THE UNIVERSITY of ADELAIDE

Nutrient Cycling Between Litters and Soil after Fire in Native Woodland and *Pinus radiata* Plantations

In partial fulfilment of requirement for the degree of Doctor of Philosophy

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May 2019

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Thesis abstract

Fire can change both the quantity and nature of soil organic matter during the event and can affect organic matter inputs after the event. These changes may affect the microbial biomass, nutrient availability and nutrient cycling, and may have flow on effects on soil carbon stocks and cycling. Post-fire effects may also affect success of established (re-sprouting) and recently recruited plants. In particular, changed organic matter inputs, such as the formation of a post-fire litter layer, represent a significant unknown in soil and fire ecology.

The aims of the work presented in this thesis focus on the effects of fire on microbial nutrient limitation, microbial decomposition of pre- and post-fire litters, and the effects of thermal alteration of pine needles on soil nitrogen cycling and the soil microbial biomass. The materials for this work were generated from forestry reserves that had recently been exposed to an uncontrolled fire. These reserves included *Eucalyptus* woodlands ('native') and *Pinus radiata* plantations ('pine'); sampling in all of these reserves initially occurred four months after the fire with further samples collected as described in Chapters 2, 3, and 6. To address the knowledge gaps, a series of field activities and experiments were conducted including: several soil surveys to determine temporal change in the soils after the fire; a study to determine microbial nutrient limitation in burnt and not burnt soils; a study to determine decomposition dynamics of native and pine litters; a study to determine decomposition of pine litters and char during a 3 month exposure; a study using thermally altered fresh pine needles to explore nitrogen cycling; and a soil microbiome study (Next Gen sequencing) using four amendments from the thermal alteration study to determine microbial responses to the post fire litter layer.

The results of these field activities and laboratory experiments indicated that the forestry reserve soils were not strongly affected by fire when nutrient availability and microbial nutrient limitation are considered. The post-fire pine litter, however, caused significant disturbances to nitrogen cycling when soils were incubated with pine litter collected from the field and when incubated with thermally altered needles generated under laboratory conditions. Post-fire pine litters and pine needles heated experimentally to temperatures $\leqslant$ 200ºC absorbed mineral nitrogen, preventing its extraction from soil and litter mixes. This ability was lost in needles heated experimentally to >200ºC. This temperature was associated with the degradation of polysaccharides and represented a step change in microbial activity and, potentially, in microbial accessibility to the added organic matter.

The major conclusions from this work are that soil nutrient content and availability were not strongly affected by the fire event in native and pine forest reserves, but that fire affected pine litter has strong nitrogen absorption properties that are likely to affect the mineral N pools available for the regeneration of forest growth. It is also clear that a post-fire litter layer in pine forests can have distinctly different effects on the soil environment depending on canopy temperature conditions during the fire: post-fire litters composed of low temperature needles absorb most mineral nitrogen that they contact while high temperature needles appear relatively inert. This relationship was affected by a thermal tipping point at approximately 200ºC (detected using a combination of soil respiration and solid state ^{13}C CP-MAS NMR spectroscopy). This research outlines an important knowledge gap in short term forest nutrient cycling and microbial responses to fire that may affect forest fire emissions estimates.

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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Erinne Stirling
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Acknowledgements

I am grateful to many people for their assistance during my PhD, and would like to thank some particularly important people.

First, and most importantly, I would like to thank Associate Professor Timothy Cavagnaro for his guidance and contributions throughout this project. I truly benefitted from his flexible and diplomatic approach to supervision. Thanks are also due to late additions to the supervisory panel: Associate Professor Ronald Smernik and Dr Lynne Macdonald. I thank Ron for encouraging interesting lines of thought and Lynne for rekindling my drive through her infectious enthusiasm.

I acknowledge Professor Petra Marschner for her contributions to the early work in this project and am extremely grateful to Dr Matthew Denton for his excellent work as Post Graduate Coordinator.

I would like to acknowledge the funding provided by The Holsworth Wildlife Research Endowment & The Ecological Society of Australia and the Nature Foundation of South Australia. The funds from these philanthropic associations allowed a more varied and interesting set of experiments than would otherwise been permitted. Travel funding was also provided by the Australian Society of Soil Science Inc. (Federal branch) for travel to the 21st World Congress of Soil Science in Rio de Janeiro, Brazil, and on three occasions was provided by the Australian Society of Soil Science Inc. (South Australia branch) to participate in Soil Judging events in Western Australia, Queensland, and Canberra.

