569.

Smith, C.J., Danilowicz, B.S., Clear, A.K., Costello, F.J., Wilson, B., Meijer, W.G., 2005. T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. FEMS Microbiology Ecology 54, 375-380.

Šnajdr, J., Dobiášová, P., Urbanová, M., Petránková, M., Cajthaml, T., Frouz, J., Baldrian, P., 2013. Dominant trees affect microbial community composition and activity in post-mining afforested soils. Soil Biology and Biochemistry 56, 105-115.

Stark, C.H., Condron, L.M., O'Callaghan, M., Stewart, A., Di, H.J., 2008. Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. Soil Biology and Biochemistry 40, 1352-1363.

Thies, J.E., 2007. Soil microbial community analysis using terminal restriction fragment length polymorphisms. Soil Science Society of America Journal 71, 579-591.

Van Der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296-310.

Van Der Putten, W.H., Bardgett, R.D., De Ruiter, P., Hol, W., Meyer, K., Bezemer, T., Bradford, M., Christensen, S., Eppinga, M., Fukami, T., 2009. Empirical and theoretical challenges in aboveground–belowground ecology. Oecologia 161, 1-14.

van der Wal, A., van Veen, J.A., Smant, W., Boschker, H.T., Bloem, J., Kardol, P., van der Putten, W.H., de Boer, W., 2006. Fungal biomass development in a chronosequence of land abandonment. Soil Biology and Biochemistry 38, 51-60.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703-707.

Vilgalys, R., Hester, M., 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172, 4238-4246.

VSN International, 2011. GenStat for Windows 14th Edition. VSN International, Hemel Hempstead.

Walker, L.R., Wardle, D.A., Bardgett, R.D., Clarkson, B.D., 2010. The use of chronosequences in studies of ecological succession and soil development. Journal of Ecology 98, 725-736.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science 37, 29-38.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629-1633.

Wardle, D.A., Giller, K.E., 1996. The quest for a contemporary ecological dimension to soil biology. Soil Biology and Biochemistry 28, 1549-1554.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18, 315-322.

Wu, J., Joergensen, R., Pommerening, B., Chaussod, R., Brookes, P., 1990. Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. Soil Biology and Biochemistry 22, 1167-1169.

Yan, D., Mills, J.G., Gellie, N.J.C., Bissett, A., Lowe, A.J., Breed, M.F., 2018. High-throughput eDNA monitoring of fungi to track functional recovery in ecological restoration. Biological Conservation 217, 113-120.

Yates, C.J., Hobbs, R.J., 1998. Temperate Eucalypt Woodlands: a review of their status, processes threatening their persistence and techniques for restoration. Australian Journal of Botany 45, 949-973.

Yunusa, I.A., Brown, G.W., Kwong, R.M., Ronnfeldt, G.R., Slater, T., Crouch, A., Unkovich, M., 2002. Integrating biodiversity and productivity on intensive farms: A potential role for shelter-belts in the Victorian Riverina, Agriculture for the Australian Environment. Proceedings of the 2002 Australian Academy of Science Fenner Conference on the Environment'. Canberra. (Eds. B Wilson and A Curtis) pp, pp. 255-277.

Paper III: Plant genus (*Acacia* and *Eucalyptus*) alters soil microbial community structure and abundance within revegetated shelterbelts

After establishing changes in community composition under shelterbelts, the research described in **Paper III** was conducted to determine if potential drivers such as plant genus affect the ecological dynamics of soil microbial communities in shelterbelts in south-eastern Australia. In this paper I investigated the effects of two commonly used overstory genera (*Acacia* and *Eucalyptus*) on soil microbial community structure compared with those under adjacent pastures. This research was conducted to obtain insight to potential drivers of change in soil microbial communities.

Carnovale, D., Bissett, A., Thrall, P. H., Baker, G. (2019). Plant genus (*Acacia* and *Eucalyptus*) alters soil microbial community structure and relative abundance within revegetated shelterbelts. Applied Soil Ecology, 133, 1-11.

Abstract

The composition of native woody vegetation communities in restored agricultural landscapes can alter plant-soil interactions and thus ecological function. This study investigated the effects of primary species groups (*Acacia*, *Eucalyptus*) used in shelterbelts in south-eastern Australia on soil microbial community structure compared with those under adjacent pastures. The ratio and structure of bacterial and fungal communities was analysed using quantitative PCR (qPCR) and terminal restriction fragment length polymorphism (T-RFLP). The fungal:bacterial ratio (copy number) was highest under *Eucalyptus* trees but did not differ between *Acacia* and pasture. Both fungal and bacterial communities varied at different depths in the soil profile and across sampling periods. The dominant tree genus was a significant factor in observed differences in bulk soil bacterial and fungal communities compared to the surrounding pasture. The effect of tree type on fungal communities was more pronounced than on bacterial communities. Fungal communities not only differed between shelterbelts and pastures, but also between *Acacia* and *Eucalyptus* dominated shelterbelts. These patterns were consistently observed at different soil depths. Shelterbelt systems which contain a mixture of different plant genera have the potential to exhibit greater microbial community heterogeneity than single species plantations. Maintaining a variety of tree species during afforestation may therefore contribute to conserving important bacterial and fungal diversity in agricultural landscapes.

Key words: *Acacia, Eucalyptus,* shelterbelts, soil microbial communities, T-RFLP, agricultural restoration

Introduction

In south-eastern Australia, agricultural areas have often been subject to restoration processes such as the establishment of shelterbelts (strips of planted native trees and shrubs) to restore altered ecosystem services (Cleugh et al., 2002; Hobbs, 1993) or to reduce land degradation (e.g. soil erosion) (Bird, 1998; Bird et al., 1993). The establishment of shelterbelts contributes to the conservation of biodiversity, in particular for mammals, birds and amphibians (Lindenmayer and Hobbs, 2004). Along with biodiversity and other benefits, conversion from agriculture to forest systems affects soil physical and chemical properties. This is particularly well studied for soil carbon storage (Hoogmoed et al., 2012; Paul et al., 2018; Paul et al., 2002; Paul et al., 2015). Much less attention has been given to how soil bacterial and fungal

community structure and function are influenced by the identity of the tree species used, or the restoration process itself (Cavagnaro et al., 2016). However, understanding the key drivers that influence microbial community composition is an important element of managing the effects of land use change.

Interactions between above and belowground elements of ecosystems has received increasing attention as awareness grows of their importance in driving ecosystem processes (Bardgett and Wardle, 2010; Bardgett et al., 1998; Wardle et al., 2004). One area of particular interest is the effect of plant community structure on soil microbial community structure and function (Prescott and Grayston, 2013). Tree species that occupy a site may alter the rate of fundamental soil processes, such as nutrient cycling and carbon dynamics, through differences in plant microbial associations, amount and quality of leaf and root litter, and root exudates (Bauhus et al., 1998; Hobbie, 1992). These differences can control the composition and function of soil communities (Bardgett and Wardle, 2010) and more specifically microbial communities (Zak et al., 2003). For example, variation in microbial communities in bulk forest floor samples can be related to different associated tree species. These differences are largely due to physio-chemical properties of plant litter from different tree types (Ndaw et al., 2009; Schutter and Dick, 2001) as is clearly illustrated for comparisons between conifers and broadleaved species (Bauhus et al., 1998; Gartzia-Bengoetxea et al., 2016; Hagen-Thorn et al., 2004; Jiang et al., 2010; Thoms et al., 2010). For example, Jiang et al., (2010) found increased catabolic diversity (CLPP, community level physiological profiles) and distinct soil bacterial and fungal communities in soils underneath broadleaf and mixed species plantations compared with those below conifers in the top 0-10 cm. In support, distinct bacterial and fungal community profiles in soil (0–5, 10–20 cm depth) in pure plots of spruce, Douglas fir, oak and beech in France were observed by Lejon et al. (2005). Further to this, several other studies have found that the largest observed effects of tree species on soil chemical properties is found within the topsoil, corresponding to the highest amount of organic matter input (Augusto et al., 2003; Hagen-Thorn et al., 2004; Thoms et al., 2010).

Much of the previous research examining effects of plant species on microbial communities has focused on comparisons between conifer and broadleaf species. In Australia, two of the most widespread and ecologically important genera are *Acacia* and *Eucalyptus*, which form dominant components of many ecosystems. Importantly, these groups are also widely used in land reclamation and restoration activities, including in the establishment of

shelterbelts in agricultural landscapes. The quality of *Acacia* and *Eucalyptus* litter varies greatly (Hoogmoed et al., 2014b). *Eucalyptus* litter tends to have low nitrogen and high phenolic content, while *Acacia* tends to have higher nitrogen concentrations (Hoogmoed et al., 2014a). These differences, along with associated dominant symbiotic relationships, i.e. *Acacia* sp. with rhizobial N-fixing bacteria (Burdon et al., 1999; Thrall et al., 2000) and *Eucalyptus* spp. with mycorrhizal fungi (Warcup, 1980) may foster different microbial communities within the bulk soil and thus impact on ecosystem function.

Disentangling the effect of different plant genus on soil microbial communities is further complicated by microbial interactions with soil edaphic properties. Soil edaphic properties such as pH, texture, organic matter content and carbon:nitrogen (C:N) ratio have demonstrated a strong relationship between soil microbial community diversity and structure at various landscape scales (Bissett et al., 2010; Brockett et al., 2012; Fierer and Jackson, 2006; Fierer et al., 2009; Rousk et al., 2010; Siciliano et al., 2014).

In order to better understand plant-soil interactions within restored agricultural landscapes, we investigated the effects of dominant plant type on soil microbial communities using shelterbelts containing *Acacia* and *Eucalyptus* trees adjacent to grazed pastures. We hypothesised that: 1) soil microbial communities would differ under different tree types, with an increase in the fungal:bacterial ratio under *Eucalyptus* relative to *Acacia* due to dominant symbiotic relationships; 2) soil microbial communities and soil edaphic properties at different depths under each tree genus would also differ, and that; 3) changes in microbial community composition would correspond to changes in soil chemical properties driven by differences in plant type.

Methods

Study sites

The study site chosen was located on agricultural land dominated by sheep grazing near Murrumbateman in New South Wales, Australia (34°57′0″S, 149°01′0″E). The soils at the site are described as Chromosol, yellow, eutrophic (Australian Soil Classification: order, sub-order, great-group; Isbell, 2016).

Two shelterbelts (rows of planted native trees and shrubs sown in 4-5 rows) were established in 1995 by property owners, one dominated by *Acacia* species, the other by

Eucalyptus species. The shelterbelts were approximately 100 metres apart. The shelterbelts and adjacent pastures were sampled for soil microbial communities in October 2011 and April 2012. The *Eucalyptus* shelterbelt was comprised mostly of *E. macarthurii, E. blakelyi, E. viminalis* and *E. melliodora*. The *Acacia* shelterbelt included *A. mearnsii, A. baileyana, A. decurrens, A. cardiophylla* and *A. cultriformis*. The two shelterbelts ran parallel to each other and were approximately 100m apart.

Soil sampling

Within each of the shelterbelts, the soil under six trees was sampled at 10 metre intervals along transects (*Acacia* spp. n=6 trees, *Eucalyptus* spp. n=6). Directly under each sampled tree, at four compass points equidistant from the trunk (50 cm out), soil cores (30cm deep x 5cm wide) were taken in the mineral soil. Soil for each individual tree was then bulked by soil depth, 0-10 cm, 10-20 cm and 20-30 cm (Fig. S1). In 2012 all six trees were sampled at 10-20 cm and 20-30 cm (6 composite samples per tree genus per soil depth). However, in 2011 only three trees of each genus were sampled at 10-20 cm and 20-30 cm due to resource constraints (six composite samples per tree for 0-10cm and three composite samples per tree genus for 10-20 and 20-30 cm soil depth). In 2011 we also sampled between tree rows. Two inter-row samples were collected per soil depth interval in the open space next to each sampled tree (Fig. S1). Three trees of each genus were sampled at 10-20 cm and 20-30 cm in the inter row. The two samples per depth interval were bulked, resulting in one composite inter row sample per depth per tree. Within the pastures transects were laid out parallel to, but approximately 15 metres away from, each shelterbelt. Soil cores were taken in the mineral soil at the same depths as in shelterbelts. A total of three pasture composite samples was taken per depth interval in 2011 and six composite samples were taken per depth interval in 2012.

Soil analysis

After the removal of a subsample for molecular analysis the remainder of the bulked soil samples were air-dried. Soil moisture content was determined gravimetrically by oven drying 10 g subsamples at 105°C until weight became stable. Analysis on soil properties such as pH and electrical conductivity (EC), total carbon (C), total nitrogen (N) particle size (sand, silt and clay), organic C, nitrate N (NO_3-N), ammonium N (NH_4^+-N), sulphur (S), phosphorus (P), potassium (K), exchangeable cations (Ca, Mg, Na, K, Al) and trace elements (Cu, Fe, Mn Zn) were conducted. A detailed description of the analysis methods is presented in Carnovale *et al*. (2015) and **Paper I** in this thesis.

DNA extraction

The 111 individual soil composite samples (57 samples for 2011 and 54 samples for 2012) were frozen at -20° C until analysis (~1 year). Total soil DNA was extracted from 0.25 g sub-samples using the Power Soil DNA Extraction Kit (MoBio Laboratories, Carlsbad, California, USA), with the following modification to manufacturer's instructions: samples were shaken in a Bio101 Savant Bead Beater on the maximum speed for 2 minutes instead of vortexing (Bissett et al., 2011). The quality of the PCR product was assessed on an agarose gel and DNA quantity was determined using PicoGreen dsDNA Quantitation Reagent (Invitrogen). This DNA was used as a template for T-RFLP and qPCR analyses of bacterial and fungal communities.

Real time qPCR for fungi and bacteria abundance

The abundances of bacterial 16S and fungal ITS rRNA genes were determined using quantitative real time PCR (qPCR) as described in Fierer et al. (2005). Primers used for bacterial ITS were Eub338 (Lane, 1991) and Eub518 (Muyzer et al., 1993) and for fungal ITS primers were 5.8s and ITS1F. The qPCR assays were performed in triplicate 10µl reactions containing 5 µl SsoAdvancedTM SYBR® green 246 Supermix (Bio-Rad, Hercules, CA), 0.4µl of forward and reverse primers (10µM), 2.2µl H_2O and 2µl template DNA. Quantification of bacterial 16S rRNA genes was carried out under the following conditions: 95ºC for 2 minutes followed by 95ºC for 10s, 53ºC for 30s and 72ºC for 20s. Fungal quantification was as follows: 95ºC for 1 minutes followed by 95ºC for 5s, 53ºC for 20s and 72ºC for 20s. Forty cycles were run for bacteria and fungi followed by a melting curve to confirm the amplified products were of the appropriate size. For each plate a standard curve was run with a 10-fold dilution series from 10^9 copies to 10^4 copies. Standards were prepared from PCR product generated using the primers above (from *Pseudomonas fluorescens* strain 5 and *Fusarium oxysporum vasinfectum*) and purified using the Agencourt PCR purification system. Standards were quantified using Picogreen (Invitrogen, Grand Island, NY, USA) and amplification efficiencies were>90% and r^2 values were>0.99 for bacterial and fungal calibration curves.

T-RFLP

Bacterial and fungal soil communities were analysed using terminal-restriction fragment length polymorphism (T-RFLP) (Thies, 2007). Amplification of bacterial 16S rRNA genes was performed using the 6-carboxyfluorescein (FAM)-labelled primer FAM27f (5′-*AGTTTGATCMTGGCTCAG*-3′) and primer BAC519r (5′-*GWATTACCGCGGCKGCTG* -3′).

Fungal internal transcribed spacer (ITS) region was amplified from soil DNA using fungal primers: 6-carboxyfluorescein (FAM)-labelled primer FAMITS1-F (5′-*CTTGGTCATTTAGAGGAAGTAA*-3′) (Gardes and Bruns, 1993) and ITS4 (5-*TCCTCCGCTTATTGATATGC*-3′) (White et al., 1990). 50µL PCR reactions contained 2ng of extracted DNA, 1U of MyTaq DNA Polymerase (bioline) and 1x supplied buffer, and 0.5µM of each of the primers. Fungi PCR mix was as above with the addition of 0.5µg BSA (Promega). Bacterial PCRs were run at 94°C for 5 min, 40 cycles of 94°C for 30s, 58°C for 30s and 72°C for 45 seconds followed by a final extension step at 72°C for 7 minutes. We confirmed amplification in a 1% agarose gel. The PCR product was then purified using the Agencourt AMPure XP system according to the manufacturer's instructions. Approximately 25ng of the FAM-labeled PCR products were digested with the restriction endonucleases *HinfI*, in a volume of 25µL that included 5U of *HinfI* and the supplied buffer at 1X. Restriction digests were incubated at 37 C for 4 hours. Digested products were isopropanol precipitated, resuspended in 10µL of formamide containing LIZ600 size standard (Applied Biosystems), then denatured at 95°C for 2 min. Profiles of T-RFLP were generated by separating the fragments on an ABI 3130xl Genetic Analyser (Applied Biosystems, UK). Terminal-restriction fragments (T-RFs) generated by the sequencer were exported and initially analysed for absolute peak areas using GeneMapper™ 4.0 (Applied Biosystems, UK). First, T-RF profiles were checked for stable baselines, voltage and calibrations and profiles were trimmed to between 50 and 600bp to eliminate T-RFs caused by primer-dimers while retaining fragments within the linear range of the internal size standard. Data were then uploaded to the online T-RFLP analysis software package T-REX (http://trex.biohpc.org/;(Culman et al., 2009)). Noise filtering was carried out by removing peaks whose area was less than twice the standard deviation computed over all peaks (Abdo et al., 2006). T-RFs were aligned where peaks with values less than the threshold (1 base pair) were identified and grouped into a T-RF (Smith et al., 2005). The relative abundance of a detected T-RF for a sample was calculated as the signal area of each peak divided by the total peak area of all the peaks of the T-RFLP profile for that sample. For statistical analysis, the relative abundance of a detected T-RF within a given T-RFLP profile was used.

Statistical analysis

An analysis comparing soil properties and microbial communities under trees compared to inter row areas was conducted on the 2011 data using two-way ANOVA and PERMANOVA. Initial analyses showed that inter row soil samples were not significantly different to those taken

from under trees (Table S1). Thus, inter row and under-tree data were combined for the 10-20cm and 20-30cm depths for 2011 in order to keep replicate numbers the same across sampling years. The data for the 0-10cm samples were disregarded. This resulted in equal numbers of replicates between sampling years (n=6 samples per depth interval per genus). Statistical analysis followed a factorial design [site type (*Acacia*, *Eucalyptus*, pasture)*soil, depth*year] for all analyses where possible. For soil edaphic properties data were log transformed to achieve homogeneity of variances and normality prior to analysis when necessary. The data were then subjected to ANOVA to test for significant differences using the factorial design above, followed by Tukey HSD post-hoc tests for differences between groups. All statistical tests were performed at p=0.05. Statistical analysis was conducted using GenStat software 16th edition package (VSN International, 2011).

Multivariate analysis was used to assess differences in microbial community composition and edaphic properties. Relative abundances of TRFs identified were used to produce Bray Curtis similarity matrices based on samples without the need for any transformations or normalisations. Edaphic data were normalised and, by using Euclidean distances, distance matrices were calculated. Some environmental variables required transformation prior to analysis as indicated from a draftsman plot (in these cases data were log transformed). ANOSIM and PERMANOVA (Anderson, 2001) were used to test the significance of site type, depth and sampling year on differences in community structure. General differences between soil microbial communities were visualised using non metric multidimensional scaling (nMDS) and canonical analysis of principle coordinates (CAP) (Anderson and Willis, 2003) computed from the similarity matrices.

Differences in bacterial and fungal community composition and how these related to predictor environmental edaphic variables were determined from Distance Based Linear Models (DistLM) (Legendre and Anderson, 1999). Co-varying edaphic variables (r^2>0.75) were identified and subsequently only one variable was run in the DistLM. Results were interpreted such that this single variable represented the behaviour of all variables with which it was found to co-vary. To identify variables explaining significant amounts of variation in bacterial and fungal communities the stepwise model selection procedure (Anderson et al., 2008) was applied. To select the predictor variables a corrected Akaike Information Criterion (AICc) was applied. The statistical significance and percentage contribution of each soil edaphic variable alone were determined by conducting marginal tests (Anderson and Cribble,

1998; Legendre and Anderson, 1999; McArdle and Anderson, 2001). For visualization of the DistLM results, distance-based redundancy *analysis* (*dbRDA*) was applied. Multivariate tests were conducted using PRIMER 6 (Clarke and Gorley, 2006; Clarke and Warwick, 2001) and PERMANOVA + (Anderson et al., 2008).

Results

Soil edaphic properties

Site type (*Acacia, Eucalyptus,* pasture) was significant for all soil edaphic properties except C:N ratio and Ca (Table S2). Soil depth alone was also significant for all soil properties, with soil chemical properties such as total C, N, P declined and C:N ratio and pH increased with increasing soil depth across all site types. Interaction effects of site type*depth and site type*year showed more variability in relation to interactions with measured soil properties (Table S2). Pairwise comparisons (Table 1) showed a significant difference between *Acacia* and *Eucalyptus* soil within the top 0-10cm for C, P, K, Cu, Fe, Zn, Al, Mg, Na, NO_3 and S. No significant difference was observed between *Acacia* and *Eucalyptus* soils at 0-10cm for total N and C (p>0.05), however the C:N ratio was significantly lower under N-fixing *Acacia* (12:2) compared to *Eucalyptus* (13:1). The pasture soils had a significantly lower C:N ratio (11:2) than either *Acacia* or *Eucalyptus*. Below 10cm effects of *Acacia* compared to *Eucalyptus* were reduced for most major soil edaphic properties (Fig. 1). PCA analysis showed a shift primarily along PC1 in many soil edaphic properties including pH, which separated the top 0-10cm soils from samples deeper in the soil profile (Fig. 1).