Gratitude also goes to my partner, aunt, friends, fellow PhD students, other members of the soil ecology group, and members of the soil science community. In particular, I thank Clint Garrett, Patricia Aller, Yanchen Zhang, Ashlea Doolette, Rebecca Stonor, Jess Mackay, Christina Asanopoulos, and Supriya Lath for support, compassion, and unending reliability.

Lastly, I would like to thank the support staff of The Waite for doing all those things that keep the place running. In particular, thanks to Janet Penhall, Nicola Featherstone, and numerous others for keeping us organized and safe, and for providing those services that we couldn't work without.

Associated presentations

Bold indicates presenting author.

EcoTAS 2017 Hunter Valley, New South Wales, Australia

Stirling, E., Macdonald, L.M., Smernik, R.J. and Cavagnaro, T.R.

Post fire soil and litter decomposition dynamics under native and pine forest reserves[1].

World Soil Congress 2018 Rio de Janeiro, Brazil

Stirling, E., Smernik, R.J., Macdonald, L.M. and Cavagnaro, T.R.

Fire influences on litter decomposition: C chemistry and N transformations in thermally altered *Pinus radiata* needles[2].

National Soils Conference 2018 Canberra, Australian Capital Territory, Australia

Stirling, E., Macdonald, L.M., Smernik, R.J. and Cavagnaro, T.R.

Fire influences on decomposition: microbial diversity in soils incubated with thermally altered *Pinus radiata* needles[3].

The University of Adelaide School of Agriculture, Food and Wine Annual Research Day 2018 Adelaide, South Australia, Australia

Stirling, E., Smernik, R.J., Macdonald, L.M. and Cavagnaro, T.R.

Forests, fire, fungi: a nitrogen story[4].

[1] Associated with Chapter 4
[2] Associated with Chapter 6
[3] Associated with Chapter 7
[4] Associated with Chapter 8

Associated grants

Research grants

Holsworth Wildlife Research Endowment and The Ecological Society of Australia

2015 $7,440 AUD
2016 $6,480 AUD
2017 $6,900 AUD

Nature Foundation of South Australia PhD/Masters Scholarship Grant

2015 $2,000 AUD
2016 $1,500 AUD

Travel grants

Australian Soil Science Society Inc. Federal Branch World Soil Congress Travel Scholarship

2018 $3,000 AUD

Australian Soil Science Society Inc. South Australia State Branch National Soils Conference Travel Award

2018 $500 AUD

Total grant funds associated with this project

2015-2018 $27,820 AUD

Chapter 1 – Introduction

This thesis is presented as a series of journal manuscripts and articles, each of which contains a more detailed literature review. However, to give context to the overall project, a short literature review prepared at the start of the project is now given. The literature review below represents the state of the literature as of May 2015. Please see individual chapters for more detailed and current literature relating to specific research activities.

1.1 Literature review

Fire is an integral part of some Australian landscapes and can be considered the primary consumer of energy in Australia's nutrient poor environments (Orians and Milewski 2007). It is an important ecological driver as burning creates a patchwork of ecosystems in varying stages of recovery (Penman et al. 2007). Fire is important for the reproduction and establishment of some native plant and fungal species (Orians and Milewski 2007, McMullan-Fisher et al. 2011). In addition to its natural functions, fire is also used to control woody weeds, and restore environmental function in Mediterranean and semi-arid environments (Blank et al. 2003, Dai et al. 2006). This literature review describes the effect of fire on soil nutrient cycling and litter decomposition.

1.1.1 General fire effects on soil nutrient resources

Fire has direct and indirect effects on the plant community through combustion, nutrient cycling, and changed soil properties. It has a strong effect on vegetation structure as even low intensity fires can change an understory shrub dominated system into a grass dominated system (Close et al. 2011). Fire enhances coarse particulate matter mineralisation through pyro-mineralisation and the absence of fire can cause organic matter accumulation (Hinojosa et al. 2012). Fire affects soil nutrient cycling directly through heat and ash deposition, and indirectly by changing the quantity and nature of organic matter (González-Pérez et al. 2004, Caon et al. 2014). Although heat does not penetrate deep into the soil profile due to the insulating effect of soils, heating may accelerate some physical nutrient transformation processes and change other processes near the soil surface (Arocena and Opio 2003). Pyro-mineralisation of organic matter during fire affects soil pH. The loss of organic matter may decrease soil pH buffering capacity, leading to large changes in pH after fire (Noble et al. 1996). Soil pH strongly affects nutrient solubilisation and precipitation and favours some microbial groups over others. Burnt sites often have a higher pH than sites that are not burnt; however, the differences are usually not significant in the field (Tongway and Hodgkinson 1992, Arocena and Opio 2003).

When soil organic matter inputs such as leaf litter are considered, litterfall and decomposition are affected by temperature, recent rainfall and fire history (Penman and York 2010). Frequent fire may decouple nitrogen and phosphorus cycling and initiate a short term shift from phosphorus limitation to nitrogen limitation post-fire (Toberman et al. 2014). Nitrogen limitation should not be an issue, however, in Australian forests that develop a legume dominated understory (Attiwill and May 2001). Burning every two years significantly lowered freshly fallen litter decomposition rates in an Australian *Eucalyptus* forests (Toberman et al. 2014). The fresh litter from these sites had higher a C:N ratio and lower C:P ratio than sites that were not burnt indicating nitrogen loss and phosphorus enrichment (Toberman et al. 2014).

Overall, literature on the effect of fire on soils and nutrient cycling in Australian forestry systems is limited and therefore our understanding of these effects is incomplete. Fire will decrease

aboveground biomass and may increase or decrease carbon, nitrogen, and phosphorus resources in the soil. Fire may increase or decrease the microbial biomass and microbial activity, which may then increase or decrease carbon, nitrogen, and phosphorus mineralisation. Litterfall after a fire may be affected by soil nutrient limitation, meaning post-fire litters may have changed nutrient profiles. Overall, the literature indicates large uncertainties in both the direction and magnitude of change in nutrient cycling after a fire.

1.1.1.1 Carbon

Carbon (C) is present as inorganic and organic forms in the soil. Inorganic C is found in high pH soils as carbonate compounds and, apart from forming insoluble precipitates with some base cations, is not an important part of nutrient cycling. Organic C is found in all organic compounds and is used as a microbial energy source. Organic C availability limits microbial activity in soils and microbes quickly react to the addition of available organic C (Hoyle et al., 2008, Mondini et al., 2006).

Fire may increase, decrease, or have no significant effect on soil organic C (SOC) depending on fire intensity, vegetation dynamics and time elapsed since the fire event (Serrasolsas and Khanna 1995, Prieto-Fernández et al. 1998, Castelli and Lazzari 2002). Fire frequency may have cumulative negative effects in otherwise stable landscapes (Bird et al. 2000, Bastias et al. 2006) while sites that are degraded may not experience significant, or measurable, changes in SOC if carbon inputs and organic matter retention are low (Tongway and Hodgkinson 1992, Guénon et al. 2013). Fire prevention may cause an increase in SOC; however, fuel accumulation is a significant fire risk and, in addition to consuming the entire litter C, a single intense fire may consume 50% of the SOC in the top 5 cm after fire suppression (Bird et al. 2000). Site specific variables such as rainfall and plant biomass recovery influence SOC post-fire and strongly influence fire effects, particularly in water limited environments (Granged et al. 2011).

Fire intensity has a significant effect on SOC content with low intensity fire increasing the SOC content through changes in primary productivity, organic matter quality, or micro-climate (Ansley et al. 2006, Dai et al. 2006), and moderate and high intensities decreasing the SOC content (Guénon et al. 2013, Heath et al. 2015). When considered in Australian landscapes, low intensity prescribed fires in Australia's eastern states and the Adelaide Hills (SA) consumed 4-9% of the aboveground C; these fires had no detectable effect on the SOC in the top 10 cm (Volkova and Weston 2015). Low intensity fires increased SOC in the top 5 cm at two NSW woodland sites, possibly due to the addition of pyrogenic organic C (Heath et al. 2015).

Pyrogenic organic C includes carbon compounds which have experienced some degree of thermal alteration leading to the substitution of organically formed carbon bonds to heat induced carbon bonds; these compounds may have long term effects on the SOC pool. Charcoal inputs may appear to compensate for organic C losses at the soil surface but may not compensate the microbial biomass if they are highly stable organic compounds resistant to decomposition (Guinto et al. 1999, Guénon et al. 2013). Thermally altered organic structures may significantly reduce decomposition rate (Baldock and Smernik 2002, Guénon et al. 2013) particularly when they have a particle size greater than 1 mm (Jenkins et al. 2014). Fine pyrogenic C particles may increase (Jenkins et al. 2014) or decrease microbial respiration (Fritze et al. 1998). Pyrogenic organic C may change future soil nitrogen cycling through lower concentrations of phenolic and labile C compounds (Guénon et al. 2013).