Table 1. Soil edaphic properties under A=*Acacia*, E= *Eucalyptus* and P=Pasture at three depth intervals (0-10, 10-20 20-30cm). Means followed by different letters indicate significant differences according to ANOVA and subsequent Tukey's test ($p<0.05$) within each depth interval across years.

	Depth (cm)	***Total N*** *%*	***Total C*** *%*	***C:N ratio***	***P*** (mg kg^{-1})	***K*** (mg kg^{-1})	***EC*** (mS)	***pH*** ***(H_2O)***	***Moisture %***	***Clay %***	***Sand %***	***Silt %***
A	***0-10***	0.18_{a}	2.17_{b}	12.2_{b}	6_{a}	119_{a}	40.5_{b}	4.85_{a}	14.2_{a}	12.44_{a}	65.69_{a}	21.86_{b}
E		0.15_{a}	2.01_{ab}	13.1_{c}	7_{b}	179_{b}	40.72_{b}	5.18 b	13.33_{a}	15.24_{b}	67.42_{b}	17.3_{a}
P		0.15_{a}	1.66_{a}	11.2_{a}	5.75_{a}	181.5_{b}	28.48_{a}	5.41_{b}	16.27_{a}	12.75_{a}	65.86_{a}	21.38_{b}
A	***10-20***	0.05_{a}	0.57_{ab}	13.2_{a}	2.75_{a}	74.5_{a}	20.34_{a}	5.18_{a}	8.91_{a}	13.35_{b}	68.10_{b}	18.55_{b}
E		0.04_{a}	0.59_{b}	13.8_{a}	3_{a}	114.5_{b}	18.61_{a}	5.37_{a}	9.69_{a}	12.16_{a}	71.57_{c}	16.28_{a}
P		0.04_{a}	0.47_{a}	15.2_{a}	2.73_{a}	119.2_{b}	18.13_{a}	5.79_{b}	13.38_{b}	14.21_{c}	59.8_{a}	25.94_{c}
A	***20-30***	0.02_{a}	0.34_{ab}	18.5_{a}	1.9_{a}	60.8_{a}	13.08_{a}	5.69_{a}	8.79_{a}	13.13_{c}	64.56_{a}	22.31c
E		0.02_{a}	0.37_{b}	20.9_{a}	1.9_{a}	80.2_{b}	15.09_{a}	5.75_{a}	8.66_{a}	11.53_{a}	67.42_{b}	21.05_{b}
P		0.02_{a}	0.25_{a}	18.8_{a}	1.9_{a}	99.8_{c}	15.76_{a}	6.05_{a}	15.72_{b}	12.04_{b}	69.71_{c}	18.26_{a}
	Depth (cm)	***Cu*** (mg kg^{-1})	***Fe*** (mg kg^{-1})	***Mn*** (mg kg^{-1})	***Zn*** (mg kg^{-1})	***Al*** ($cmol_c$ kg^{-1})	***Ca*** ($cmol_c$ kg^{-1})	***Mg*** ($cmol_c$ kg^{-1})	***Na*** ($cmol_c$ kg^{-1})	***NH4+*** (mg kg^{-1})	***NO3-*** (mg kg^{-1})	***S*** (mg kg^{-1})
A	***0-10***	1.13_{b}	248.9_{c}	100.61_{c}	1.51_{b}	0.65_{b}	1.67_{a}	0.5_{b}	0.06_{a}	10_{a}	6_{c}	7.6_{c}
E		0.81_{a}	225_{b}	96.03_{b}	1.8_{c}	0.36_{a}	1.63_{a}	0.79_{c}	0.07_{b}	9_{a}	2_{a}	6.7_{b}
P		1.32_{b}	200.1_{a}	70.68_{a}	1.35_{a}	0.35_{a}	1.68_{a}	0.39_{a}	0.05_{a}	11.75_{a}	3_{b}	5.55_{a}
A	***10-20***	0.88_{b}	102.1_{b}	60.71_{b}	0.60_{b}	0.35_{b}	1.07_{b}	0.27_{b}	0.07_{c}	7_{b}	1.18_{b}	3.9_{b}
E		0.74_{a}	95_{b}	74.74_{c}	0.54_{b}	0.25_{ab}	1.01_{ab}	0.43_{c}	0.06_{b}	5_{a}	0.9_{a}	3.58_{b}
P		1.03_{c}	74.2_{a}	31.34_{a}	0.31_{a}	0.27_{a}	0.82_{a}	0.16_{a}	0.04_{a}	6_{ab}	1_{ab}	2.98_{a}
A	***20-30***	0.69_{a}	36.4_{b}	24.68_{a}	0.25_{b}	0.13_{c}	1.29_{a}	0.40_{b}	0.07_{b}	3.75_{b}	0.9_{a}	2.33_{a}
E		0.68_{a}	37.5_{b}	24.88_{a}	0.45_{c}	0.10_{b}	1.19_{a}	0.45_{c}	0.09_{c}	2.5_{a}	0.9_{a}	2.45_{b}
P		1.36_{b}	21.1_{a}	21.9_{a}	0.13_{a}	0.08_{a}	1.18_{a}	0.24_{a}	0.04_{a}	3_{b}	0.9_{a}	2.43_{b}

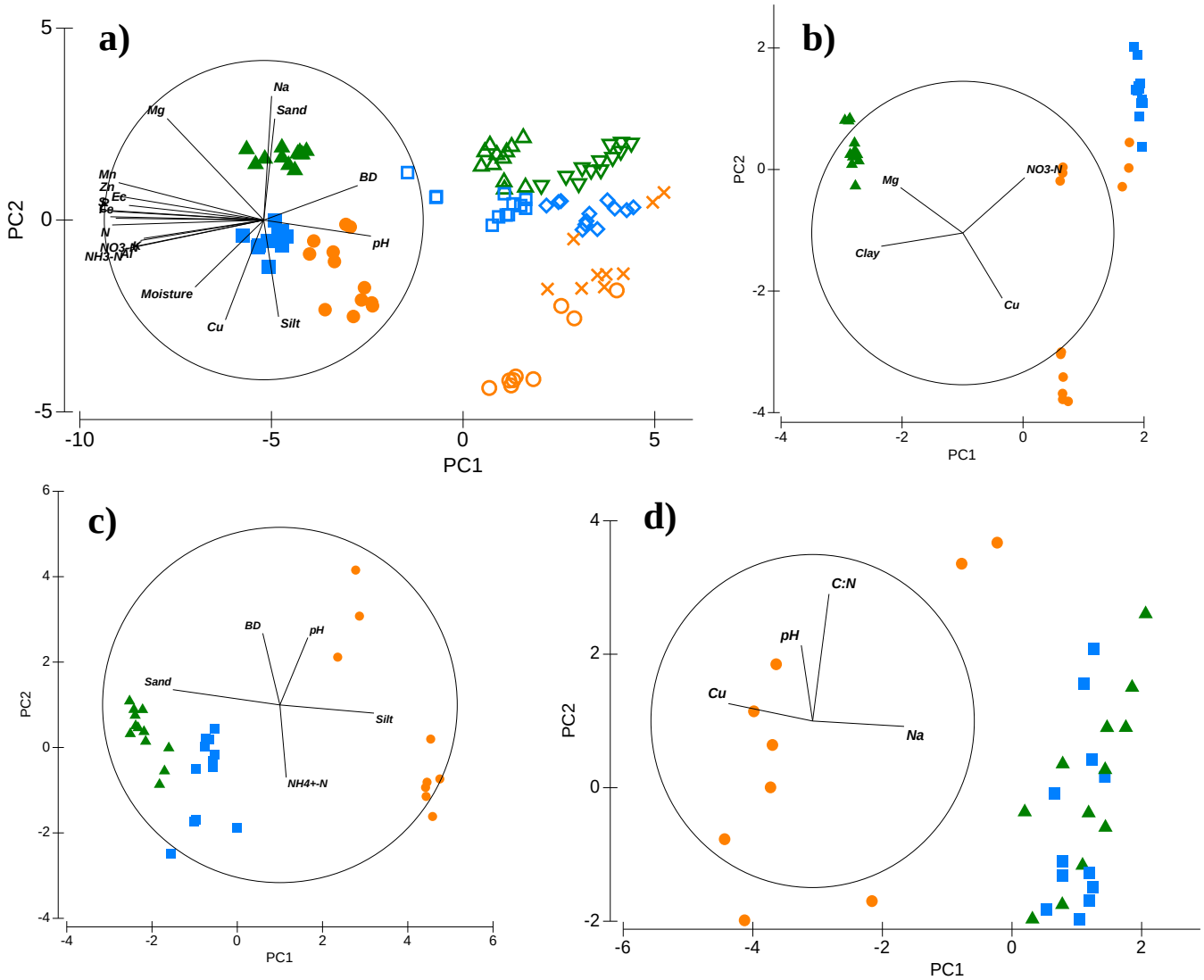


Figure 1. PCA soil physio-chemical data showing: a) site type×depth for all soil depths - colours refer to site type, green=*Acacia*, blue=*Eucalyptus*, orange=pasture; filled symbols =0-10cm depth; hollow symbols = 10-30cm depths; b) 0-10cm, ● pasture, ■ *Acacia*, ▲ *Eucalyptus*; c)10-20cm, ● pasture, ■ Acacia, ▲ *Eucalyptus*; d) 20-30cm. ● pasture, ■ *Acacia*, ▲ *Eucalyptus*. Correlations of soil edaphic variables with PCA axes for correlations>0.4. BD= Bulk density.

Fungal:bacteria ratio

Fungal:bacteria (F:B) ratios were primarily influenced by site type (p=0.002) and year (p<0.001) (Fig.2). The F:B ratio was highest under *Eucalyptus* trees but did not differ between *Acacia* and pasture (Fig. 2). Across years, the F:B ratios were overall higher in 2011 than in 2012.

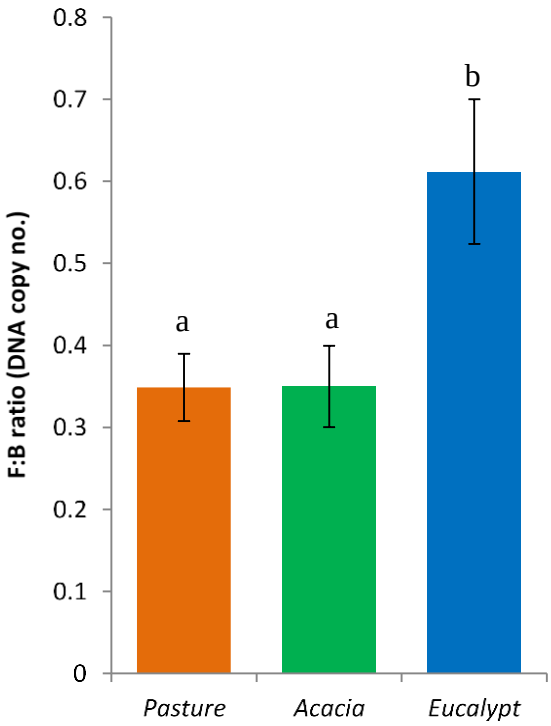


Figure 2. Fungal:bacterial gene copy ratio +/-SE determined by qPCR by site type across all soil depths for combined sampling times. The letters above the bars indicate a significant difference based on Tukey's test ($p<0.05$).

Bacterial and fungal T-RFLP community profiles

The composition of fungal and bacterial communities as determined by T-RFLP differed significantly between site types ($p=0.001$), soil depth (fungi: $p=0.002$ fungi; bacteria: $p=0.001$) and year ($p=0.001$) (Table 2, Fig. S2). To investigate treatment effects further, bacterial and fungal community profiles were analysed separately for each sampling year (Table 3) as bacterial and fungal community data indicated treatment*year effects (Table 2). Fungal communities under all treatments differed from each other across both sampling years (2011 and 2012: $p=0.001$) (Table 3, Fig. 3). Differences between fungal communities were also influenced by depth*site type interactions where in each depth profile fungal community, composition between site type differed (Table 3, Fig. 3).

There were no significant differences in the composition of soil bacterial communities under *Acacia* versus *Eucalyptus* within either sampling year (Table 3). However, in 2012, bacterial community composition under *Acacia* and *Eucalyptus* trees differed to those in the pasture (Table 4); and there was a significant interaction between depth and year for bacterial

communities (Fig. 4). Bacterial community composition between soil depths varied within each year, as well as within the same soil depth across years.

Table 2. PERMANOVA testing microbial community structure in response to site type (*Acacia*, *Eucalypt*, pasture), soil depth (0-10cm, 10-20cm, 20-30cm) and sampling year (2011, 2012). *p*-values less than 0.05 are in bold. MC= Monte Carlo *p*.

	Fungi			**Bacteria**		
	Pseudo f	*p*	*p*(MC)	Pseudo f	*p*	*p*(MC)
Site type	14.95	0.001	**0.001**	2.58	0.001	**0.001**
Depth	2.23	0.001	**0.002**	19.1	0.001	**0.001**
Year	3.28	0.001	**0.001**	10.9	0.001	**0.001**
Site type x depth	1.30	0.045	**0.053**	0.99	0.502	0.513
Site type x year	1.52	0.03	**0.039**	2.14	0.002	**0.002**
Depth x year	0.91	0.624	0.622	2.16	0.001	**0.002**
Site type x depth x year	0.85	0.807	0.793	0.77	0.902	0.884

Table 3. ANOSIM for pair-wise tests of time*site type (fungi and bacteria), depth*site type (for fungi) and depth*year (for bacteria) combinations. Tests were performed between site types for each time and within each site type for times, between each site type within each soil depth and between each soil depth within each year and within each soil depth for each year. A=*Acacia*, E=*Eucalyptus*, P=pasture.

	Fungi		**Bacteria**	
Pairwise Tests	**R Statistic**	*p*	**R Statistic**	*p*
Between Site types				
2011				
A, E	0.483	0.001	0.015	0.30
A, P	0.849	0.001	0.091	0.06
E, P	0.753	0.001	0.027	0.24
2012				
A, E	0.276	0.001	0.066	0.066
A, P	0.623	0.001	0.1	0.21
E, P	0.802	0.001	0.148	0.013
Within Site types				
A 2011, A 2012	0.114	0.01	0.198	0.005
E 2011, E 2012	0.042	0.14	0.332	0.001
P 2011, P 2012	0.059	0.15	0.304	0.002
Depth*site type				
0-10				
A, E	0.389	0.002		
A, P	0.785	0.001		
E, P	0.759	0.001		
10-20				
A, E	0.353	0.001		
A, P	0.829	0.001		
E, P	0.826	0.001		
20-30				
A, E	0.385	0.001		
A, P	0.484	0.001		
E, P	0.933	0.001		
Depth Year				
2011				
0-10, 10-20			0.642	0.001
0-10, 20-30			0.84	0.001
10-20, 20-30			0.277	0.002
2012				
0-10, 10-20			0.449	0.001
0-10, 20-30			0.759	0.001
10-20, 20-30			0.101	0.013
2011-2012				
0-10-0-10			0.174	0.001
10-20-10-20			0.402	0.001
20-30-20-30			0.503	0.001

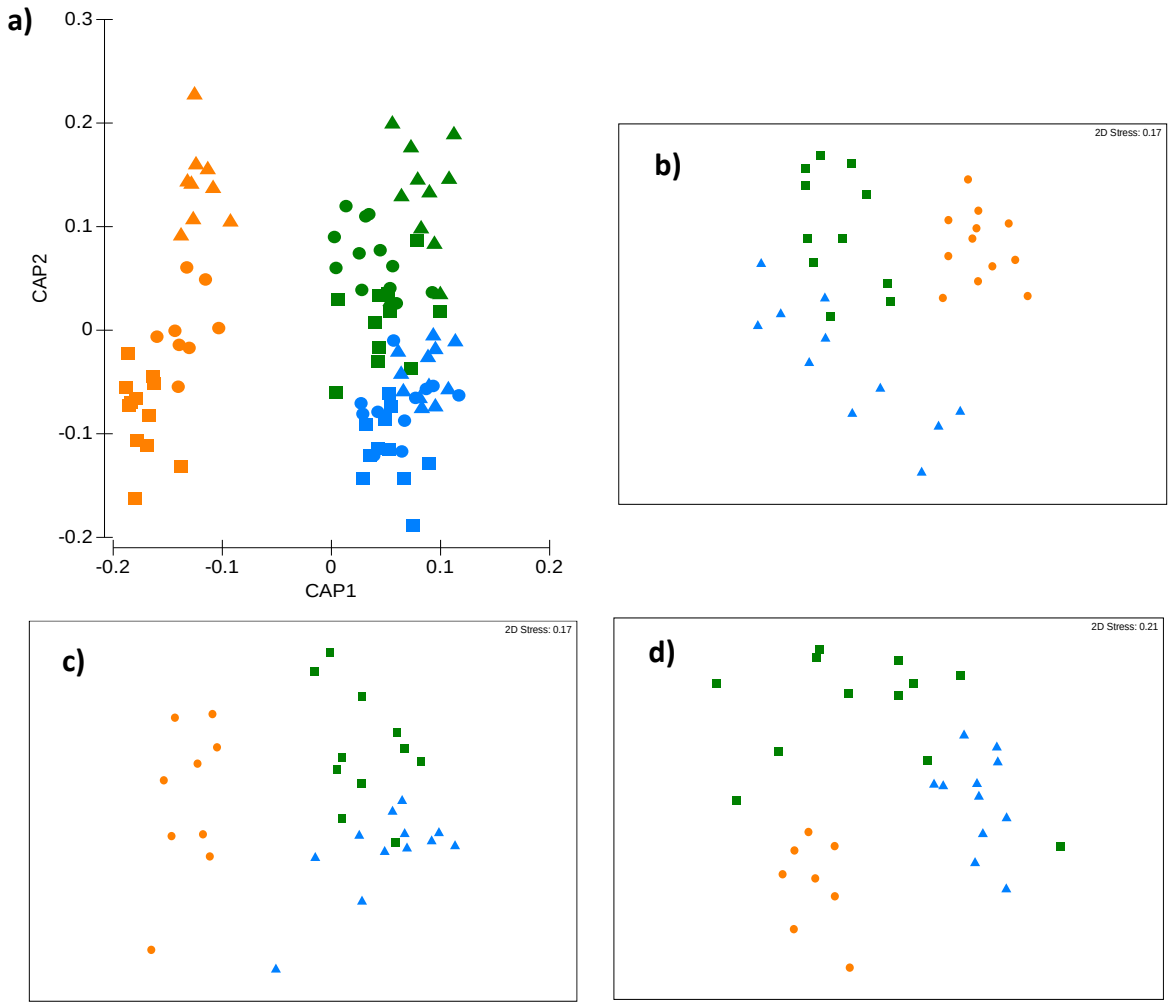


Figure 3. a) CAP fungal community by site type and soil depth for combined sampling times, colours refer to site type, green = *Acacia*, blue = *Eucalyptus*, orange= Pasture, symbols refer to depth (■ 0-10, ▲ 10-20, ● 20-30). **b)** nMDS based on Bray Curtis similarity measure of fungi community composition at 0-10cm, **c)** 10 -20cm, **d)** 20-30 cm (● pasture, ■ *Acacia*, ▲ *Eucalyptus*). Points closest to each other in the nMDS plots indicate community compositions with a higher similarity.

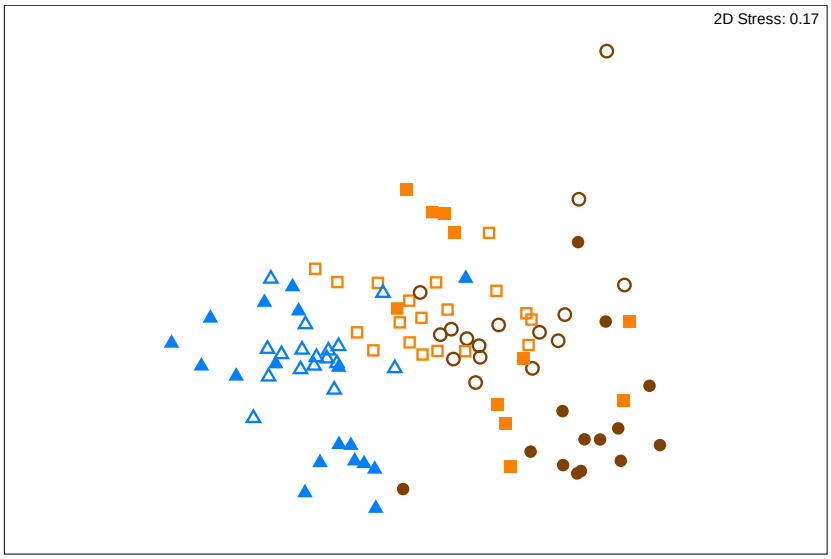


Figure 4. nMDS analysis based on Bray Curtis similarity measure of bacteria by soil depths for site types combined (■ 0-10cm ▲ 10-20cm, ● 20-30cm), and year. Hollow symbols = 2011 sampling period; filled symbols = 2012 sampling period.

Soil edaphic properties and bacterial and fungal communities

The relationships between soil bacterial and fungal communities were assessed using DistLM and visualised with dbRDA. Co-varying variables (r>0.75) removed included total N, P, NO_3-N, NH_4^+-N, Fe, Mn, Zn, Al and EC. Marginal tests indicated that Cu (8.03%, p=0.001), Na (7.62%, p=0.001), Mg (6.23%, p=0.001) strongly correlated to fungal community composition on their own (Table 4). In contrast, total C (21.45%, p=0.001), K (14.11%, p=0.001), pH (14.06 %, p=0.001), Mg (7.86%, p=0.001) and C:N ratio (7.51%, p=0.001) most strongly correlated to bacterial community composition (Table 5). Using AICc and a stepwise procedure for fungi, five soil physiochemical variables (Cu, silt, K total C, pH and Mg) accounted for 26% of the variation in fungal community composition. Fungal communities primarily separated out by Cu and silt. These variables also differentiated pasture communities from those under *Acacia* and *Eucalyptus* (Fig. 5). For bacteria, six soil physiochemical variables (total C, pH, moisture, bulk density, clay and Mg) accounted for 30% of the variation in community composition (Fig. 5). Bacterial community composition primarily separated out by total C and pH which differentiated communities in the 0-10 cm layer from those at depths >10cm.