1.1.1.2 Nitrogen

Nitrogen (N) is required by all organisms for cellular reproduction and protein synthesis. A small amount of nitrogen may be provided through mineral weathering with some sedimentary and metasedimentary rocks containing environmentally significant quantities of nitrogen (Holloway and Dahlgren 2002). However, most nitrogen must be converted from atmospheric N or recycled through the soil from organic matter decomposition before it can be used by living organisms. Nitrogen is found in oxidised and reduced inorganic forms in the soil and within organic molecules in soils and litters; the majority of soil N is in organic forms (95%) (Schulten and Schnitzer 1998). Nitrogen content and availability relies on organic matter breakdown by microorganisms and therefore relies on organic C availability. Soil N may be lost from these forestry systems through leaching, erosion, and volatilisation.

Fire does not usually have a significant effect on total soil N as fire induced changes are small compared to the amount of nitrogen in the soil (Wan et al. 2001). However, studies have found that fire increases or decreases soil total N content depending on intensity and the time elapsed since a fire (Bastias et al. 2006, Dai et al. 2006). Fires can volatize approximately 90% of biomass N, with approximately a third of total N volatilising at temperatures up to 250°C (Lobert et al. 1990, Badía and Martí 2003). Fire may also change the C:N ratio of soil as nitrogen containing organic matter is more resistant to heating than C (Badía and Martí 2003, Duguy et al. 2007, Knicker 2010).

Fire can have measurable effects on mineral N (N_{min}) by increasing soil ammonium and nitrate/nitrite concentrations through pyromineralisation. Changes in N_{min} concentration vary strongly with time elapsed after fire. For example, in Mediterranean climate zones, ammonium concentration increases immediately after fire and then decreases asymptotically while nitrate concentration lags behind ammonium and is more variable (Bauhus et al. 1993, Wan et al. 2001, Caon et al. 2014). Combustion increases N_{min} through physical mineralisation and burning can enhance N_{min} concentrations for several seasons (Prober et al. 2008). If the O-horizon and ash remain *in situ*, soil organisms can take advantage of the increased availability.

In Australian wet and dry sclerophyllous forests burnt every two or four years there was a significant effect on N_{min} pools when averaged for all sampling times; burn frequency had a significant negative effect on cumulative N mineralisation (Guinto et al. 1999). Infrequent fire events can increase net N mineralisation (Carreira et al. 1994, Wang et al. 2014) while increasing fire frequency can decrease it (Bastias et al. 2006). Nitrification may be suppressed post-fire because of low microbial biomass, SOC, or changed organic matter quality (Guénon et al. 2013). However, nitrification may appear to increase after fire because of decreased nitrate uptake by the microbial biomass (Koyama et al. 2010). In ecologically similar systems, results from solid-state ^{15}N NMR indicate fire causes an accumulation of pyrrole-type N; fire can introduce new chemical compounds to the environment (Knicker et al. 2005).

1.1.1.3 Phosphorus

Phosphorus (P) is a structural component of genetic information, cell membranes, and cellular energy transfer. Phosphorus cycling is a largely a closed system, with atmospheric and leaching losses small compared to the overall phosphorus soil pool (Stevenson and Cole 1999). Native soil P is derived from apatite minerals and is usually present in an oxidised form (orthophosphate) that is negatively charged and may be sorbed to soil particle surfaces via cations such as calcium,

iron, and aluminium (Smits et al. 2012). Additional phosphorus may be introduced into the environment through fertilizer application.

Fire can increase or decrease plant available P concentrations through its effects on mineralisation and the activity of phosphatase enzymes. Some studies have found that burning increases plant available P concentration with the effect increasing with fire frequency (Tongway and Hodgkinson 1992, Prober et al. 2008) while other studies found that burning has no measurable effect on phosphorus availability (Arocena and Opio 2003, Guénon et al. 2013). Hinojosa et al. (2012) found that fire increases phosphorus mineralisation in pools with short turnover time but does not affect concentrations of mid and slow turnover phosphorus pools. The effect of fire on phosphorus availability appears to be temperature sensitive as Badía and Martí (2003) reported that available P concentrations increased when soils were exposed to temperatures ranging from 150°C to 500°C. Fire may also affect future mineralisation potential through its effects on phosphatase enzymes with burnt sites having reduced phosphatase activity (Blank et al. 2003, Hinojosa et al. 2012). While there has been relatively little research in similar native ecological systems in Australia, phosphorus responses may be important in our strongly phosphorus limited systems.

1.1.2 Fire and soil biology

Soil biological properties include the living organisms in the soil and their biological processes. Fire affects biological soil properties by reducing the quantity and changing the quality of organic nutrients, changing the chemical properties, and perturbing the microbial population (Palese et al. 2004). The magnitude and direction of these effects are influenced by initial soil conditions, inherent soil properties, fire intensity and soil depth (Hamman et al. 2007). For example, moist heat is a more effective biocide than dry heat; therefore microbial death during fire is greater in moist soils (Mataix-Solera et al. 2011). The overall effect of heating is modulated by the heat capacity of the soil, the heat penetration, and the vertical profile distribution of the microbes. In some cases, long term conditions are more important than direct fire effects as vegetation type and management practices that affect pH and soil organic matter are important regulators of gross microbial composition (Bååth et al. 1995, Cookson et al. 2007).

Fire has been found to affect soil microbial community structure, microbial density and population fluctuations, and to have no observable effect on species richness in Mediterranean forests and shrublands (Vázquez et al. 1993, Bååth et al. 1995, Goberna et al. 2012). Fire frequency has a significant negative relationship with soil heterotrophic respiration in Australian woodlands (Greene et al. 1990, Tongway and Hodgkinson 1992, Holden and Treseder 2013). Palese et al. (2004) concluded that fire-induced increases in mineral nutrient availability may be too short-lived to influence microbial populations. Furthermore, the lack of soil organic matter will affect microbial populations until it is replenished.

Fungi are often more strongly affected by fire than other microorganisms, with 30 years of prescribed fire every two or four years resulting in a significantly altered soil fungal community structure in a wet sclerophyll forest (Bastias et al. 2006, Guénon et al. 2013). However, the relationships between fire and fungi are variable with some fungi experiencing local extinctions after fire and others requiring fire to complete their life cycle (McMullan-Fisher et al. 2011). In a review of the relationships between fire and fungi, McMullan-Fisher et al. (2011) found that fire interval studies of microbial diversity do not show a consistent relationship, and that there is

a major problem with quantifying and understanding these relationships due to uncertainties in fungal species identification and classification.

1.1.3 Study materials, aims and objectives

All soils, litters, and foliage used in this work were collected from Old Kersbrook Forest in the Adelaide Hills, South Australia. This forestry system is described in detail in Chapter 2 but will be briefly introduced here. Old Kersbrook Forest contains 'native' forest reserves which are maintained for conservation and recreational use and 'pine' forest reserves maintained for *Pinus radiata* timber production. While surrounding areas of the Adelaide Hills are relatively productive, this site is constrained by light textured, skeletal soils formed on ancient residuum (the Gawler Complex; >1 000 Ma). Due to the age of the parent material, these soils are severely phosphorus limited; the native vegetation of the region is low open woodlands (Figure 1.1). Commercial forestry at this site uses fertilisers to supplement the poor soil conditions.