Table 4. Tests for relationships between the community structure of fungi and soil physio-chemical variables, using the non-parametric multivariate multiple regression analysis (DistLM). On the left are the marginal tests of individual sets. On the right are the partial (conditional) tests, where amount explained by each variable added to model is conditional on variables already in the model. *p*-values less than 0.05 are in bold (%Var: the percentage of the multivariate variance in the community structure explained by that variable; %Cumul: cumulative percentage of variance explained).

	Marginal test			Sequential tests				
Variable	Pseudo-F	*p*	% Var	Variable	Pseudo-F	*p*	% Var	% Cumul.
pH	3.58	**0.001**	Cu	cu	8.29	**0.001**	8.03	8.03
BD	2.16	**0.013**	2.22	silt	5.08	**0.001**	4.72	12.74
Moisture	3.83	**0.001**	3.87	k	4.12	**0.001**	3.71	16.45
C%	2.14	**0.024**	2.20	C%	5.87	**0.001**	5.02	21.46
K	4.94	**0.001**	4.94	pH	2.85	**0.003**	2.39	23.85
Clay	1.77	**0.046**	1.83					
Silt	4.57	**0.001**	4.59					
Na	7.83	**0.001**	7.62					
Mg	6.31	**0.001**	6.23					
Ca	1.50	0.089	1.56					
Cu	8.29	**0.001**	8.03					
C:N ratio	1.37	0.167	1.43					
Best solution	AICc =748.68, R^2= 0.2385, 5 variables (Cu, Silt, K, total C, and pH)							

Table 5. Tests for relationships between the community structure of bacteria and soil physio-chemical variables, using the non-parametric multivariate multiple regression analysis (DistLM). On the left are the marginal tests of individual sets, on the right are the partial (conditional) tests, where amount explained by each variable added to model is conditional on variables already in the model. *p*-values less than 0.05 are in bold (%Var: the percentage of the multivariate variance in the community structure explained by that variable; %Cumul: cumulative percentage of variance explained).

	Marginal test			***Sequential tests***				
Variable	Pseudo-F	*p*	%Var	Variable	Pseudo-F	*p*	Prop.	% Cumul.
pH	15.22	**0.001**	14.06	C	27.05	**0.001**	22.53	22.53
Bd	9.21	**0.001**	9.01	pH	5.01	**0.001**	4.00	26.53
Moisture	4.20	**0.004**	4.32	BD	2.74	**0.001**	2.15	28.68
C	27.05	**0.001**	22.53	Mg	2.33	**0.006**	1.80	30.48
C:N ratio	7.55	**0.001**	7.51	moisture	2.62	**0.002**	1.99	32.47
K	15.28	**0.001**	14.11	clay	2.27	**0.013**	1.70	34.16
Cu	3.88	**0.002**	4.00					
Ca	4.43	**0.002**	4.55					
Mg	7.94	**0.001**	7.86					
Clay	4.09	**0.002**	4.21					
Na	1.45	0.138	1.54					
Silt	0.86	0.549	0.92					
Best solution	AICc and Cu	645.14 R^2	0.3416	6 variables = Total C, pH, bulk density, Mg, soil Moisture				

BD=bulk density

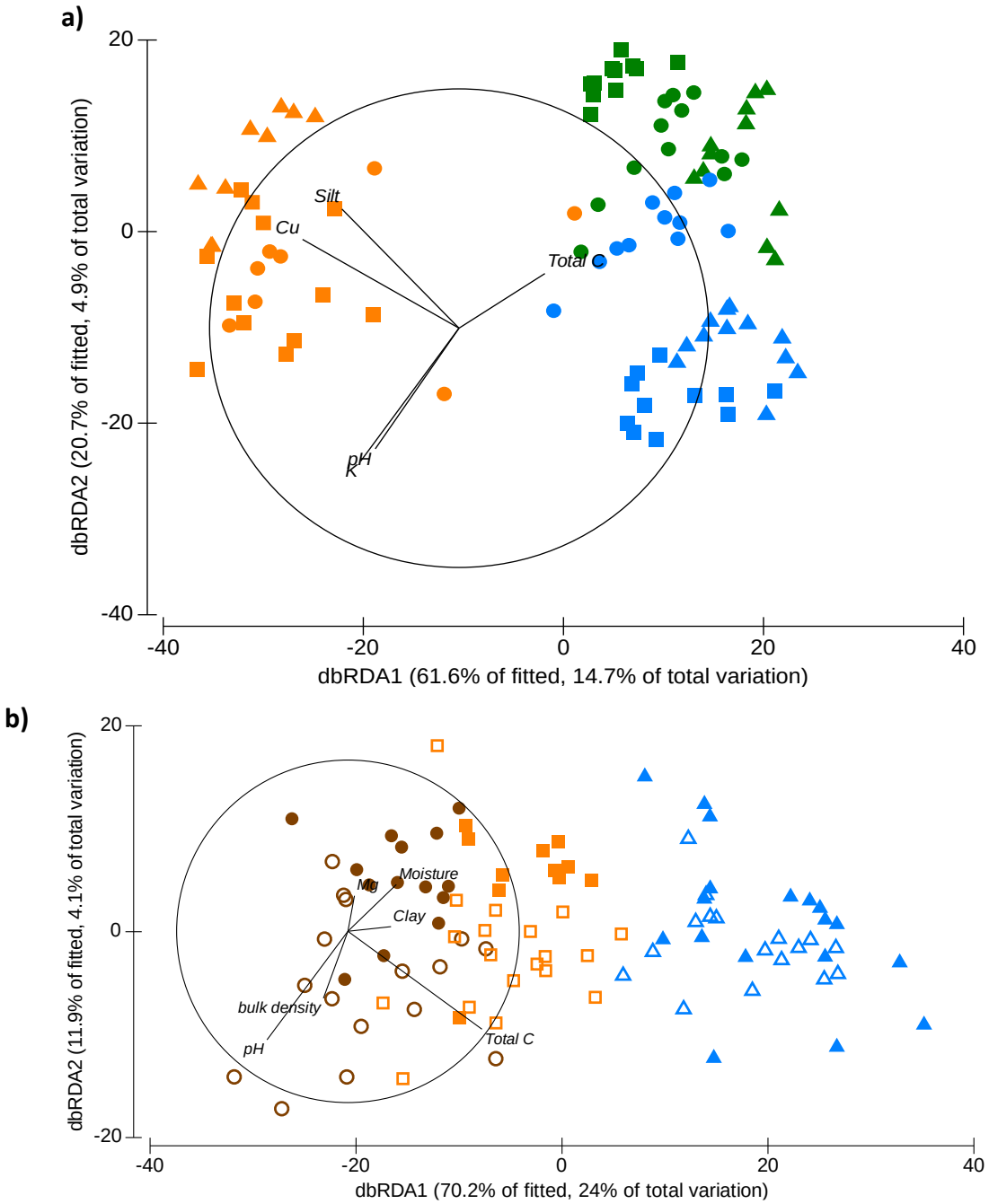


Figure 5. Plot of dbRDA ordination of: **a)** fungal structure by site type and depth for combined sampling times; colours refer to site type, green = *Acacia*, blue = *Eucalyptus*, orange= Pasture; symbols refer to depth, ■ 0-10, ▲ 10-20, ● 20-30. **b)** measured soil physio-chemical properties and changes in bacterial community by depth and year depths for site types combined, ■ 0-10, ▲ 10-20, ● 20-30; hollow symbols=2011, filled symbols=2012. Only variables with correlations >0.4 are reported.

Discussion

Overall, our results demonstrate that microbial community composition can be significantly influenced by the presence of different dominant tree genera in revegetated shelterbelt systems, and that these communities in turn differ from those occurring in adjacent pastures. However, the effect of tree genus on fungal communities was more pronounced than on bacterial communities. Our original hypothesis that F:B ratios would be higher under *Eucalyptus* than under *Acacia* was supported. Plant type effects within shelterbelts were most dominant within the first 0-10cm of the soil profile, after which microbial communities were influenced to a greater extent by edaphic properties. This is consistent with other studies showing that organic inputs and plant effects are greatest in the upper soil stratum (Hoogmoed et al., 2012; Li et al., 2018).

With regard to revegetation using woody plants, soil development has been shown to be largely influenced by the dominant tree species which includes plant inputs such as the quantity and quality of the litter (Šnajdr et al., 2013). For example, a meta-analysis by Paul et al. (2002) quantifying changes in soil C under large-scale afforestation or reforestation found that soil C stocks were greatly affected by land use, climate and the type of forest established. Thus, when softwood plantations (e.g. *Pinus radiata)* were established on previously improved pastoral land in Australian temperate regions, most soil C was lost in comparison to the establishment of deciduous hardwoods, or N_2-fixing species (either as an understorey or as a plantation). They found that on average within the 0-10cm soil profile soil C generally decreased relative to the initial soil C content during the first 5 years. This decline was followed by soil C recovery over about 30 years to levels found in agricultural soils. In our study, we detected no significant differences in total N, K or C between pasture and *Eucalyptus* despite 17 years since establishment. This may indicate that the decline in soil properties may have reached its peak and nutrients are starting to recover. Mendham et al. (2003) and O'Connell et al. (2003) also found soil organic C and N were not significantly influenced by afforestation 7-11 years after plantation establishment in south-western Australia.

We were also unable to detect significant differences in total N and C levels between *Acacia* and *Eucalyptus* stands. There was, however, a trend of increasing N, NO_3-N and NH_4^+-N under *Acacia* and a slight increase in C, resulting in a significantly lower C:N ratio in the top 0-10cm under *Acacia* as compared to *Eucalyptus*. This may be indicative of higher N concentrations in *Acacia* litter and increased N in root exudates from legumes (Warembourg et

al., 2003) resulting in increased available soil N (Hoogmoed et al., 2014a). It is also possible that lower C:N ratio under *Acacia* (N-fixers) may favour bacterial over fungal decomposers (Fierer et al., 2009). Such a shift towards bacterial dominance may in turn slow the rate of decomposition of organic matter and increase soil carbon sequestration rates (Hoogmoed et al., 2014a).

The increase in the F:B ratio observed under *Eucalyptus* shelterbelts compared to pasture soil is similar to observed higher F:B ratios in forest soils when compared to agricultural or grassland soils (Bailey et al., 2002; Bossuyt et al., 2001; Högberg et al., 2007; Treseder, 2004). Forest systems generally have litter which is relatively more recalcitrant than the litter in pastures and grassland systems, leading to an increased abundance of fungi which can more readily break down low quality substrates (Six et al., 2006). Six et al. (2006) showed that fungal-dominated soils resulted in slower C turnover rates because fungi incorporate more C into biomass than bacteria. This incorporation of C into fungi cell walls also facilitates C stabilisation (Busse et al., 2009), while bacteria in general require more N than fungi per unit biomass C accumulation (De Deyn et al., 2008). The higher available NO_3-N and NH_4^+-N under *Acacia* may thus support higher bacterial abundance contributing to lower F:B ratios relative to *Eucalyptus.* Fungal to bacterial gene ratios have been shown to be significantly correlated to soil C:N ratio and pH at the field scale (Fierer et al., 2009; Rousk et al., 2010). However, at finer spatial scales (i.e. plot based shelterbelts) we were unable to link variation in the F:B gene ratio with any soil edaphic variables, including the C:N ratio.

Plant species that vary in eco-physiological traits (e.g. biochemistry, metabolism, structure and function, nutrient and biomass allocation) will exert an influence on soil biota through differential rhizo-deposition and litter quality (Bardgett et al., 1999). For example, legumes are known to produce root exudates rich in N (Warembourg et al., 2003) thus increasing available N in the soil. N rich systems have been shown to decrease fungal abundance (de Vries et al., 2012), with several recent studies comparing the influence of N-fixing legumes versus other functional plant groups on soil microbial communities (Bini et al., 2013; Hoogmoed et al., 2014a; Milcu et al., 2013; Strecker et al., 2015). Our data support the idea that under functionally different dominant tree genera within a revegetated shelterbelt system, there are changes in the bulk soil fungal and bacterial communities compared to the surrounding pasture; although in our study *Acacia* and *Eucalyptus* were more similar to one another than to pastures.

The responses to different plant types in shelterbelts relative to the pasture system were more pronounced in the fungal community. This may possibly be attributed to bacteria being

generally less adapted to decompose recalcitrant litter (e.g. from *Acacia* and *Eucalyptus*) than fungi (Henriksen and Breland, 1999; Six et al., 2006; Van Der Heijden et al., 2008). As a consequence, the effect of plant type on soil fungal communities may be greater, particularly within the organic and first few centimetres of the mineral soil. A higher C:N ratio under *Eucalyptus* may also promote fungal species capable of degrading low quality substrates such as phenolic compounds (Six et al., 2006; Swift, 1982). The less pronounced changes in bacterial communities we observed may also indicate resistance to land use change within the bacterial community due to their rapid life cycles (Vries et al., 2012).

Like Thoms et al. (2010), our sampling scheme to a depth of 30cm offered further insight into the soil layers that may be to a greater degree altered by the active rooting zone in shelterbelt systems. The most notable changes in soil properties were within the 0-10cm layer. It has been shown that the principal factor governing composition of the microbial community across soil profiles is resource availability (Fierer et al., 2003), which is mainly characterised by decreasing C concentration and a reduction in C quality with increasing soil depth (Richter and Markewitz, 1995). Decreases in C with depth and the rise in C: N ratio within our study across site types is consistent with this, and as a result we observed a significant effect of site type and depth on fungi (Table 3, Fig. 3). These conclusions are consistent with the study of Lejon et al. (2005) which showed that fungal communities up to a depth of 30 cm could be separated out by plant genus. Our results indicate that discrimination of bacteria communities under each plant genus between soil depths was not as robust beyond a depth of 10 cm, where deeper samples were more similar to each other than to communities in the 0-10 cm samples.

Temporal shifts in microbial community composition may arise due to variation in soil temperature and moisture (Cao et al., 2010; Moore-Kucera and Dick, 2008; Myers et al., 2001). Not surprisingly, soil moisture was lower under shelterbelt trees than in pastures. This may be partly because the presence of *Acacia* and *Eucalyptus* species have been related to high water repellence in soils (Crockford et al., 1991; Doerr et al., 1998). Stand density may also influence soil moisture in these systems where high aboveground biomass exerts a large demand for water (Bréda et al., 1995) and as such we observed reduced soil moisture with increasing depth compared to within pasture systems. The soil surface is likely to experience wider variation in soil temperatures and moisture (daily and seasonally) than soils found at greater depth (Brady and Weil, 2002). However, we found that between sampling years, soil moisture availability did not differ with soil depth with soils <10cm deep experiencing no greater variation in soil moisture than those deeper down. This is also reflected in the fact that we found no statistical

differences in fungal communities between sampling years within each soil depth. Contrary to this, bacterial community composition varied considerably between sampling periods within soil depths, indicating that factors other than environmental conditions may influence bacterial communities in these shelterbelt systems.

The effect of plant genus is further complicated as several other studies (Fierer et al., 2009; Girvan et al., 2003; Singh et al., 2008) have suggested that soil characteristics are the most important factors shaping microbial community structure. Unlike other studies which have shown that pH plays a significant role in explaining variation in soil microbial communities at various spatial scales from distances <1m to field and continental scales (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010), in our study pH did not show the highest correlation with community composition (accounting for 3.6% and 14% of the variation in fungal and bacterial community composition respectively). There was, however, a range of soil physiochemical factors important for shaping microbial community structure under *Acacia*, *Eucalyptus* and pastures (Fig. 5). Among the soil physiochemical factors we investigated, multivariate analysis of data suggested that variation in bacterial communities was associated with total C (including co-varying variables such as total N, NO_3-N and NH_4^+-N, and P). These variables collectively accounted for 21.5% of the variability in bacterial community composition. The fungal community responded to different physiochemical factors with copper explaining the greatest precent of the variation (8.03%); soil moisture had little influence on fungal communities.

Conclusion

The results of this study, comparing revegetated shelterbelt and pasture sites under the same environmental conditions, indicate that plant genus can strongly influence fungal and, to a lesser extent, bacterial community composition. Changes in fungal soil communities were seen at different depths under *Acacia* and *Eucalyptus*. Within these shelterbelt systems, the effect of tree genus on soil microbial communities was stronger than that of individual soil properties. Overall, the effect of plant genus on soil fungal and bacterial communities highlights the importance of mixed genus plantings as well as which species are included in revegetation mixtures. These can lead to increased heterogeneity within shelterbelt systems compared to pastures which may be vital for ecosystem functions such as nutrient cycling and decomposition.

Acknowledgements

We are grateful to the land owners who allowed access to their property. We also thank Greening Australia for access to shelterbelt data. We would like to thank Tim Goodwin and James Tyrrell for their assistance with field sampling. We thank the anonymous reviewer for their constructive comments and feedback. This work was supported by CSIRO Agriculture and Food and the Fenner School of Environment and Society (The Australian National University).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.apsoil.2018.09.001.

References

Abdo, Z., Schüette, U.M.E., Bent, S.J., Williams, C.J., Forney, L.J., Joyce, P., 2006. Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. Applied and Environmental Microbiology 8, 929–938.

Anderson, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Can. J. Fish. Aquatic Sciences 58, 626–639.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA + for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth.

Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84, 511–525.

Augusto, L., Dupouey, J.-L., Ranger, J., 2003. Effects of tree species on understory vegetation and environmental conditions in temperate forests. Ann. Forest Scince 60, 823–831.

Bailey, V.L., Peacock, A.D., Smith, J.L., Bolton Jr, H., 2002. Relationships between soil microbial biomass determined by chloroform fumigation–extraction, substrate induced respiration, and phospholipid fatty acid analysis. Soil Biology and Biochemistry 34, 1385–1389.

Bardgett, R.D., Shine, A., 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biology and Biochemistry 31, 317–321.

Bardgett, R.D., Wardle, D.A., 2010. Aboveground-belowground Linkages: Biotic Interactions, Ecosystem Processes, and Global Change. Oxford University Press, Oxford.

Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and belowground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry 30, 1867–1878.

Bauhus, J., Paré, D., Côté, L., 1998. Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biology and Biochemistry 30, 1077–1089.

Bini, D., Santos, C.A.d., Bouillet, J.-P., Gonçalves, J.L.d.M., Cardoso, E.J.B.N., 2013. *Eucalyptus grandis* and *Acacia mangium* in monoculture and intercropped plantations: Evolution of soil and litter microbial and chemical attributes during early stages of plant development. Applied Soil Ecology. 63, 57–66.

Bird, P., 1998. Tree windbreaks and shelter benefits to pasture in temperate grazing systems. Agroforestry Systems 41, 35–54.

Bird, P., Bicknell, D., Bulman, P., Burke, S., Leys, J., Parker, J., Van der Sommen, F., Voller, P., 1993. The role of shelter in Australia for protecting soils, plants and livestock. In: The Role of Trees in Sustainable Agriculture. Springer, pp. 59–86.

Bissett, A., Richardson, A., Baker, G., Wakelin, S., Thrall, P., 2010. Life history determines biogeographical patterns of soil bacterial communities over multiple spatial scales. Molecular Ecology 19, 4315–4327.

Bissett, A., Richardson, A.E., Baker, G., Thrall, P.H., 2011. Long-term land use effects on soil microbial community structure and function. Applied Soil Ecology 51, 66–78.

Bossuyt, H., Denef, K., Six, J., Frey, S., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16, 195–208.

Brady, N., Weil, R., 2002. The Nature and Proprieties of Soils. Prentice Hall, New Jersey, USA.

Bréda, N., Granier, A., Aussenac, G., 1995. Effects of thinning on soil and tree water relations, transpiration and growth in an oak forest (*Quercus petraea* (Matt.) Liebl.). Tree Physiology 15, 295–306.

Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven bio-geoclimatic zones in western Canada. Soil Biology and Biochemistry 44, 9–20.

Burdon, J., Gibson, A., Searle, S.D., Woods, M., Brockwell, J., 1999. Variation in the effectiveness of symbiotic associations between native rhizobia and temperate Australian Acacia: within-species interactions. Journal of Applied Ecology. 36, 398–408.

Busse, M.D., Sanchez, F.G., Ratcliff, A.W., Butnor, J.R., Carter, E.A., Powers, R.F., 2009.

Soil carbon sequestration and changes in fungal and bacterial biomass following incorporation of forest residues. Soil Biology and Biochemistry 41 (2), 220–227.

Cao, Y., Fu, S., Zou, X., Cao, H., Shao, Y., Zhou, L., 2010. Soil microbial community composition under Eucalyptus plantations of different age in subtropical China. European Journal of Soil Biology 46, 128–135.

Carnovale, D., Baker, G., Bissett, A., Thrall, P., 2015. Earthworm composition, diversity and biomass under three land use systems in south-eastern Australia. Applied Soil Ecology. 88, 32–40.

Cavagnaro, T.R., Cunningham, S.C., Fitzpatrick, S., 2016. Pastures to woodlands: changes in soil microbial communities and carbon following reforestation. Applied Soil Ecology. 107, 24–32.

Clarke, K., Gorley, R., 2006. PRIMER v6: User Manual/Tutorial (Plymouth Routines in Multivariate Ecological Research). Primer-E Ltd, Plymouth.

Clarke, K., Warwick, R., 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. PRIMER-E, Plymouth, UK.