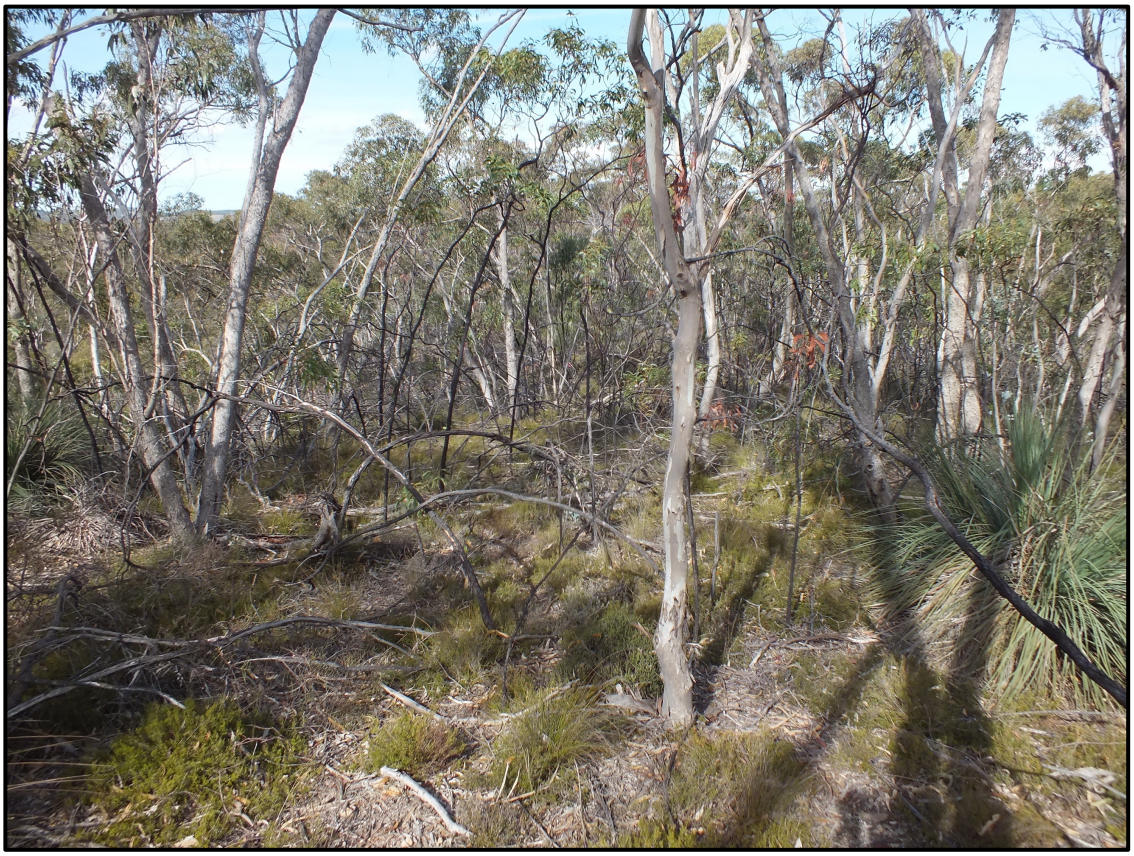

Figure 1.1 View from site 1 in Old Kersbrook Forest, during the autumn of 2015. This view is dominated by *Eucalyptus fasciculosa* ('pink gum'; small tree) with occasional *Xanthorrhoea semiplana* ('yakka' or 'grass tree'; medium shrub).

Aims of this project include:

- Determine the effects of fire on soil nutrient content.
- Determine the effects of fire on microbial nutrient limitation.
- Explore decomposition dynamics of native and pine litters affected by fire.
- Determine the effects of heating temperature on litter decomposition.
- Determine the effects of needle heating temperature on subsequent microbial decomposition.

One of the challenges faced during this project include the inherent heterogeneity of forest soils, particularly as the study sites contain many rocky outcrops (Zhou et al. 2010). Heterogeneity is imposed at small and large scales; for example, through site history (such as fire history) and root architecture (Cohn et al. 2015, Lin and Zeng 2017). The main sampling method in this project used to reduce soil heterogeneity included taking many subsamples of soil to bulk per plot or site (as explained in individual chapters); these 'bulked' soils where then used in the experiments. One negative effect of using this method includes the potential for pseudoreplication; this issue is discussed in Chapter 8.

1.2 Overall structure of the narrative

This body of work examines the relationships between soil, litter, and fire, and the role of fire affected litters in soil nutrient cycling in two forestry reserves. This is achieved in six chapters written in manuscript style (Figure 1.1)[5]. The first two manuscripts focus on direct fire effects on soil nutrient contents and subsequent nutrient limitation. Chapter 2 focusses on soil change over time via analyses of carbon and nutrient contents in soils from native and pine reserves that were not burnt or were burnt at two different severity ratings. The soils collected during the first survey (May 2015) were then used to determine microbial nutrient limitation in Chapter 3. Building on the finding that the soils of Old Kersbrook Forest were resilient against the effects of fire, the following chapters used more complex substrates to investigate the fire effects on litters. Therefore, Chapter 4 used a subset of soils and litters collected from the first survey to investigate litter decomposition dynamics. Results from Chapter 4 indicated significant differences between fire affected and not fire affected pine needles; these differences opened new avenues of investigation that were pursued in Chapters 5, 6, and 7. Chapter 5 investigates pine litters over a longer incubation period with the presence or absence of char as an additional factor. This experiment showed nitrogen cycling was strongly affected by fire history of the litter. The final two manuscripts used fresh pine needles that had been thermally altered experimentally to determine the effects of heating in the canopy on post-fire litter decomposition and nitrogen cycling. Chapter 6 investigates soil nitrogen cycling of thermally altered pine needles intensively over two weeks, wherein I found a thermal tipping point at approximately 200ºC by using a combination of soil respiration and solid state ^{13}C CP-MAS NMR spectroscopy. Characterisation of the needles and the results of the experiment in Chapter 6 informed the decision on which needles to include in the final experiment. Chapter 7 investigates the response of soil microbes to a subset of thermally altered pine needles over a period of 43 days using Next Gen Illumina sequencing. The thesis concludes with a general discussion of the main findings and relationships found herein (Chapter 8).