Cleugh, H.A., Prinsley, R., Bird, P.R., Brooks, S.J., Carberry, P.S., Crawford, M.C., Jackson, T.T., Meinke, H., Mylius, S.J., Nuberg, I.K., Sudmeyer, R.A., Wright, A.J., 2002. The Australian National Windbreaks Program: overview and summary of results. Australian Journal of Experimental Agriculture 42, 649–664.

Crockford, H., Topalidis, S., Richardson, D., 1991. Water repellency in a dry sclerophyll eucalypt forest—measurements and processes. Hydrological Processes 5, 405–420.

Culman, S.W., Bukowski, R., Gauch, H.G., Cadillo-Quiroz, H., Buckley, D.H., 2009. T-REX: software for the processing and analysis of T-RFLP data. BMC Bioinformatics 10, 171.

De Deyn, G.B., Cornelissen, J.H., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters 11, 516–531.

de Vries, F.T., Manning, P., Tallowin, J.R., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., Hobbs, P.J., Quirk, H., Shipley, B., Cornelissen, J.H., 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters 15, 1230–1239.

Doerr, S.H., Shakesby, R.A., Walsh, R.P., 1998. Spatial variability of soil hydrophobicity in fire-prone eucalyptus and pine forests, Portugal. Soil Sci. 163, 313–324.

Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Applied and Environmental Microbiology. 71, 4117–4120.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. PNAS 103, 626–631.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35, 167–176.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecology Letters. 12, 1238–1249.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.

Gartzia-Bengoetxea, N., Kandeler, E., Martínez de Arano, I., Arias-González, A., 2016. Soil microbial functional activity is governed by a combination of tree species composi- tion and soil properties in temperate forests. Applied Soil Ecology. 100, 57–64.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl. Applied and Environmental Microbiology. 69, 1800–1809.

Hagen-Thorn, A., Callesen, I., Armolaitis, K., Nihlgård, B., 2004. The impact of six European tree species on the chemistry of mineral topsoil in forest plantations on former agricultural land. For. Ecol. Manage. 195, 373–384.

Henriksen, T.M., Breland, T.A., 1999. Decomposition of crop residues in the field: evaluation of a simulation model developed from microcosm studies. Soil Biology and Biochemistry 31, 1423–1434.

Hobbie, S.E., 1992. Effects of plant species on nutrient cycling. Trends Ecol. Evol. 7, 336–339.

Hobbs, R.J., 1993. Can revegetation assist in the conservation of biodiversity in agricultural areas? Pacific Conservation Biology 1, 29.

Högberg, M.N., Högberg, P., Myrold, D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590–601.

Hoogmoed, M., Cunningham, S., Thomson, J., Baker, P., Beringer, J., Cavagnaro, T., 2012. Does afforestation of pastures increase sequestration of soil carbon in Mediterranean climates? Agric. Ecosyst. Environ. 159, 176–183.

Hoogmoed, M., Cunningham, S.C., Baker, P., Beringer, J., Cavagnaro, T.R., 2014a. N- fixing trees in restoration plantings: effects on nitrogen supply and soil microbial communities. Soil Biology and Biochemistry 77, 203–212.

Hoogmoed, M., Cunningham, S.C., Baker, P.J., Beringer, J., Cavagnaro, T.R., 2014b. Is there more soil carbon under nitrogen-fixing trees than under non-nitrogen-fixing trees in mixed-species restoration plantings? Agric. Ecosyst. Environ. 188, 80–84.

Isbell, R., 2016. The Australian Soil Classification. CSIRO Publishing.

Jiang, Y.M., Chen, C.R., Liu, Y.Q., Xu, Z.H., 2010. Soil soluble organic carbon and ni trogen pools under mono-and mixed species forest ecosystems in subtropical China. J. Soils Sediments 10, 1071–1081.

Lane, D., 1991. 16S/23S rRNA Sequencing. Nucleic Acid Techniques in Bacterial Systematics, 125–175.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Applied and Environmental Microbiology. 75, 5111–5120.

Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69, 1–24.

Lejon, D.P., Chaussod, R., Ranger, J., Ranjard, L., 2005. Microbial community structure and density under different tree species in an acid forest soil (Morvan, France). Microb. Ecol. 50, 614–625.

Li, J., Tong, X., Awasthi, M.K., Wu, F., Ha, S., Ma, J., Sun, X., He, C., 2018. Dynamics of soil microbial biomass and enzyme activities along a chronosequence of desertified land revegetation. Ecol. Eng. 111, 22–30.

Lindenmayer, D., Hobbs, R., 2004. Fauna conservation in Australian plantation forests–a review. Biol. Conserv. 119, 151–168.

Mendham, D.S., O'Connell, A.M., Grove, T.S., 2003. Change in soil carbon after land clearing or afforestation in highly weathered lateritic and sandy soils of south-western Australia. Agric. Ecosyst. Environ. 95, 143–156.

Milcu, A., Allan, E., Roscher, C., Jenkins, T., Meyer, S.T., Flynn, D., Bessler, H., Buscot, F., Engels, C., Gubsch, M., 2013. Functionally and phylogenetically diverse plant communities key to soil biota. Ecology 94, 1878–1885.

Moore-Kucera, J., Dick, R.P., 2008. PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microb. Ecol. 55, 500–511.

Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Applied and Environmental Microbiology. 59, 695–700.

Myers, R.T., Zak, D.R., White, D.C., Peacock, A., 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Sci. Soc. Am. J. 65, 359–367.

Ndaw, S., Gama-Rodrigues, A., Gama-Rodrigues, E., Sales, K., Rosado, A., 2009.Relationships between bacterial diversity, microbial biomass, and litter quality in soils under different plant covers in northern Rio de Janeiro State, Brazil. Can. J. Microbiol. 55, 1089–1095.

O'connell, A., Grove, T., Mendham, D., Rance, S., 2003. Changes in soil N status and N supply rates in agricultural land afforested with eucalypts in south-western Australia. Soil Biology and Biochemistry 35, 1527–1536.

Paul, K.I., England, J.R., Baker, T.G., Cunningham, S.C., Perring, M.P., Polglase, P.J., Wilson, B., Cavagnaro, T.R., Lewis, T., Read, Z., Madhavan, D.B., Herrmann, T., 2018. Using measured stocks of biomass and litter carbon to constrain modelled estimates of sequestration of soil organic carbon under contrasting mixed-species environ- mental plantings. Sci. Total Environ. 615, 348–359.

Paul, K.I., Polglase, P.J., Nyakuengama, J.G., Khanna, P.K., 2002. Change in soil carbon following afforestation. For. Ecol. Manage. 168, 241–257.

Paul, K.I., Roxburgh, S.H., England, J.R., de Ligt, R., Larmour, J.S., Brooksbank, K., Murphy, S., Ritson, P., Hobbs, T., Lewis, T., Preece, N.D., Cunningham, S.C., Read, Z., Clifford, D., John Raison, R., 2015. Improved models for estimating temporal changes in carbon sequestration in above-ground biomass of mixed-species environmental plantings. For. Ecol. Manage. 338, 208–218.

Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. For. Ecol. Manage. 309, 19–27.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4, 1340–1351

Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4, 1340–1351.

Schutter, M., Dick, R., 2001. Shifts in substrate utilization potential and structure of soil microbial communities in response to carbon substrates. Soil Biology and Biochemistry 33, 1481–1491.

Siciliano, S.D., Palmer, A.S., Winsley, T., Lamb, E., Bissett, A., Brown, M.V., van Dorst, J., Ji, M., Ferrari, B.C., Grogan, P., Chu, H., Snape, I., 2014. Soil fertility is associated with fungal and bacterial richness, whereas pH is associated with community com- position in polar soil microbial communities. Soil Biology and Biochemistry 78, 10–20.

Singh, B.K., Nunan, N., Ridgway, K.P., McNicol, J., Young, J.P.W., Daniell, T.J., Prosser, J.I., Millard, P., 2008. Relationship between assemblages of mycorrhizal fungi and bacteria on grass roots. Applied and Environmental Microbiology. 10, 534–541.

Six, J., Frey, S., Thiet, R., Batten, K., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555–569.

Smith, C.J., Danilowicz, B.S., Clear, A.K., Costello, F.J., Wilson, B., Meijer, W.G., 2005. T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. FEMS Microbiol. Ecol. 54, 375–380.

Šnajdr, J., Dobiášová, P., Urbanová, M., Petránková, M., Cajthaml, T., Frouz, J., Baldrian, P., 2013. Dominant trees affect microbial community composition and activity in post-mining afforested soils. Soil Biology and Biochemistry 56, 105–115.

Strecker, T., Barnard, R.L., Niklaus, P.A., Scherer-Lorenzen, M., Weigelt, A., Scheu, S., Eisenhauer, N., 2015. Effects of plant diversity, functional group composition, and fertilization on soil microbial properties in experimental grassland. PLoS One 10, e0125678.

Swift, M., 1982. Basidiomycetes as components of forest ecosystems, Symposium Series-British. Mycological Society.

Thies, J.E., 2007. Soil microbial community analysis using terminal restriction fragment length polymorphisms. Soil Sci. Soc. Am. J. 71, 579–591.

Thoms, C., Gattinger, A., Jacob, M., Thomas, F.M., Gleixner, G., 2010. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biology and Biochemistry 42, 1558–1565.

Thrall, P.H., Burdon, J., Woods, M.J., 2000. Variation in the effectiveness of symbiotic associations between native rhizobia and temperate Australian legumes: interactions within and between genera. J. Appl. Ecol. 37, 52–65.

Treseder, K.K., 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. New Phytol. 164, 347–355.

Van Der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters. 11, 296–310.

Vries, F.T., Manning, P., Tallowin, J.R., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., Hobbs, P.J., Quirk, H., Shipley, B., Cornelissen, J.H., 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters. 15, 1230–1239.

VSN International, 2011. GenStat for Windows, 14th ed. VSN International, Hemel Hempstead.

Warcup, J., 1980. Ectomycorrhizal associations of Australian indigenous plants. New Phytol. 85, 531–535.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.

Warembourg, F., Roumet, C., Lafont, F., 2003. Differences in rhizosphere carbon-partitioning among plant species of different families. Plant Soil 256, 347–357.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18, 315–322.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84, 2042–2050.

Paper IV The effect of litter type on decomposition rates and soil microbial communities in a shelterbelt system.

After I established that plant genus can strongly influence fungal and, to a lesser extent, bacterial community composition, the question was raised as to how such changes in soil communities alter ecosystem function. To explore this, in **Paper IV** I examined the effect of litter type and litter mixing on decomposition rates and extracellular enzyme activities. In particular, I explored the effects of *Acacia*, *Eucalyptus* litter and mixed litter in a litter bag experiment in the field. I established a litter decomposition experiment using a reciprocal design (litter x dominant overstory genus) in revegetation shelterbelts to establish if litter type affects decomposition rates, microbial community and enzyme activities associated with carbon degradation.

In review

Carnovale, D., Richardson, A.E., Thrall, P.H., Bissett, A., Baker, G., The effect of litter type on decomposition rates and soil microbial communities in a shelterbelt system. Pedobiologia.

Abstract

Acacia and *Eucalyptus* species are commonly used for revegetation in Australia and other parts of the world, yet little is known regarding how their litter might differentially impact decomposition rates and extracellular enzyme activities. To investigate this, a litter decomposition experiment was established using a reciprocal design (litter type x dominant overstory genus) in *Acacia* and *Eucalyptus* revegetation shelterbelts on a common soil to assess rates of decomposition and C degradation associated with differences in enzyme activities and microbial communities. Litter bags with two mesh sizes were used to either exclude (<2 mm) or allow access (>2mm) by soil macrofauna. Bags were filled with either *Eucalyptus*, *Acacia* or an equal mix of *Acacia* and *Eucalyptus* litter. Decomposition, enzyme activities and bacterial and fungal community profiles were determined following recovery of surface-placed bags at 40, 96, 187, 307 days and 395 days and a buried control at day 395. Our findings did not support that mixed litter and litter under the same overstory genus would decay at a faster rate than that under a different overstory. In our study litter decomposition was greatest in *Acacia* litter irrespective of overstory genus and there was thus no evidence of a home field advantage (HFA). The abundance and structure of bacterial and fungal communities were assessed by terminal restriction fragment length polymorphism (T-RFLP) of 16S rRNA genes and quantified by qPCR. We were able to demonstrate that distinct fungal and bacterial communities occupied each litter type, and these also differed between dominant overstory genus. However, we still lack a predictive framework for understanding more generally the impact of different plant species mixes on revegetation outcomes, including rates of return of contribution to ecosystem function.

Key words: *Acacia*, *Eucalyptus*, litter mixing, microbial communities, extracellular enzyme activities

Introduction

The establishment of shelterbelts is common practice on agricultural land in mixed farming systems generally associated with decreased levels of soil organic carbon (C) and increased demand for nutrient inputs (Cleugh et al., 2002; Paul et al., 2002). Decomposition of plant material returned to soil is a key ecosystem process governed by soil microorganisms and is integral for the carbon storage potential of terrestrial ecosystems (Wallenstein et al., 2013; Ward et al., 2015). Decomposition is therefore an important consideration in ecosystem restoration. The rate of decomposition has been shown to be influenced by litter quality, the physical and

chemical environments, and the resident soil biota (Bardgett and Wardle, 2010; Hobbie, 1992; Zheng et al., 2010). For example, it is well known that changes in vegetation composition in ecosystem restoration can alter the quality and quantity of litter inputs to soil and thus decomposition rates (Bardgett, 2005; Hobbie, 1992). Understanding relationships between plant litter diversity and soil biological function in restored agricultural landscapes has the potential to help improve rehabilitation methods and achieve increased ecosystem services, such as carbon sequestration and nutrient cycling.

Acacia and *Eucalyptus* species are commonly used for revegetation in Australia and other parts of the world, yet little is known regarding how their litter might differentially impact on decomposition rates, extracellular enzyme activities and bacterial and fungal communities in soil. Numerous studies have examined the effect of litter type and litter mixing on decomposition rates, largely focusing on deciduous and northern hemisphere species (Chapman and Newman, 2010; Jiang et al., 2013; Slade and Riutta, 2012). Nitrogen-fixing species, such as *Acacia,* often have higher litter N concentrations (Koutika et al., 2014; Voigtlaender et al., 2012), and higher initial decomposition rates (Toky and Singh, 1993). However, previous work indicates that the long-term rates of decomposition may not be faster in N-fixing species because N enriched litter may delay the latter stages of lignin breakdown (Berg, 2000; Berg and Ekbohm, 1991; Resh et al., 2002). *Eucalyptus* species produce litter with lower nutrient content and higher C:N and C:P ratios. This low-quality litter has been shown to result in low decomposition rates compared to species with higher nutrient status (Guo and Sims, 2001; Paul and Polglase, 2004). As such, it is suggested that *Eucalyptus* litter stores a high proportion of nutrients in undecomposed litter over longer periods of time (Adams and Attiwill, 1986). There is evidence in plantation forests that the presence of *Acacia* species within shelterbelts has potential to alter or modify *Eucalyptus* decomposition rates and in turn influence rates of nutrient cycling within the system (Bachega et al., 2016; Bini et al., 2013). In natural *Eucalyptus marginata* forests, O'Connell (1986) found that the presence of *Acacia* increased the nutrient status of *Eucalyptus* litter through decomposition, and in turn accumulated significantly higher amounts of N and P in decomposing litter. This represents the accumulation of N and P from sources outside the litter bags. In contrast, Bachega et al. (2016) found that decomposition rates for *Acacia* litter were slower than for *Eucalyptus* litter, with no effect of tree species in the monoculture system (i.e. no comparative advantage) in which litter was placed. Further to this, the initial N concentrations were 1.9 to 2.9 times higher and initial P concentrations were 1.5

to 3.3 times higher in the *Acacia* residues, indicating that C:N and C:P ratios were not associated with higher rates of decomposition for up to 724 days.

The decomposition of litter in relation to the chemistry of the plant material has been extensively explored. Less well studied is how changes in soil microorganisms and microbial mediated decomposition processes are governed by the quality and quantity of plant litter, by its spatial and temporal distribution and by the ratio of aboveground to belowground inputs. Plant litter quality effects on decomposition may be through the indirect influence of litter on the decomposer community (Wardle et al., 2002). Fanin et al. (2016) showed that litter decomposition was mainly influenced by litter quality but found that the local microbial communities and interactions with their substrates also explained a small (<5%), but significant, part of the variation in C mineralization. Microbial communities in soil vary in their substrate preferences and strategies of nutrient acquisition (Goldfarb et al., 2011). Extracellular enzymes secreted by soil microorganisms are fundamental to the rates of decomposition and nutrient cycling and alteration of biogeochemical properties of ecosystems. Extracellular enzyme activity (EEA) can be used to indicate how microbial communities and ecosystems respond to environmental changes (Sinsabaugh et al., 1993). For example, an increase in N-enriched litter has been associated with increased glycosidase activities (Saiya-Cork et al., 2002; Waldrop et al., 2004). Litter bag experiments have demonstrated that macrofauna may also influence decomposition and are suggested to increase the availability of litter to soil microorganisms and subsequent rates of degradation (Bradford et al., 2002; Brussaard, 1998).

Due to specialisation of soil decomposer communities that have been shown to differ according to different litter types, it has been proposed that litter will decompose faster in its environment of origin compared to other environments. This is referred to as the home field advantage (HFA) (Ayres et al., 2006; Ayres et al., 2009b; Gholz et al., 2000; Strickland et al., 2009a; Strickland et al., 2009b). HFA theory proposes that there is specialisation of local decomposer communities that are more closely associated with the characteristics of the litter they typically encounter, possibly because the decomposers in the litter environment have specific metabolic capacities (Wickings et al., 2012). However, whether or not HFA occurs will depend on the extent to which decomposer communities are composed of generalists with wide metabolic function as compared to specialists. As such, reports of HFA in decomposition are inconsistent (Chapman and Koch, 2007; Gießelmann et al., 2011; Prescott et al., 2000; Veen et al., 2015). In instances where litter substrates were of similar quality, the findings have

generally been unsupportive of the HFA theory (Ayres et al., 2006). Studies that have demonstrated HFA largely took place in ecosystems dominated by one species, where litters were transplanted into ecosystems with highly contrasting litter qualities (Strickland et al., 2009; Jacob et al., 2010; Veen et al., 2015b). In systems with higher litter diversity the decomposer community is likely to either contain a more diverse array of microbial species or include generalist species that are able to decompose a range of litters (Prescott et al., 2000; Chapman and Koch 2007). Moreover, soil communities in mixed stands may contain greater diversity in functional groups. However, it is the relative proportion of these groups (e.g., fungi to bacteria) that determines the overall efficiency of the decomposer community. To include systems with higher litter diversity, Freschet et al. (2012) proposed the substrate-matrix quality interaction (SMI) hypothesis, which takes into account the contrasting litter qualities which may co-exist within the same litter matrix. Under the SMI hypothesis a continuum from positive to negative interactions between specific litters (substrates) and decomposer communities occurs as specific litters and the ecosystem litter layer (matrix) become increasingly dissimilar in quality.

In this study the effects of both litter type (*Acacia*, *Eucalyptus* and Mixed) and overstory genus (*Acacia* and *Eucalyptus*) on litter decomposition, enzyme activities and litter associated fungal and bacterial communities was investigated using litter bags placed in the field in a reciprocal design. The following hypotheses were tested: (1) litter decomposition will be greatest in mixed litter treatments due to chemical and physical changes in leaf mixes; (2) litter under the same overstory genus will decay at a faster rate than that under a different overstory due to the home field advantage (HFA); (3) enzyme activities will differ between litter types and dominant overstory and will correlate with litter decomposition rates; and (4) distinct fungal and bacterial communities will occupy each litter type and this will differ with respect to the dominant overstory genus.

Methods

Site description

A single site on agricultural land dominated by grazing was selected near Murrumbateman in New South Wales, Australia, an area with a temperate climate. The site consisted of two shelterbelts (linear rows of planted trees and shrubs sown in 4 to 5 rows) established in 1995 on a common soil type. One shelterbelt contained an overstory dominated by *Acacia* spp. (*A.*

mearnsii, A. baileyana, A. decurrens, A. cardiophylla and *A. cultriformis)* and the other an overstory dominated by *Eucalyptus* spp. (*E. macarthurii*, *E. blakelyi*, *E. viminalis* and *E. melliodora*). A more detailed description of the site and soil properties are presented in Carnovale et al. (2018), and **Paper III** in this thesis.

Decomposition experiment

Litter decomposition was measured by determining mass loss of plant materials contained in litter bags. Litter from *Acacia* and *Eucalyptus* species were initially collected within the respective shelterbelts in August 2012. Within the centre of the shelterbelts, litter was collected using nets suspended approximately 30cm above the ground to prevent any colonisation by soil organisms. The litter was collected every 2 weeks and air dried until used. Any additional litter required was collected by shaking trees and removal of freely abscising senesced leaves. Both litter types were thoroughly mixed. Nylon litter bags were constructed (15cm x15cm) using 2 mesh sizes and then heat sealed. A small mesh aperture (≤2mm) was used to exclude macrofauna and a large mesh size (>2mm) was used to allow access by macrofauna. Bags were made using 1.5 x 2 mm fiberglass coated nylon mesh, for the large mesh-sized bags several holes 5 mm in diameter were made in the nylon mesh. The bags were filled with either *Eucalyptus* litter, *Acacia* litter or a 50/50 (weight/weight) mix of *Acacia* and *Eucalyptus* litter. Ten grams of litter were accurately recorded and placed into each bag. The total C and N content of the litter was determined on a subsample that was finely milled and analysed by dry combustion using an isotope ratio mass-spectrometry (Europa Scientific, Model 20-20). Litter pH was determined by suspension of milled material (1:5 suspension) and shaking for 30 mins in 0.01 M $CaCl_2$.

Litter bags were deployed at the two sites (one each with an *Acacia* or *Eucalyptus* overstory) from October 2012 to December 2013. The two sites were approximately 100 m apart and located on a common soil type (Carnovale et al. 2019). With respect to the design, six replicate plots 10 m apart were established within each site. At each of the 6 plots within each shelterbelt, existing litter was brushed away, one bag of each treatment (litter type by mesh size) for each collection time point was placed directly on top of the A0 soil horizon and secured with a metal peg. At one location, a set of bags were also buried in soil to a depth of ~5cm. The total number of litter bags was 432 (2 sites x 3 litter types x 2 mesh sizes x 6 retrieval time points x 6 plots, plus one set of bags for burial that were retrieved at the final collection). At one site six replicates of each treatment were collected immediately after deployment to

quantify litter loss during transportation and to serve as the day 0 control. One set of litter bags was then collected from each plot at 40 days, 96 days, 187 days, 307 days and 395 days (corresponding to approximately 1, 3 6, 9 and 13 months). Following collection, each litter bag was placed in a sealable plastic bag to limit the amount of loss associated with transport. Each litter bag was then hand sorted in the laboratory to remove macrofauna, soil particles and plant roots where required. A weighed subsample of fresh material from each bag was taken for molecular analysis and stored at -20ºC until analysis. Samples of 0.25g were similarly taken for enzyme analysis and stored in sealed vials at room temperature for 24h. The remaining litter was then dried at 60ºC until the mass of litter became stable. The weight of the oven dried litter was recorded and loss of litter mass calculated.

DNA extraction

Individual litter samples, both surface and buried, were frozen at -20° C for a maximum of 18 months prior to analysis. Litter was pulverised using a bead beater prior to extraction. Total DNA was extracted from subsamples of approximately 0.12 g litter using a Power Soil DNA extraction kit (MoBio Laboratories, Carlsbad, California, USA), with the following modification; samples were shaken in a Bio101 Savant on the maximum speed for 2 min instead of vortexing (Bissett et al., 2011). DNA quality was assessed on an agarose gel and concentrations of purified DNA were determined by nanodrop spectrophotometry (NanoDrop, Wilmington, DE). This DNA was used as a template for further T-RFLP and qPCR for bacteria and fungal analysis.

Real time qPCR for fungi and bacteria abundance

The abundance of the bacterial 16S rRNA gene and fungal internal transcribed spacer (ITS) were determined using quantitative real time PCR (qPCR). Universal primers for bacteria were Eub338 (ACTCCTACGGGAGGCAGCAG) and Eub518 (ATTACCGCGGCTGCTGG) (Lane, 1991, Muyzer et al., 1993). For fungi, primers used were 5.8S (CGCTGCGTTCTTCATCG) and ITS1F (CTTGGTCATTTAGAGGAAGTAA) (Fierer et al., 2005). Assays of qPCR were performed in triplicate using 10 µL reactions containing iQ SYBR Green Supermix (Bio-Rad), primers, and template DNA. Quantification of bacterial 16S rRNA genes was carried out under the following conditions: 95ºC for 2 minutes followed by 95ºC for 10s, 53ºC for 30s and 72ºC for 20s. Fungal quantification was as follows: 95ºC for 1 minute followed by 95ºC for 5s, 53ºC for 20s and 72ºC for 20s. A total of 40 cycles were run for bacteria and fungi, followed by a melting curve to confirm the amplified products were of the

appropriate size. For each plate a standard curve was run with a 10-fold dilution series from 10^9 copies to 10^4 copies of PCR product generated using the primers above from *Pseudomonas fluorescens* strain 5 and *Fusarium oxysporum vasinfectum*. Standards were purified through an Isolate PCR and Gel Kit (Bioline) and quantified using Picogreen (Invitrogen, Grand Island, NY, USA). Amplification efficiencies were>90% and r^2 values were>0.99 for bacterial and fungal calibration curves.

T-RFLP

T-RFLP fragments of the bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS) regions were amplified from litter DNA. Primers used were 27f-519r (bacteria) and ITS1f-ITS4r (fungi) as previously described (Bissett et al., 2011; Singh et al., 2006). Each 50µL reaction contained 2ng of extracted DNA, 1 U of MyTaq DNA Polymerase (Bioline) and 1x supplied buffer, and 0.5µM of each of the oligonucleotide primers. The fungal PCR mix was as above with the addition of 0.5 µg BSA (Promega). Bacterial PCRs were run at 95°C for 5 min, 30 cycles of 95°C for 35s, 53°C for 30s and 72°C for 30s followed by a final extension step at 72°C for 10 min. Fungal PCRs were run at 95°C for 2 min, 40 cycles of 94°C for 30s, 53°C for 30s and 72°C for 45s followed by a final extension step at 72°C for 10 min. Amplification was confirmed in a 1% agarose gel, and the PCR product was then purified using Multiscreen$_{HTS}$ (Millipore) according to the manufacturer instructions. PCR products were quantified using Picogreen (Invitrogen, Grand Island, NY, USA) and diluted to 2ng/µL. Approximately 25ng of the FAM-labelled PCR products were digested with the restriction endonuclease *Hinf*I in a volume of 40 µL, that included 5U of *Hinf*I and the supplied buffer at 1X. Restriction digests were incubated at 37°C for 4 h. T-RFLP profiles were generated by separating the fragments on an ABI 3130xl Genetic Analyzer (Applied Biosystems, UK).

Raw T-RFLP profile data were initially analysed for absolute peak areas using the size-calling software GeneMapper™ 4.0 (Applied Biosystems, UK). T-RFLP profiles were first checked for stable baselines, voltage and calibrations and profiles were trimmed to between 50 and 600 bp to avoid T-RFs caused by primer-dimers and to obtain fragments within the linear range of the internal size standard (Singh et al., 2007). Data were then uploaded to the online T-RFLP analysis software package T-REX (http://trex.biohpc.org/; Culman et al., 2009) and evaluated for acceptable peak intensity. Noise filtering was carried out by removing peaks whose area was less than twice the standard deviation computed over all peaks (Abdo et al. 2006). T-RFs were aligned where peaks with values less than the threshold (1 base pair) were

identified and grouped into a T-RF (Smith et al., 2005). The relative abundance of a detected T-RF for a sample was calculated as the signal area of each peak divided by the total peak area of all the peaks of the T-RFLP profile for that sample. For statistical analysis, the relative abundance of a detected T-RF within a given T-RFLP profile was used.

Litter extracellular enzyme activity

Litter extracellular enzyme activity (EEA) assessments were undertaken within 24 h of sample collection according to established methods (Sinsabaugh et al., 2002; Sinsabaugh et al., 2003). Assays were performed to assess the activities of acid phosphatase (AP), chitin-degrading N-acetylglucosamine (NAG) and cellulose-degradation by β-glucosidase (βG), and cellobiohydrolase (CBH). Enzyme activities were determined using 4-methylumbelliferyl (MUB) containing substrates (MUB-ß-d-cellobioside for cellobiohydrolase, MUB-1,4-ß-d-glucopyranoside for ß-glucosidase, MUB-phosphate for phosphatase and MUB-N-acetyl glucosaminide for chitinase). Reagents were obtained from Sigma-Aldrich (Germany). For each sample, 0.25 g of litter in 3 ml of acetate buffer was homogenised in a Bio101 Savant at the maximum speed for two 30 second intervals. This was then diluted to 10 ml using acetate buffer. Assays were conducted using 96 well microplates where 100µl of sample suspension and 100µl of 200 µM substrate solution was added to each sample well. Three replicates per sample were used along with blank (no sample) negative controls and a quench standard curve were prepared. Microplates were incubated in the dark at 25ºC for 2 h for acid phosphatase and glucosidase or 4 h for cellobiohydrolase. Reactions were stopped by addition of 10 µL of 1.0 M NaOH added to each well. Fluorescence was measured using a micro plate fluorometer (Victor 2, Multifunction plate reader, PerkinElmer, USA) at 365 nm excitation and 450 nm emission. After correcting for negative controls and quenching, activities were expressed in units of n mol h^{-1} g^{-1}.

Statistical analysis

The litter decomposition rate constant based on loss of mass over the 395 day study for each treatment combination was calculated using a single negative exponential model (Jenny et al. 1949; Olson 1963)

$$X_t/X_0 = e^{-kt}$$

where *Xt/Xo* is the proportion of original mass remaining at time *t*, and *k* is the decomposition rate constant.

This model produces a single decay rate constant (k value). To test for significant differences in rates of litter decomposition, F:B ratios and litter enzyme activities, data were subjected to restricted maximum likelihood (REML) models, where replicated plots were considered a random effect. In the first instance, statistical analysis was conducted using the following fixed model design [litter type* mesh, size*overstory, genus*sampling time]. No effects of mesh size were found except for NAG activity. Thus, subsequent analyses were simplified using a litter type*overstory genus*sampling time design, except for NAG activity which included mesh size. F:B ratio data was log-transformed to achieve homogeneity of variances and normality prior to analysis. The 13 month buried litter bags were excluded from this analysis and directly compared only to the 13 month litterbags placed on the soil surface. Univariate analysis of variance (ANOVA) with post hoc Tukey HSD tests was carried out to look for significant differences in rates of litter decomposition, F:B ratios and litter enzyme activities within each sampling time. Pearson correlations were used to examine relationships among enzyme activities and litter decomposition (litter loss and decay rate constant). ANOVA was used to determine differences in initial litter and soil chemistry. All REML, ANOVA and regression analyses were conducted with GenStat software 16th edition package (VSN International, 2011). All statistical tests were performed at $p = 0.05$.

Multivariate analysis was applied to assess changes in fungal and bacterial community composition. The relative abundance of TRFs identified was used to produce Bray Curtis similarity matrices. Analysis of similarities (ANOSIM) and permutational analysis of variance (PERMANOVA) (Anderson, 2001) were used to test significance of litter type and overstory genus on microbial assemblages. A two-way crossed orthogonal PERMANOVA was used to test the effect of sampling time, litter type and overstory type. For each time point, ANOSIM based on the Bray–Curtis coefficient was performed to evaluate differences in microbial assemblages with respect to litter type (transect) and overstory genus. When significant effects were found, further assessments were done using pairwise comparisons. General differences between soil microbial communities were visualised using non-metric multidimensional scaling (nMDS) and canonical analysis of principle coordinates (CAP) (Anderson and Willis, 2003). Multivariate tests were conducted using PRIMER 6 (Clarke and Gorley, 2006; Clarke and Warwick, 2001) and PERMANOVA + (Anderson et al., 2008).

Results

Initial soil chemical properties between *Acacia* and *Eucalyptus* overstories were varied. While there was no significant difference in total soil C and N content, the C:N ratio was significantly higher in soils under *Eucalyptus* (Table 1). Soil pH and P were significantly lower in soils from *Acacia* compared to the *Eucalyptus* overstory. There was no significant difference in initial litter C between *Acacia* and *Eucalyptus* litter, whereas total N was significantly higher in *Acacia* litter.

No effects of mesh size were found for decomposition, soil bacterial and fungal communities and enzyme activities, with the exception for NAG activity. Litter decomposition, F:B ratio, and extracellular enzyme activities were subsequently analysed as a 3-way factorial design (3× litter types, 2× dominant overstory, 3 or 5 sampling time points). In all tests the interaction term litter×overstory genus was not statistically significant (Table 2). Therefore, the results are discussed in terms of the main factors (litter type, overstory type and sampling time) and interactions of litter type and overstory type with time.

Table 1. Mean values (± SE) for site descriptors (soil properties 0-10 cm, n=12) and initial litter (n=3) chemistry (nd=not determined). Values not followed by the same superscript are not significantly different.

	C %	**N%**	**C:N ratio**	**pH**	***P* (mg kg^{-1})**
Acacia overstory shelterbelt	2.15 ± 0.07 a	0.17 ± 0.005 a	12.3 ± 0.14 b	4.86 ± 0.03 b	6.0 ± 0.1 b
Eucalyptus overstory shelterbelt	1.95 ± 0.12 a	0.15 ± 0.006 a	13.1 ± 0.23 a	5.19 ± 0.05a	7.0 ± 0.1 a
Acacia litter	50.1 ± 0.14 a	2.13 ± 0.04 a	23.9 ± 1.04 b	4.30 ± 0.01 a	nd
Eucalyptus litter	50.8 ± 0.12 a	1.71 ± 0.06 b	29.3 ± 0.45 a	4.33 ± 0.003 a	nd

Table 2. : Summary of REML, showing the effect of sampling time, litter type and dominant overstory on litter decomposition (% mass remaining), litter fungal to bacteria (F:B) ratio and extracellular enzyme activities (acid phosphatase (AP); ß-glucosidase (βG); cellobiohydrolase (CBH) and N-acetylglucosamine (NAG))

	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Litter % mass remaining					
Litter	10.65	2	5.33	321.1	**0.005**
Overstory genus	53.49	1	53.49	321.4	**<0.001**
Sampling time	709.18	4	177.3	321.1	**<0.001**
Litter* overstory genus	3.12	2	1.56	321.1	0.211
Litter * sampling time	15.11	8	1.89	321.3	0.061
Overstory genus *sampling time	8.93	4	2.23	321.2	0.065
Litter*overstory genus* sampling time	10.99	8	1.37	321.2	0.207
Decay rate constant					
Litter	14.77	2	7.39	377.3	**<0.001**
Overstory genus	30.35	1	30.35	377.8	**<0.001**
Sampling time	93.09	5	18.62	377.4	**<0.001**
Litter* overstory genus	2.84	2	1.42	377.3	0.243
Litter * sampling time	22.74	10	2.27	377.5	**0.014**
Overstory genus *sampling time	8.45	5	1.69	377.4	0.136
Litter*overstory genus* sampling time	7.93	10	0.79	377.4	0.636
F: B ratio *					
Litter	14.39	2	7.2	189.1	**<0.001**
Overstory genus	3.79	1	3.79	189.1	0.055
Sampling time	47.74	2	23.87	189.3	**<0.001**
Litter* overstory genus	3.17	2	1.58	189.2	0.208
Litter * sampling time	11.6	4	2.9	189.1	**0.023**
Overstory genus *sampling time	2.85	2	1.43	189.1	0.243
Litter*overstory genus* sampling time	6.12	4	1.53	189.2	0.195
acid phosphatase (AP)					
Litter	15.57	2	7.79	321	**<0.001**
Overstory genus	7.89	1	7.89	321.1	**0.005**
Sampling time	1974.37	4	493.59	321	**<0.001**
Litter* overstory genus	6.36	2	3.18	321	**0.043**
Litter * sampling time	6.79	8	0.85	321.1	0.56
Overstory genus *sampling time	7.76	4	1.94	321	0.104
Litter*overstory genus* sampling time	9.54	8	1.19	321	0.303
β-glucosidase (βG)					
Litter	24.82	2	12.41	321.1	**<0.001**
Overstory genus	20.46	1	20.46	321.2	**<0.001**
Sampling time	528.9	4	132.22	321.1	**<0.001**
Litter* overstory genus	1.96	2	0.98	321.1	0.376
Litter * sampling time	97.54	8	12.19	321.1	**<0.001**
Overstory genus *sampling time	47.2	4	11.8	321.1	**<0.001**
Litter*overstory genus* sampling time	6.63	8	0.83	321.1	0.578
Cellobiohydrolase (CBH)					
Litter	46.66	2	23.33	321.1	**<0.001**
Overstory genus	21.9	1	21.9	321.2	**<0.001**
Sampling time	579	4	144.75	321.1	**<0.001**
Litter* overstory genus	2.04	2	1.02	321.1	0.361
Litter * sampling time	81.76	8	10.22	321.1	**<0.001**
Overstory genus *sampling time	28.43	4	7.11	321.1	**<0.001**
Litter*overstory genus* sampling time	9.8	8	1.22	321.1	0.284
N-acetylglucosamine (NAG)					
Litter	35.6	2	17.8	291	**<0.001**
Mesh	4.5	1	4.5	291	**0.035**
Overstory genus	13.75	1	13.75	291.1	**<0.001**
Sampling time	1025.9	4	256.48	291	**<0.001**
Litter* mesh	4.17	2	2.09	291.1	0.126
Litter* overstory genus	2.31	2	1.15	291	0.317
overstory genus* mesh	2.24	1	2.24	291	0.136
Litter * sampling time	7.81	8	0.98	291.1	0.455
Mesh * sampling time	5.1	4	1.27	291	0.28
Overstory genus *sampling time	41.18	4	10.3	291	**<0.001**
Litter* mesh * overstory genus	1.57	2	0.79	291.1	0.457
Litter*overstory genus* sampling time	8.06	8	1.01	291.1	0.43
Litter * mesh* sampling time	8.84	8	1.1	291.1	0.36
Litter* mesh* overstory genus* sampling time	3.16	4	0.79	291.1	0.532

Litter decomposition

At 40 days there was little difference in decomposition of differing litter types or by dominant overstory with respect to the remaining mass (88.6% to 95.3%). After 395 days in the field, litter mass remaining ranged from 52.6% to 69.3% across all treatments, and decomposition was greatest for *Acacia* litter compared to *Eucalyptus* and mixed litters across both overstory genus (Table 3). Moreover, as decomposition progressed, the mass loss was greater for litter under *Eucalyptus* dominated shelterbelts compared to *Acacia* shelterbelts, although the difference between like litters across each site was variable with no consistent patterns. Litter bags that were buried had significantly less litter remaining at 395 days compared to those placed on top of the soil surface (Table 3). However, there was no significant difference between buried and non-buried decomposition for each treatment type (litter*genus overstory). There was a high standard error of means among the buried samples (±5.1 to 7.8 %).

Negative exponential decay models resulted in decay rate constants ranging from 0.15 g g-1yr-1 (Mixed litter) to 0.27 g g-1yr-1 (*Acacia* litter). There was a significant effect of litter type on the decay rate constant ($P < 0.005$), but there was no significant difference in the decay rate constant between like litter under differing dominant overstories ($P > 0.05$) or any interactions. There was no clear correlation between enzyme activity for each enzyme and decay rate constant (supplementary table 1).

Table 3. Effects of overstory genus, litter type on litter decomposition indicted as mean percentage litter biomass remaining (mean ± SE). For each time point means not followed by a common letter were significantly different as determined by post hoc Tukey HSD tests.

Overstory genus	Litter type	Percentage of litter remaining						Decomposition constant K
		40 days	96 days	187 days	307 days	395 days	395 days buried	
Acacia	*Acacia*	90.8±1.5abc	90.1±1.2ab	85.1±2.0c	72.6±3.9ab	60.1±3.5ab	42.2±7.7	0.21±0.03ab
	Eucalyptus	95.3±1.2c	88.0±1.1a	80.3±1.3bc	72.5±1.7ab	69.3±2.9b	41.8±7.1	0.15±0.02a
	Mixture	93.0±0.9abc	95.3±1.4b	79.3±2.9bc	72.8±2.3b	66.0±3.9ab	47.0±6.9	0.18±0.03ab
Eucalyptus	*Acacia*	88.6±1.8a	84.0±2.3a	69.2±3.1a	64.2±3.1a	52.6±3.6a	46.2±5.1	0.27±0.02b
	Eucalyptus	94.2±0.6bc	87.7±0.8a	72.9±1.9ab	66.9±1.7a	60.6±2.8ab	43.8±6.7	0.21±0.02ab
	Mixture	90.2±0.8ab	84.3±2.2a	72.8±2.0ab	68.3±2.1a	64.1±2.8ab	43.3±5.8	0.18±0.02ab

Enzyme activity

Litter type had a significant effect on all extracellular enzyme activity associated with decomposing litter (Table 2). Across most sampling points NAG, βG and CBH were highest in *Eucalyptus* litter and lowest in *Acacia* litter. AP activity was highest under *Acacia* litter with *Eucalyptus* and mixed litter showing similar AP activity levels (Fig. 1). Generally enzyme activities at 40 and 187 days where higher than that at 96 and 395 days. CBH, βG and AP activity were significantly higher ($p<0.001$) in litter buried compared to litter placed on the A0 horizon, whereas there was no difference in NAG activity between buried and unburied litter (Figure 10). Overall, all enzyme activities were highest in litters under the *Acacia* dominated shelterbelt compared to the site with the *Eucalyptus* overstory (Fig. 2). Interactions between litter type and time were significant for CBH, NAG and βG (Table 2).

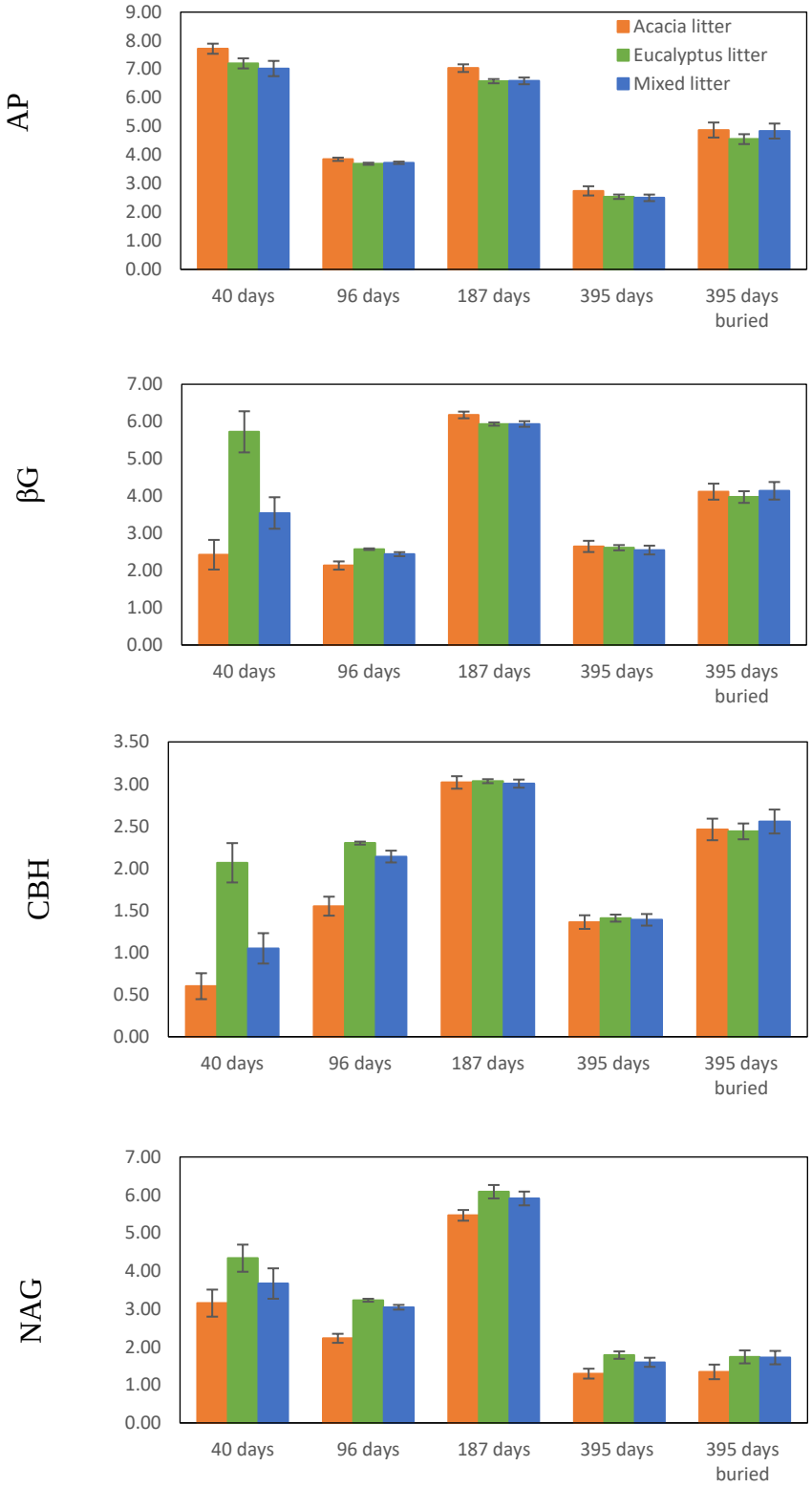


Figure 1. Enzyme activities (umol g^{-1} h^{-1}) in recovered litters according to litter type (A=*Acacia*, E=*Eucalyptus* and M=mixed) for acid phosphatase (AP), cellulose-degrading β-glucosidase (βG), cellobiohydrolase (CBH) and chitin-degrading N-acetylglucosamine (NAG). Statistical differences for main effects and interactions are shown in Table 2). Error bars indicate ± SE.

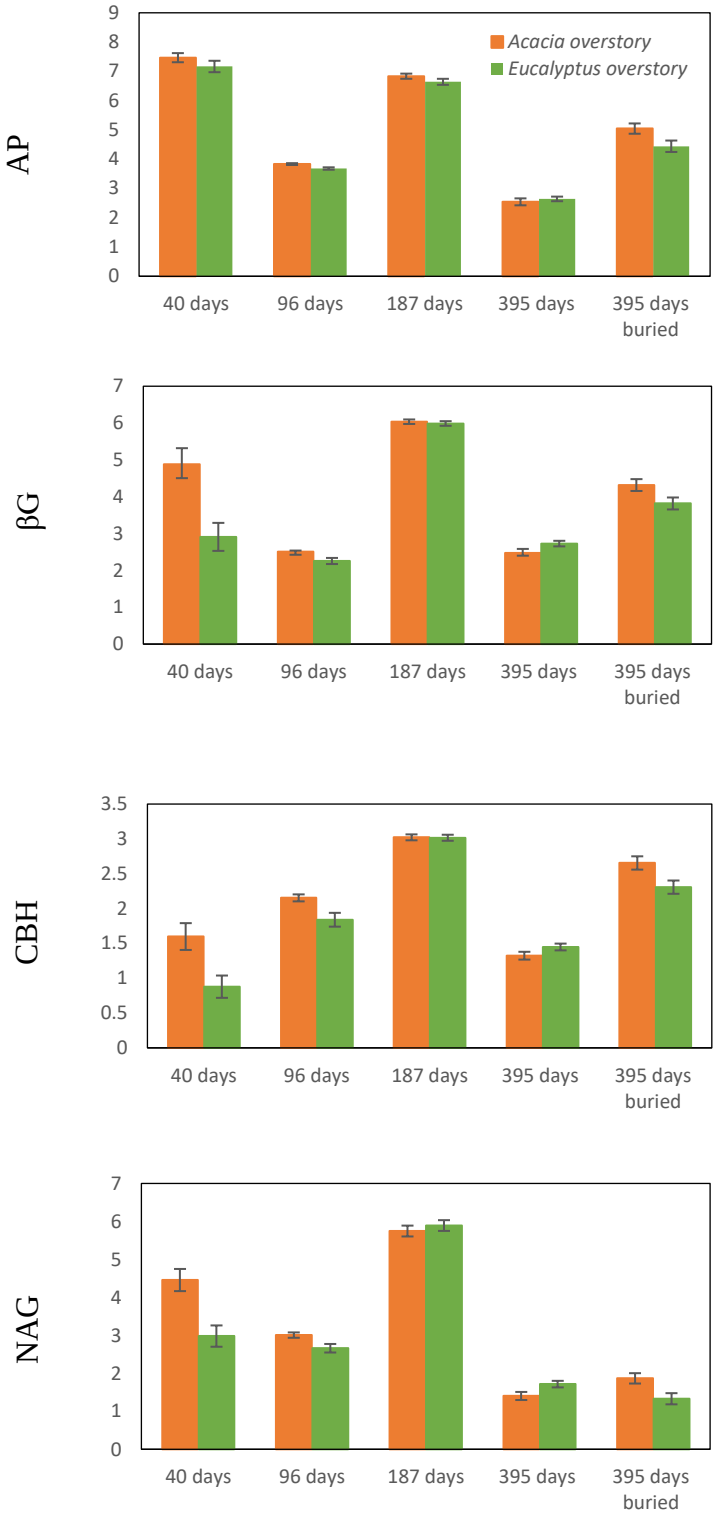


Figure 2. Enzyme activities (umol g^{-1} h^{-1}) in recovered litters according to overstory (A=*Acacia*, E=*Eucalyptus*) for acid phosphatase (AP), cellulose-degrading β-glucosidase (βG), cellobiohydrolase (CBH) and chitin-degrading N-acetylglucosamine (NAG). Statistical differences for main effects and interactions are shown in Table 2.. Error bars indicate ± SE.

Fungi to bacteria ratio

The dominant overstory had no significant effect on the fungi:bacteria (F:B) ratio as determined by qPCR, thus the two overstory treatments were combined when discussing litter type effects. Across all sampling points, the F:B ratio in *Acacia* litter was higher (0.63) than *Eucalyptus* (0.51) and mixed litter (0.50), with no significant difference in F:B ratio between *Eucalyptus* and mixed litter (Table 2, Fig. 3). There was a significant litter type by sampling time interaction. In general, over time the F:B ratio decreased at 187 days, but then increased again at 395 days. At 40 days there was no significant difference in the F:B ratio across litter types. At day 187, the mixed litter treatment exhibited a significantly lower F:B ratio than *Acacia* litter.

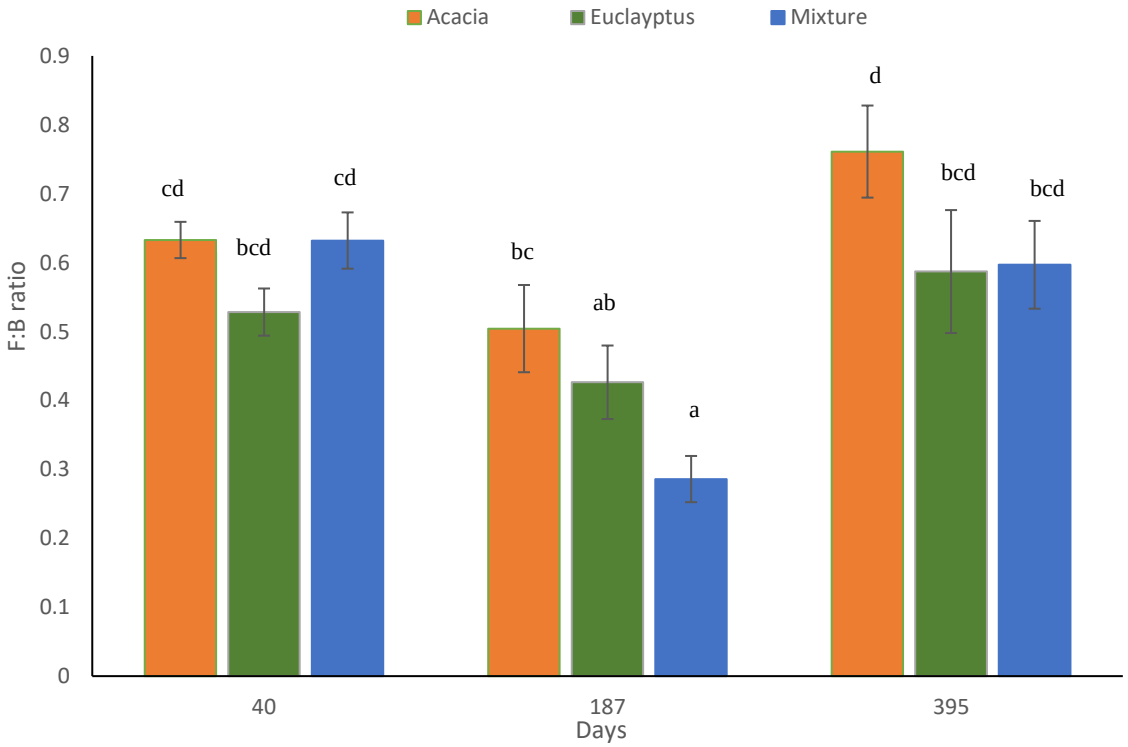


Figure 3. Fungal:bacterial (F:B) gene copy ratio determined by qPCR of litter types across all plots at three time points. The letters above the bars indicate significant differences based on Tukey's test ($p<0.05$). Error bars indicate ± SE.

Bacterial and fungal T-RFLP community profiles

PERMANOVA analysis of the fungal and bacterial T-RFLP community profiles indicated that soil microbial communities changed in relation to all factors (litter type, dominant overstory and sampling time; Table 4). In preliminary analysis mesh size had no significant effect on litter microbial communities and was removed from subsequent analyses. There was also a significant 3-way interaction between litter type*overstory*time for both bacteria (p=0.032) and for fungi (p=0.016). The effects of litter type and dominant overstory on fungal and bacteria community structure was assessed for each sampling timepoint (Fig. 4 and 5) At 40 days there was a significant difference between *Acacia* and *Eucalyptus* litter for fungal and bacterial communities (p=0.001). For bacterial communities there was no significant difference between *Eucalyptus* litter and mixed litter across all sampling times. There was a significant difference in the bacterial community between *Acacia* and mixed litters only after 395 days. Across all time points there was a significant difference in fungal communities between *Acacia* and mixed litters. At both 40 and 395 days, there were significant differences in fungal communities between *Eucalyptus* and mixed litter treatments. After 40 days the fungal community structure was similar under *Acacia* and *Eucalyptus* overstories (Table 4, Fig. 4). There was a significant effect of dominant overstory at 187 and 395 days for both fungi and bacteria. Litter*overstory interactions within each time point varied where, in general, fungal communities showed the greatest differentiation in community structure between treatments compared to bacteria (Table 7). For *Acacia* litter under the *Acacia* overstory compared to under *Eucalyptus* overstory there was a significant difference between fungal and bacterial communities after 395 days. Prior to this, community similarity was variable.

Table 4. PERMANOVA analysis of bacterial and fungal communities according to litter type, dominant overstory and sampling time. P-values less than 0.05 are in bold. MC= Monte Carlo P.

	Fungi			Bacteria		
	Pseudo-F	*P*	P(MC)	Pseudo-F	*P*	P(MC)
litter	6.9051	**0.001**	0.001	2.6594	**0.001**	0.001
overstory genus	16.047	**0.001**	0.001	2.7733	**0.001**	0.001
sampling date	19.984	**0.001**	0.001	38.972	**0.001**	0.001
litter*overstory genus	1.2631	0.109	0.143	1.3732	0.059	0.09
litter*date	2.8167	**0.001**	0.001	2.1491	**0.001**	0.001
genus*date	3.0773	**0.001**	0.001	2.2175	**0.001**	0.001
litter* overstory genus* date	1.3933	**0.016**	0.019	1.3543	**0.032**	0.031

Table 5. Summary of ANOSIM results for pair-wise tests for fungal and bacterial communities according to litter type and overstory genus within each sampling time. Tests were performed between litter type within sampling time and for overstory genus within sampling time (A= *Acacia*, E= *Eucalyptus*, M = mixture). *p*-values less than 0.05 are in bold

	Fungi			**Bacteria**	
Pairwise tests	**R Statistic**	***p***		**R Statistic**	***p***
Litter type					
40 days					
A ,E	0.526	**0.001**		0.23	**0.001**
A, M	0.272	**0.001**		0.068	0.066
E, M	0.091	**0.013**		0.036	0.175
187 days					
A ,E	0.217	**0.001**		0.255	**0.001**
A, M	0.107	**0.007**		0.077	0.055
E, M	0.014	0.298		0.074	0.089
395 days			0		
A, E	0.248	**0.001**		0.221	**0.001**
A, M	0.233	**0.001**		0.207	**0.001**
E, M	0.068	**0.038**		0	0.457
Overstory genus			0		
40 days					0
A, E	0.013	0.276		0.081	**0.021**
187 days					
A, E	0.45	**0.001**		0.107	**0.013**
365 days					
A, E	0.338	**0.001**		0.111	**0.004**

Table 6. Summary of ANOSIM results for pair-wise tests for fungal and bacterial communities for litter type plus overstory genus within each sampling time. Tests were performed between litter type with overstory type within sampling time (A= *Acacia*, E= *Eucalyptus*, M = Mixture), the first letter represents litter type, and the second letter represents dominant overstory. *p*-values less than 0.05 are in bold

	Fungi		**Bacteria**	
Pairwise tests	**R Statistic**	***p***	**R Statistic**	***p***
Litter by overstory				
40 days				
AE, EE	0.644	**0.001**	0.291	**0.001**
AE, ME	0.265	**0.001**	0.04	0.189
AE, AA	0.034	0.209	0.159	**0.004**
AE, EA	0.59	**0.001**	0.113	**0.04**
AE, MA	0.376	**0.001**	0.097	0.105
EE, ME	0.142	**0.003**	0.079	0.075
EE, AA	0.504	**0.001**	0.309	**0.001**
EE, EA	-0.013	0.550	0.063	0.124
EE, MA	0.153	**0.011**	0.061	0.191
ME, AA	0.204	**0.005**	0.091	0.064
ME, EA	0.091	**0.046**	0.021	0.283
ME, MA	0.024	0.256	0.012	0.402
AA, EA	0.385	**0.001**	0.162	**0.008**
AA, MA	0.282	**0.001**	0.111	0.094
EA, MA	0.031	0.236	-0.019	0.553
187 days				
AE, EE	0.165	**0.009**	0.129	0.072
AE, ME	0.071	0.102	0.104	0.076
AE, AA	0.195	**0.008**	0.05	0.159
AE, EA	0.41	**0.001**	0.211	**0.004**
AE, MA	0.36	**0.001**	-0.051	0.748
EE, ME	-0.028	0.615	0.049	0.246
EE, AA	0.603	**0.001**	0.247	**0.005**
EE, EA	0.526	**0.001**	0.205	**0.02**
EE, MA	0.63	**0.001**	0.147	0.059
ME, AA	0.517	**0.001**	0.23	**0.004**
ME, EA	0.651	**0.001**	0.123	**0.037**
ME, MA	0.628	**0.001**	0.081	0.126
AA, EA	0.268	**0.001**	0.368	**0.001**
AA, MA	0.143	**0.011**	0.039	0.239
EA, MA	0.056	0.154	0.106	0.079
395 days				
AE, EE	0.193	**0.007**	0.104	0.063
AE, ME	0.032	0.240	0.151	**0.032**
AE, AA	0.192	**0.008**	0.151	**0.005**
AE, EA	0.389	**0.001**	0.299	**0.002**
AE, MA	0.425	**0.001**	0.251	**0.001**
EE, ME	0.022	0.312	0.055	0.147
EE, AA	0.482	**0.001**	0.256	**0.003**
EE, EA	0.363	**0.001**	0.123	**0.024**
EE, MA	0.573	**0.001**	0.095	0.054
ME, AA	0.306	**0.001**	0.297	**0.002**
ME, EA	0.292	**0.001**	0.174	**0.001**
ME, MA	0.464	**0.001**	0.067	0.094
AA, EA	0.289	**0.002**	0.316	**0.001**
AA, MA	0.401	**0.001**	0.265	**0.003**
EA, MA	0.11	**0.019**	-0.055	0.921

Table 7. Summary of ANOSIM results for pairwise tests for fungal and bacterial communities for litter type and overstory genus within each sampling time. Tests were performed between litter type with overstory type and sampling time within litter types (A= *Acacia*, E= *Eucalyptus*, M = mixture). *p*-values less than 0.05 are in bold

	Fungi		**Bacteria**	
Pairwise tests	**R Statistic**	***p***	**R Statistic**	***p***
Litter*soil* time				
within litter type				
40 days				
AE, AA	0.034	0.209	0.159	**0.004**
EE, EA	-0.013	0.55	0.063	0.124
ME, MA	0.024	0.256	0.012	0.402
187 days				
AE, AA	0.195	**0.008**	0.05	0.159
EE, EA	0.526	**0.001**	0.205	**0.020**
ME, MA	0.628	**0.001**	0.081	0.126
395 days				
AE, AA	0.192	**0.008**	0.151	0.005
EE, EA	0.363	**0.001**	0.123	**0.024**
ME, MA	0.464	**0.001**	0.067	0.094

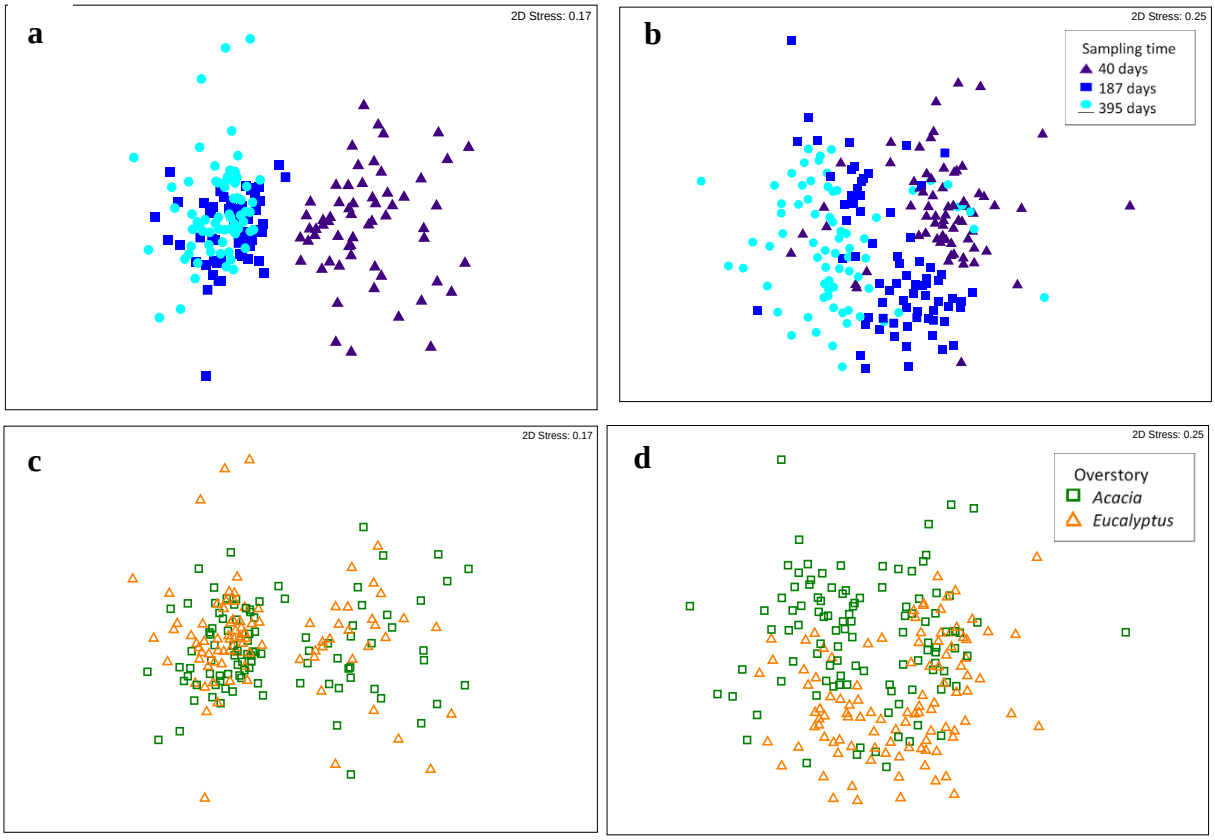


Figure 4. Non metric dimensional scaling (nMDS) of microbial community structure based on Bray Curtis similarity measures: a) bacterial community composition by month sampled; b) fungal community composition by month sampled; c) bacterial community composition by dominant overstory; d) fungal community composition by dominant overstory. Data in panels a and b is shown for 40 days (▲), 187 days (■) and 395 days (●) and panels c and d for dominant overstory soil (△ =*Acacia* and □ =*Eucalyptus*). Points closest to each other in the nMDS plots indicate community compositions with higher similarity.

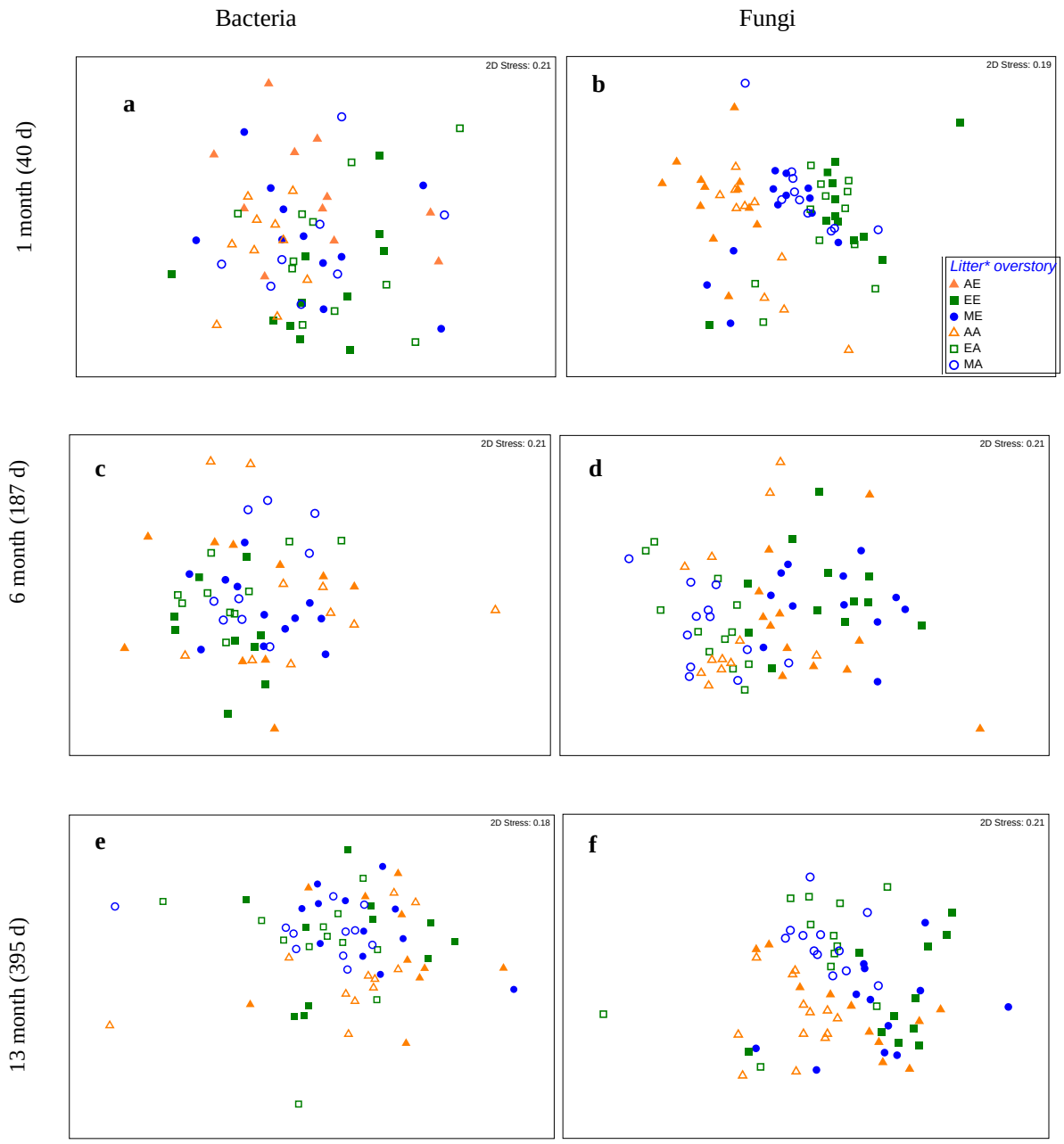


Figure 5. Non-metric dimensional scaling (nMDS) of microbial community structure in surface litter based on Bray Curtis similarity measures. Shows fungal and bacterial community composition at 40 days (1 month, panels a,b), 187 days (6 months, panels c,d) and 395 days (13 months, panels e,f)) Triangles = *Acacia* litter, squares = *Eucalyptus* litter, circles = Mixed litter. Hollow symbols indicate *Acacia* overstory, while solid symbols indicate *Eucalyptus* overstory. Points closest to each other in the nMDS plots indicate community compositions with higher similarity.

Discussion

Litter decomposition

Litter decomposition (up to 395 days) was influenced by litter genus, with the greatest effects being observed after 96 days, when mass loss was greatest for *Acacia* and least for *Eucalyptus* litter, which was most likely due to differences in litter quality (i.e., higher N in the *Acacia* litter) (Dorrepaal et al., 2007; Ward et al., 2010). It has however been shown elsewhere that decomposition rates of *Acacia* litter may slow down over longer timeframes, given that N enriched litter may delay the late stages of lignin decay (Berg, 2000; Berg and Ekbohm, 1991; Resh et al., 2002). Macrofauna have been shown to increase decomposition rates (Bradford et al., 2002). In our study, litter mesh size had no significant effect on most of the variables measured, indicating that decomposition was not being altered by the exclusion of macrofauna in this system. Buried litter bags showed greater decomposition than those placed on the soil surface at the end of the field decomposition experiment. This is likely due to the presence of more favourable environmental conditions experienced in the soil (e.g., smaller temperature and moisture changes) compared to the soil surface which would be expected to experience wider fluctuations in temperature and moisture.

Two factors have been shown to be important in the formation of a HFA. Firstly, the litter material should be of low quality (e.g. low N and P) (Ayres et al., 2009a; Ayres et al., 2009b; Strickland et al., 2009a; Strickland et al., 2009b). Secondly, the decomposer community should be conservative with respect to the traits responsible for decomposition of certain chemical substances leading to some degree of specialisation of decomposer species (Ayres et al., 2009a; Ayres et al., 2009b; Gholz et al., 2000). According to this theory it was predicted that a HFA would be seen in *Acacia* and *Eucalyptus* litter decomposition, however there was no evidence that litter decomposition was increased by a home field advantage (HFA). This lack of HFA may suggest that the decomposer communities within these restored ecosystems are more likely to be dominated by generalists. As no HFA was observed in our experiment, decomposition may be more in line with the substrate quality/matrix quality interaction (SMI) hypothesis. As such, this implies that low-quality substrates will decompose faster than expected when incubated in the decomposition matrix of poor quality, but in contrast will decompose slightly slower than expected in intermediate-quality matrices and substantially slower in matrices composed of high-quality litter (Freschet et al., 2012).

Despite the quality difference that was observed between the *Acacia* and *Eucalyptus* litters, it may be that both were of low quality compared to other studies that have used more nutrient rich plant species (Allison and Vitousek, 2004). Both litter types were shown to have relative slow decomposition rates, average *k* values of 0.36, 0.35 and 1.46 in temperate, Mediterranean and tropical regions respectively (Aerts 1997). The matrix of poor quality (*Eucalyptus* overstory) may have contributed to the accelerated decomposition rates of *Acacia* litter, rather than the direct mixing of two litters within the same litter bag. This is further supported by the fact that mixing *Acacia* and *Eucalyptus* litter within litter bags did not accelerate the decomposition rate compared to the individual litters. Our results are contrary to the findings by Slade and Riutta (2012) using deciduous tree species, which indicated no difference in mass loss between mixed and single species litter, when single species were placed in a mixed stand forest. Their findings suggested that any observed differences between single species and mixed species litter bags were due to the direct contact of neighbouring species inside the litterbag, rather than the litter environment in which they were placed (Slade and Riutta, 2012). The effects of litter mixing on various functional attributes such as decomposition rate was varied, in the present study, which is consistent with that reported by (Jiang et al., 2013) where litter mixing had differing effects on decomposition depending on sampling time.

Enzyme activities

For CBH, NAG and βG there was an initial increase in in enzyme activities at 40 days compared to 196 days. Whereas at 395 days, there was a rapid decline in enzyme activities. Decreases in enzyme activities at 395 days may be attributed to environmental variables. For instance, Prescott (2010) proposed that microbial activity is uniformly low at temperatures below 10°C regardless of other factors, which is consistent with our findings from sampling that was conducted at the end of winter. CBH, βG and AP activities in litter buried compared to litter placed on the A0 horizon were significantly higher ($p < 0.001$). The higher activity in buried litter samples may be indicative of greater decomposition due to the more stable and favourable environment compared to the soil surface.

AP activity was highest in *Acacia* litter with *Eucalyptus* and mixed litter showing similar AP activities. This is contradictory to the model presented by (Sinsabaugh and Moorhead, 1994) which predicted that plant tissues with low concentrations of N or P relative to C should exhibit greater activities of N- and P-degrading enzymes such as acid phosphatase (AP). The reasoning for this is that when N and P are more abundant microorganisms can allocate more resources

to C-degrading enzymes (e.g. βG and CB), thus increasing potential decomposition rates. Contrary to this expectation and that of (Waring, 2013), we found decomposition was highest in *Acacia* litter despite a higher microbial allocation to AP.

Correlations between litter decay rates and enzyme activities were not strong (Supplementary Table 1, Appendix 3) within our study. This is contrary to some studies which have showed correlations between enzyme activities and litter loss (Rietl and Jackson, 2012; Sinsabaugh and Findlay, 1995), but is in support of other work that found no strong relationships (Allison and Vitousek, 2004; Keeler et al., 2009). Keeler et al., 2009 attributed the lack of correlation to the high temporal heterogeneity of enzyme activities. For example, it has been shown that seasonal and inter-annual dynamics of enzyme activities may hinder the ability to detect significant relationships between enzyme activity and the integrated process of decomposition that occurs over much longer time scales (Sinsabaugh et al., 2008).

Microbial community profiles and fungal to bacterial ratios

Previous studies have shown that fungi are primarily responsible for decomposing complex compounds with higher C:N ratios (Fierer et al., 2009) and N deficiencies (Bossuyt et al., 2001). In these studies, the highest litter C:N ratio was at least twice as high as the low quality litter. We found significantly lower C:N ratios for *Eucalyptus* litter, however the difference was not an order of magnitude smaller. After 395 days the F:B ratio was greatest in *Acacia* litter (0.63) compared to *Eucalyptus* (0.51) and mixed litter (0.50), indicating that other C substrates may potentially be affecting this ratio. C substrates within the litter were not quantified, however it has been shown that relative proportions of lignin, cellulose, hemicellulose, phenolic compounds, and water soluble carbohydrates in litter can strongly influence the decomposition rate (e.g., Allison and Vitousek, 2004; Coq et al., 2010; Talbot and Treseder, 2012).

The short generation times of bacteria as well as the potential of fungi to react via rapid growth of the mycelium are traits that allow microbial communities to adjust over short time scales to varying substrates, leading to shifts in community composition (Goddard and Bradford, 2003; Goldfarb et al., 2011; Hanson et al., 2008). This is evidenced by the changes in community composition seen in our study even after 40 days. By this timepoint there was a significant difference between *Acacia* and *Eucalyptus* litter fungal and bacterial communities (P=0.001). For bacterial communities there was no significant difference between *Eucalyptus* litter and mixed litter across all sampling times.

The dominant overstory resulted in differing litter microbial communities, which is in line with earlier work (Carnovale et al., 2019), where it was shown that soil microbial communities differed between *Acacia* and *Eucalyptus* trees in the same shelterbelt systems. The observed changes in fungal community composition between litters at 187 and 395 days suggests that specialised fungi are associated with decomposition of each litter type in each overstory. However, this specialisation did not result in differences in decomposition rate or result in a HFA. Overall, the importance of microbial decomposers for the formation of HFA is still poorly understood and inoculation experiments have yielded conflicting results (Ayres et al., 2006; Strickland et al., 2009a).

Little is known about the functional diversity of enzyme activity in natural environments, specifically the groups of organisms responsible for the production of different types of enzymes, how production and efficiency are regulated among different groups of organisms, and what happens to the effectiveness of extracellular enzymes once they are released into the heterogeneous soil matrix (Nannipieri et al., 2002). Several authors have shown that there is substantial functional redundancy within microbial communities (Wertz et al., 2007; Nannipieri et al., 2003). Therefore, changes in soil microbial community structures may not always result in differences in biogeochemical functioning of microbial communities**.** To some degree, this was supported by observations in our study where distinct changes in fungal community composition did not necessarily coincide with changes in functional measures that included decomposition rate and various enzyme activities.

Conclusions

Characterisation of the decomposition process for litter from key revegetation species (*Eucalyptus* and *Acacia* in this study) can increase understanding of how plant community structure may drive soil function, as well as provide information regarding the role of revegetation in mediating soil nutrient cycling. It was hypothesised that mixed litter and litter under the same overstory genus would decay at a faster rate than that under a different overstory. Our findings did not support this prediction, as in our study litter decomposition was greatest in *Acacia* litter irrespective of overstory genus. Higher decomposition rates of the *Acacia* litter may therefore also result in increased nutrient cycling within revegetated agricultural land. Furthermore, we were able to demonstrate that distinct fungal and bacterial communities occupied each litter type and these also differed between dominant overstory genus. However, we still lack a predictive framework for understanding more generally the impact of different

plant species mixes on revegetation outcomes, including rates of return of contribution to ecosystem function. Further research on how revegetation species influence litter microbial community structure, and in turn how changes in microbial communities alter key functional processes is required. This could include studies that relate microbial community profiles to functional genes using controlled abiotic conditions to directly assess the effect of changes in microbial community composition on decomposition rates in shelterbelt systems. Furthermore, work to better understand how the initial mix of revegetation species influences microbial community function is needed, with the aim of ultimately being able to provide guidelines that improve the effectiveness of restoration efforts.

Acknowledgements

We would like to thank Shamsul Hoque and Liam Thammavongsa for assistance in sample analysis. We are thankful to Pauline Carnovale and Michael Carnovale who assisted in litter bag preparation. We are grateful to the farmers who allowed access to their property and Greening Australia for access to shelterbelts. This research was jointly supported by CSIRO Agriculture and Food and the Australian National University.

References

Adams, M., Attiwill, P., 1986. Nutrient cycling and nitrogen mineralization in eucalypt forests of south-eastern Australia. Plant and Soil 92, 341-362.

Allison, S.D., Vitousek, P.M., 2004. Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica 36, 285-296.

Ayres, E., Dromph, K.M., Bardgett, R.D., 2006. Do plant species encourage soil biota that specialise in the rapid decomposition of their litter? Soil Biology and Biochemistry 38, 183-186.

Ayres, E., Steltzer, H., Berg, S., Wall, D.H., 2009a. Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them. Journal of Ecology 97, 901-912.

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C., Wall, D.H., 2009b. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biology and Biochemistry 41, 606-610.

Bachega, L.R., Bouillet, J.-P., de Cássia Piccolo, M., Saint-André, L., Bouvet, J.-M., Nouvellon, Y., de Moraes Gonçalves, J.L., Robin, A., Laclau, J.-P., 2016. Decomposition of *Eucalyptus grandis* and *Acacia mangium* leaves and fine roots in tropical conditions did not meet the Home Field Advantage hypothesis. Forest Ecology and Management 359, 33-43.

Bardgett, R., 2005. The Biology of Soil: A community and ecosystem approach Oxford University Press, United States.

Bardgett, R.D., Wardle, D.A., 2010. Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford University Press Oxford.

Berg, B., 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133, 13-22.

Berg, B., Ekbohm, G., 1991. Litter mass-loss rates and decomposition patterns in some needle and leaf litter types. Long-term decomposition in a Scots pine forest. VII. Canadian Journal of Botany 69, 1449-1456.

Bini, D., Dos Santos, C.A., Bouillet, J.-P., de Morais Goncalves, J.L., Cardoso, E.J.B.N., 2013. Eucalyptus grandis and *Acacia mangium* in monoculture and intercropped plantations: Evolution of soil and litter microbial and chemical attributes during early stages of plant development. Applied Soil Ecology 63, 57-66.

Bissett, A., Richardson, A.E., Baker, G., Thrall, P.H., 2011. Long-term land use effects on soil microbial community structure and function. Applied Soil Ecology 51, 66-78.

Bossuyt, H., Denef, K., Six, J., Frey, S., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16, 195-208.

Bradford, M.A., Tordoff, G.M., Eggers, T., Jones, T.H., Newington, J.E., 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. Oikos 99, 317-323.

Brussaard, L., 1998. Soil fauna, guilds, functional groups and ecosystem processes. Applied Soil Ecology 9, 123-135.

Carnovale, D., Bissett, A., Thrall, P.H., Baker, G., 2019. Plant genus (*Acacia* and *Eucalyptus*) alters soil microbial community structure and relative abundance within revegetated shelterbelts. Applied Soil Ecology 113, 1-11.

Chapman, S., Newman, G., 2010. Biodiversity at the plant–soil interface: microbial abundance and community structure respond to litter mixing. Oecologia 162, 763-769.

Chapman, S.K., Koch, G.W., 2007. What type of diversity yields synergy during mixed litter decomposition in a natural forest ecosystem? Plant and Soil 299, 153-162.

Cleugh, H.A., Prinsley, R., Bird, P.R., Brooks, S.J., Carberry, P.S., Crawford, M.C., Jackson, T.T., Meinke, H., Mylius, S.J., Nuberg, I.K., Sudmeyer, R.A., Wright, A.J., 2002. The Australian National Windbreaks Program: overview and summary of results. Australian Journal of Experimental Agriculture 42, 649-664.

Coq, S., Souquet, J.-M., Meudec, E., Cheynier, V., Hättenschwiler, S., 2010. Interspecific variation in leaf litter tannins drives decomposition in a tropical rain forest of French Guiana. Ecology 91, 2080-2091.

Culman, S., Bukowski, R., Gauch, H., Cadillo-Quiroz, H., Buckley, D., 2009. T-REX: software for the processing and analysis of T-RFLP data. BMC Bioinformatics 10, 1-10.

Dorrepaal, E., Cornelissen, J.H., Aerts, R., 2007. Changing leaf litter feedbacks on plant production across contrasting sub-arctic peatland species and growth forms. Oecologia 151, 251-261.

Fanin, N., Fromin, N., Bertrand, I., 2016. Functional breadth and home-field advantage generate functional differences among soil microbial decomposers. Ecology 97, 1023-1037.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecology Letters 12, 1238-1249.

Freschet, G.T., Aerts, R., Cornelissen, J.H.C., 2012. Multiple mechanisms for trait effects on litter decomposition: moving beyond home-field advantage with a new hypothesis. Journal of Ecology 100, 619-630.

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E., Parton, W.J., 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Global Change Biology 6, 751-765.

Gießelmann, U.C., Martins, K.G., Brändle, M., Schädler, M., Marques, R., Brandl, R., 2011. Lack of home-field advantage in the decomposition of leaf litter in the Atlantic Rainforest of Brazil. Applied Soil Ecology 49, 5-10.

Goddard, M.R., Bradford, M.A., 2003. The adaptive response of a natural microbial population to carbon-and nitrogen-limitation. Ecology Letters 6, 594-598.

Goldfarb, K.C., Karaoz, U., Hanson, C.A., Santee, C.A., Bradford, M.A., Treseder, K.K., Wallenstein, M.D., Brodie, E.L., 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Frontiers in Microbiology 2, 94.

Guo, L.B., Sims, R.E.H., 2001. Eucalypt litter decomposition and nutrient release under a short rotation forest regime and effluent irrigation treatments in New Zealand: I. External effects. Soil Biology and Biochemistry 33, 1381-1388.

Hanson, C.A., Allison, S.D., Bradford, M.A., Wallenstein, M.D., Treseder, K.K., 2008. Fungal taxa target different carbon sources in forest soil. Ecosystems 11, 1157-1167.

Hobbie, S.E., 1992. Effects of plant species on nutrient cycling. Trends in Ecology and Evolution 7, 336-339.

Jiang, Y., Yin, X., Wang, F., 2013. The influence of litter mixing on decomposition and soil fauna assemblages in a *Pinus koraiensis* mixed broad-leaved forest of the Changbai Mountains, China. European Journal of Soil Biology 55, 28-39.

Keeler, B.L., Hobbie, S.E., Kellogg, L.E., 2009. Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter decomposition. Ecosystems 12, 1-15.

Koutika, L.-S., Epron, D., Bouillet, J.-P., Mareschal, L., 2014. Changes in N and C concentrations, soil acidity and P availability in tropical mixed acacia and eucalypt plantations on a nutrient-poor sandy soil. Plant and Soil 379, 205-216.

O'Connell, A., 1986. Effect of legume understorey on decomposition and nutrient content of eucalypt forest litter. Plant and Soil 92, 235-248.

Paul, K.I., Polglase, P.J., 2004. Prediction of decomposition of litter under eucalypts and pines using the FullCAM model. Forest Ecology and Management 191, 73-92.

Paul, K.I., Polglase, P.J., Nyakuengama, J.G., Khanna, P.K., 2002. Change in soil carbon following afforestation. Forest Ecology and Management 168, 241-257.

Prescott, C., Zabek, L., Staley, C., Kabzems, R., 2000. Decomposition of broadleaf and needle litter in forests of British Columbia: influences of litter type, forest type, and litter mixtures. Canadian Journal of Forest Research 30, 1742-1750.

Resh, S.C., Binkley, D., Parrotta, J.A., 2002. Greater soil carbon sequestration under nitrogen-fixing trees compared with *Eucalyptus* species. Ecosystems 5, 217-231.

Rietl, A.J., Jackson, C.R., 2012. Effects of the ecological restoration practices of prescribed burning and mechanical thinning on soil microbial enzyme activities and leaf litter decomposition. Soil Biology and Biochemistry 50, 47-57.

Saiya-Cork, K., Sinsabaugh, R., Zak, D., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biology and Biochemistry 34, 1309-1315.

Singh, B.K., Nazaries, L., Munro, S., Anderson, I.C., Campbell, C.D., 2006. Use of multiplex terminal restriction fragment length polymorphism for rapid and simultaneous analysis of different components of the soil microbial community ▿. Applied and Environmental Microbiology 72, 7278-7285.

Sinsabaugh, R., Carreiro, M., Repert, D., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1-24.

Sinsabaugh, R., Findlay, S., 1995. Microbial production, enzyme activity, and carbon turnover in surface sediments of the Hudson River estuary. Microbial Ecology 30, 127-141.

Sinsabaugh, R., Moorhead, D., 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26, 1305-1311.

Sinsabaugh, R.L., Antibus, R., Linkins, A., McClaugherty, C., Rayburn, L., Repert, D., Weiland, T., 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology 74, 1586-1593.

Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75, 201-215.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252-1264.

Sinsabaugh, R.L., Saiya-Cork, K., Long, T., Osgood, M.P., Neher, D.A., Zak, D.R., Norby, R.J., 2003. Soil microbial activity in a Liquidambar plantation unresponsive to CO_2-driven increases in primary production. Applied Soil Ecology 24, 263-271.

Slade, E.M., Riutta, T., 2012. Interacting effects of leaf litter species and macrofauna on decomposition in different litter environments. Basic and Applied Ecology 13, 423-431.

Smith, C.J., Danilowicz, B.S., Clear, A.K., Costello, F.J., Wilson, B., Meijer, W.G., 2005. T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. FEMS Microbiology Ecology 54, 375-380.

Strickland, M.S., Lauber, C., Fierer, N., Bradford, M.A., 2009a. Testing the functional significance of microbial community composition. Ecology 90, 441-451.

Strickland, M.S., Osburn, E., Lauber, C., Fierer, N., Bradford, M.A., 2009b. Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. Functional Ecology 23, 627-636.

Talbot, J.M., Treseder, K.K., 2012. Interactions among lignin, cellulose, and nitrogen drive litter chemistry–decay relationships. Ecology 93, 345-354.

Toky, O., Singh, V., 1993. Litter synamics in short-rotation high density tree plantations in an arid region of India. Agriculture, Ecosystems and Environment 45, 129-145.

Veen, G., Freschet, G.T., Ordonez, A., Wardle, D.A., 2015. Litter quality and environmental controls of home-field advantage effects on litter decomposition. Oikos 124, 187-195.

Voigtlaender, M., Laclau, J.-P., Gonçalves, J.L.d.M., Piccolo, M.d.C., Moreira, M.Z., Nouvellon, Y., Ranger, J., Bouillet, J.-P., 2012. Introducing *Acacia mangium* trees in *Eucalyptus grandis* plantations: consequences for soil organic matter stocks and nitrogen mineralization. Plant and Soil 352, 99-111.

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecological Applications 14, 1172-1177.

Wallenstein, M.D., Haddix, M.L., Ayres, E., Steltzer, H., Magrini-Bair, K.A., Paul, E.A., 2013. Litter chemistry changes more rapidly when decomposed at home but converges during decomposition–transformation. Soil Biology and Biochemistry 57, 311-319.

Ward, S.E., Orwin, K.H., Ostle, N.J., Briones, M.J., Thomson, B.C., Griffiths, R.I., Oakley, S., Quirk, H., Bardgett, R.D., 2015. Vegetation exerts a greater control on litter decomposition than climate warming in peatlands. Ecology 96, 113-123.

Ward, S.E., Ostle, N.J., McNamara, N.P., Bardgett, R.D., 2010. Litter evenness influences short-term peatland decomposition processes. Oecologia 164, 511-520.

Wardle, D., Bonner, K., Barker, G., 2002. Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. Functional Ecology 16, 585-595.

Waring, B.G., 2013. Exploring relationships between enzyme activities and leaf litter decomposition in a wet tropical forest. Soil Biology and Biochemistry 64, 89-95.

Wickings, K., Grandy, A.S., Reed, S.C., Cleveland, C.C., 2012. The origin of litter chemical complexity during decomposition. Ecology letters 15, 1180-1188.

Zheng, J.Q., Han, S.J., Wang, Y., Zhang, C.G., Li, M.H., 2010. Composition and function of microbial communities during the early decomposition stages of foliar litter exposed to elevated CO_2 concentrations. European Journal of Soil Science 61, 914-925.

Appendix 1: Paper II supplementary data

Table S1: Similarity matrix between soil edaphic properties

	pH	Ec mS	moisture %	MBC	N	MBN	C	C:N	NH_4^+-N	NO_3-N	P	K	S	Cu	Fe	Mn	Zn	Al	Ca	Mg	Exc. Na	Clay	Sand	Silt
pH																								
Ec mS	-0.66																							
moisture %	0.31	-0.22																						
MBC	-0.25	0.44	-0.23																					
N%	-0.16	0.30	-0.10	0.28																				
MBN	0.01	0.36	0.05	0.65	0.30																			
C%	-0.11	0.26	0.03	0.20	***0.83***	0.22																		
C:N	0.13	-0.04	0.33	-0.10	-0.27	-0.05	0.28																	
NH_4^+-N	-0.30	0.20	0.04	0.05	0.10	-0.14	0.04	-0.15																
NO_3-N	-0.27	0.24	-0.38	0.05	0.59	-0.08	0.54	-0.17	0.16															
P	-0.22	0.20	0.05	0.28	0.48	0.12	0.47	-0.05	0.31	0.32														
K	-0.19	0.22	-0.25	-0.01	0.19	-0.03	0.05	-0.29	0.30	0.17	-0.01													
S	-0.11	0.24	0.07	0.18	0.67	0.16	***0.75***	0.14	0.10	0.49	***0.81***	-0.08												
Cu	0.34	0.06	0.47	-0.15	0.04	0.11	0.24	0.42	-0.10	-0.18	-0.05	-0.14	0.32											
Fe	-0.31	0.14	-0.02	0.21	0.42	0.04	0.40	-0.08	0.39	0.42	***0.88***	-0.20	0.64	-0.25										
Mn	0.18	0.01	-0.04	-0.26	-0.18	-0.12	-0.28	-0.14	-0.04	0.01	-0.69	0.16	-0.42	0.33	-0.67									
Zn	0.21	0.02	0.07	0.02	0.35	0.24	0.44	0.21	-0.36	0.12	-0.04	-0.17	0.39	0.44	-0.11	0.05								
Al	-0.33	-0.04	-0.18	0.10	0.05	-0.22	0.01	-0.16	0.37	0.31	0.20	-0.02	-0.11	-0.65	0.44	-0.31	-0.64							
Ca	0.28	0.09	0.14	-0.03	0.32	0.25	0.48	0.35	-0.50	0.13	0.06	-0.21	0.53	0.68	-0.13	0.09	***0.85***	***-0.73***						
Mg	0.20	0.07	0.31	0.01	0.40	0.17	0.66	0.52	-0.18	0.15	0.21	-0.19	0.66	***0.72***	0.03	-0.07	***0.74***	-0.45	***0.83***					
Exc. Sodium	0.08	0.04	0.43	0.13	-0.06	0.08	0.06	0.25	0.36	-0.24	0.52	-0.21	0.29	0.32	0.41	-0.23	-0.07	-0.04	0.03	0.23				
% Clay	0.33	0.01	0.19	-0.16	-0.12	0.07	-0.02	0.20	-0.31	-0.14	-0.30	-0.22	0.06	***0.75***	-0.44	0.51	0.44	***-0.71***	0.61	0.36	-0.03			
% Sand	-0.13	0.00	0.02	0.26	0.35	0.07	0.38	0.10	0.13	0.17	0.48	0.08	0.39	-0.29	0.35	-0.58	0.07	0.36	-0.03	0.30	0.24	-0.69		
% Silt	0.00	-0.05	-0.13	-0.25	-0.42	-0.15	-0.53	-0.25	-0.04	-0.19	-0.51	0.03	-0.61	-0.08	-0.28	0.49	-0.36	-0.05	-0.34	-0.63	-0.33	0.30	***-0.90***	

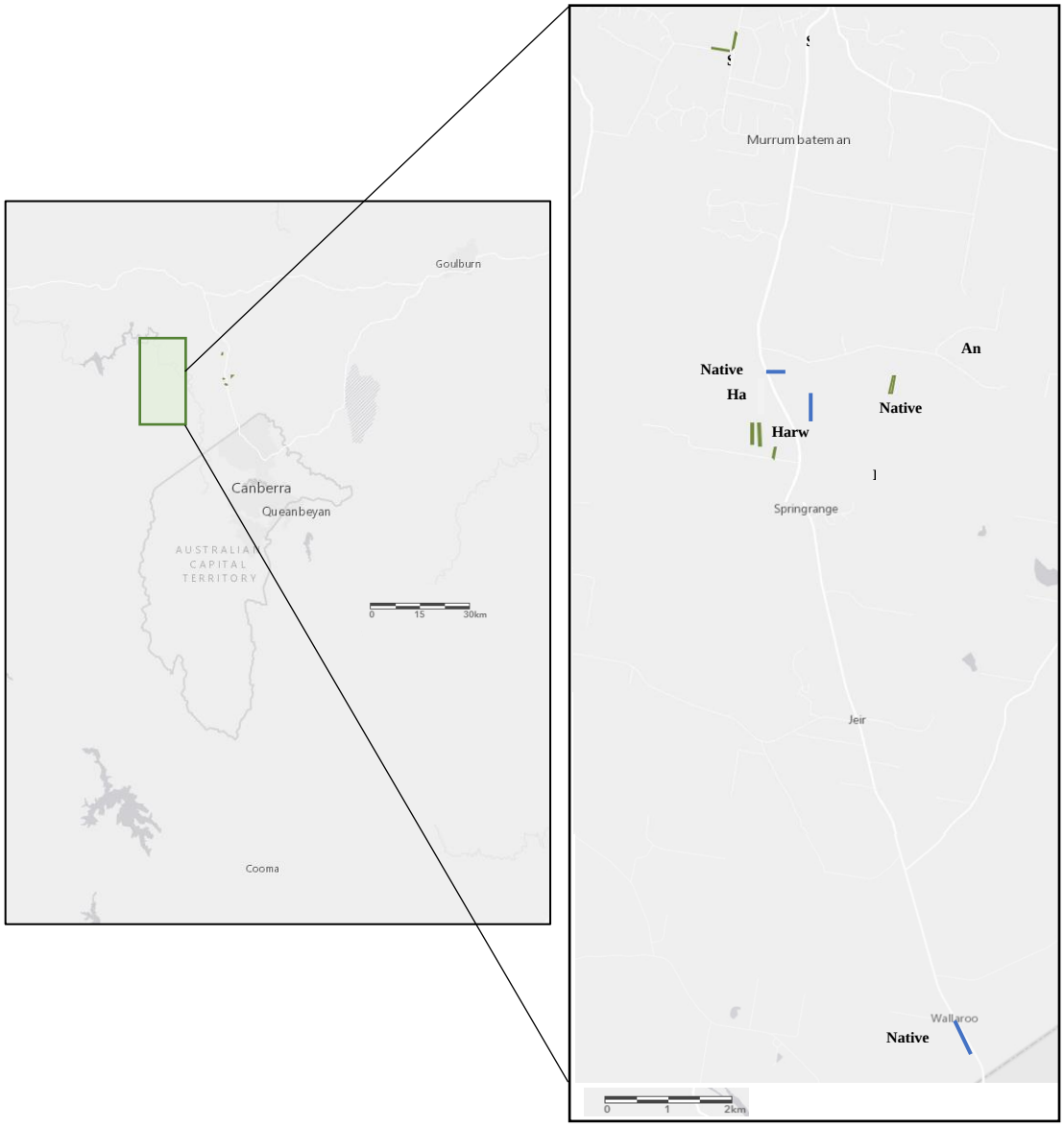
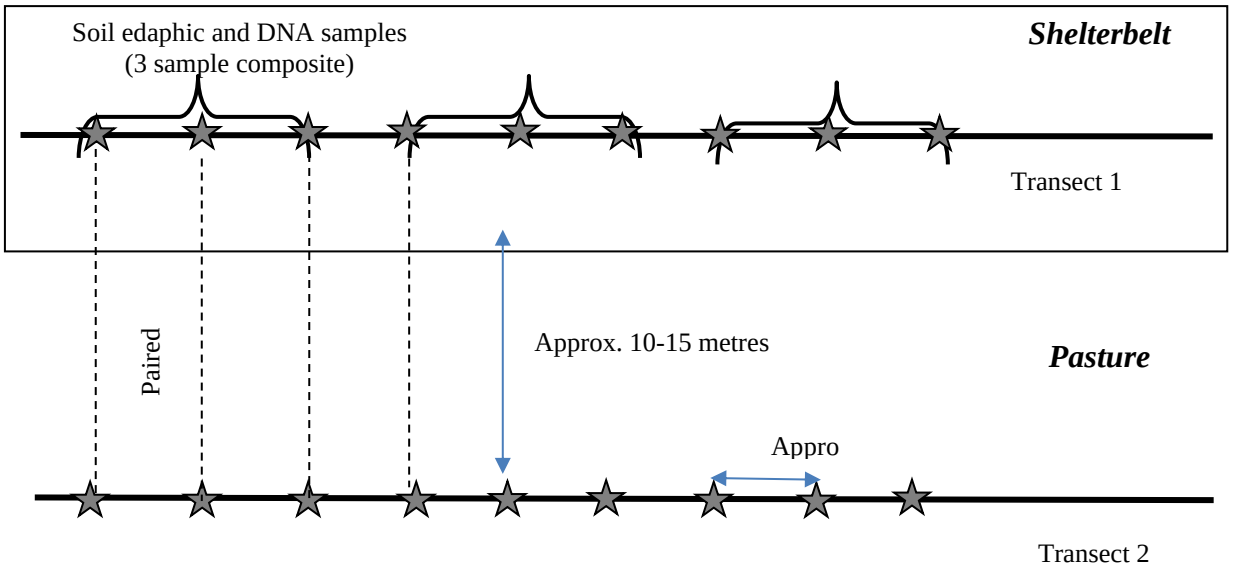


Figure S1. Study sites located with the Murrumbateman NSW region. Shelterbelts and paired pasture sites are shown in green, and native remnant sites are shown in blue.

Figure S2. Field experimental design for shelterbelt and pasture sites conducted at six sites.



Appendix 2: Paper III supplementary figures

Table S1. PERMANOVA pairwise testing microbial community structure in response to sample location (tree v interrow) by soil depth (0-10, 10-20 and 20-30cm).

Tree v interrow	**Fungi** ***P***	**Bacteria** ***P***
0-10cm	0.23	0.20
10-20cm	0.96	0.53
20-30cm	0.78	0.58

Table S2. The effect of site type (*Acacia*, *Eucalyptus* and pasture), soil depth (0-10, 10-20, 20-30) and sampling year (2011, 2012) on soil edaphic properties (ANOVA).

	Fe	Mn	Al	S	Zn	Mg	P	K	Na	*NH 4+*	EC	Cu	pH	moisture	*NO 3-*	N%	Ca	C:N	C%
site type	***	***	***	***	***	***	***	***	***	***	*	***	***	***	***	*	ns	ns	**
Depth	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Year	**	**	*	*	Ns	ns	**	***	***	**	**	ns	***	ns	ns	*	*	***	*
site type.depth	*	***	***	***	***	***	***	***	***	ns	**	***	ns	ns	***	ns	ns	ns	ns
site type.year	***	***	***	*	**	*	ns	*	**	*	ns	ns	ns	***	ns	ns	***	ns	ns
depth.year	ns	**	**	*	**	*	*	ns	ns	***	ns	*	ns	ns	ns	ns	ns	***	ns
site type.depth.year	*ns	ns	ns	ns	**	*	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Transformed	Y		Y		Y					Y									Y

*p<0.05,** p<0.01 and *** p<0.001

Sampling design		2011			2012		
Depth	Location	A	E	P	A	E	P
0-10	Under tree	6	6	3	6	6	6
	Inter row	6	6				
10-20	Under tree	3	3	3	6	6	6
	Inter row	3	3				
20-30	Under tree	3	3	3	6	6	6
	Inter row	3	3				

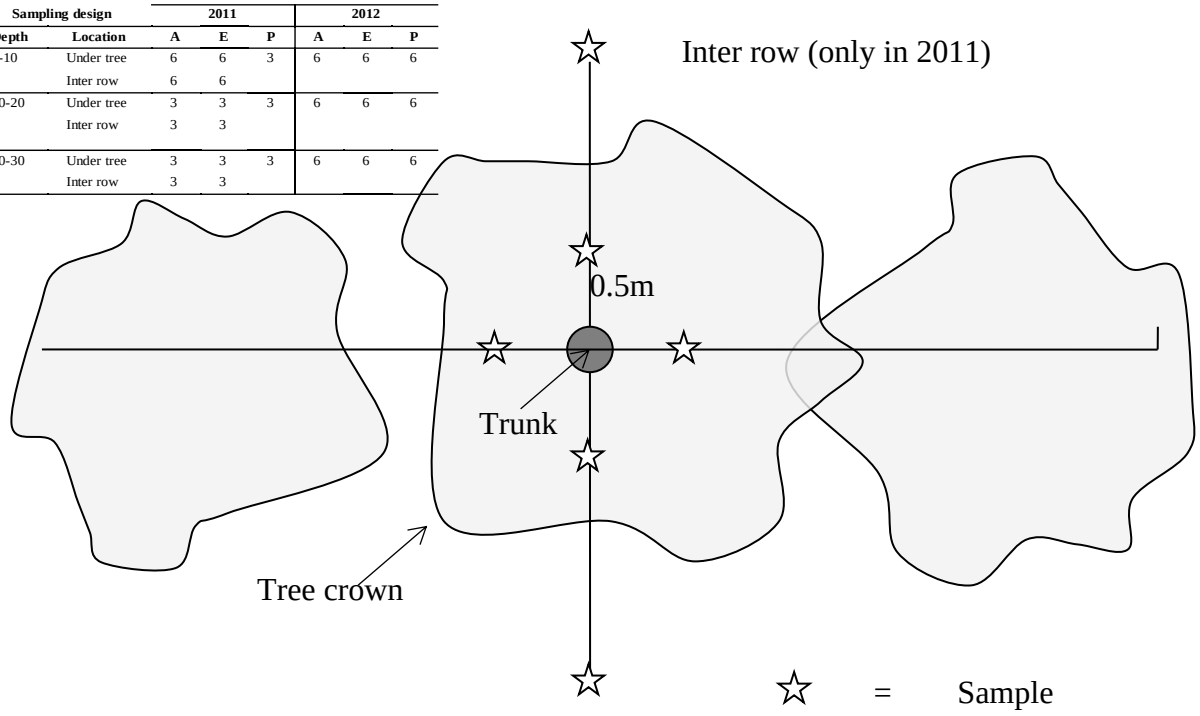


Figure S1. Soil sampling regime per tree (*Eucalyptus* n =6, *Acacia* n =6). Samples were located 0.5 m from the trunk, at 4 compass points equidistant from the trunk. Inter row samples were collected from two additional locations to a depth of 30cm only in the 2011 sampling period. A=*Acacia*, E=*Eucalyptus*, orange=pasture. Numbers in table indicate the number of composite samples per treatment (57 samples in 2011 and 54 samples in 2012).

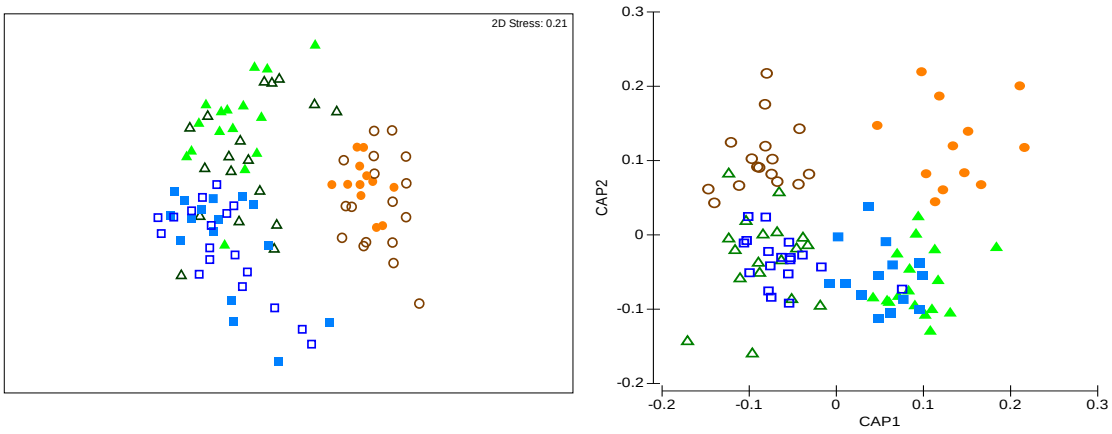


Figure S2. nMDS and CAP analysis based on Bray Curtis similarity measure of a) nMDS fungi community composition by site type and year across all soil depths. b) Cap analysis of bacteria by site type and year across all soil depths. ● pasture, ■ *Acacia*, ▲ *Eucalyptus*, hollow symbols=2011 sampling period; filled symbols=2012 sampling period.

Appendix 3: Paper IV supplementary table

Table S1: Pearson's correlation coefficients relating soil enzyme activities to litter decomposition.

	CBH	NAG	βG	AP	Litter decay constant	Litter loss %
CBH	-					
NAG	0.7577	-				
βG	0.8248	0.8546	-			
AP	0.2634	0.7099	0.6021	-		
Litter decay constant	-0.2983	-0.0401	-0.1404	0.3046	-	
Litter loss %	-0.0087	-0.2531	-0.0713	-0.4545	0.1955	-