[5] Please note that the preliminary nature of Chapters 3 and 4 mean that these manuscripts are not suitable for publishing.

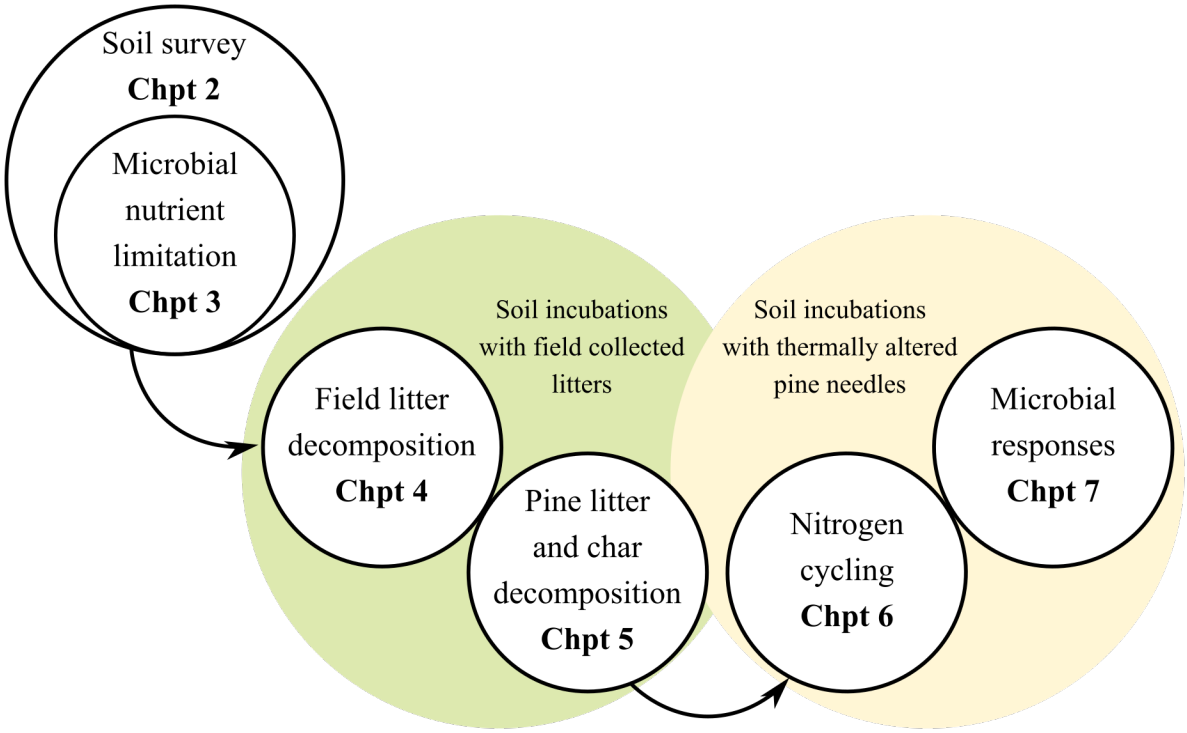

Figure 1.2 Contextual diagram of narrative flow for manuscripts within this thesis. 'Field litter' indicates both native and pine litters.

Overall, the narrative within this thesis starts broadly with the field survey, then focusses in on soil responses to increasingly complex inorganic and organic amendments. It finishes with synthesised post-fire litters (in the form of thermally altered pine needles), which give some indications of the role of fire in post-fire nutrient cycling in native and pine forests.

Statement of Authorship

Title of paper	Fire influences on soil nutrient cycling: A soil survey in the Eucalyptus woodlands and *Pinus radiata* plantations of Old Kersbrook Forest, South Australia
Publication status	☐ Published ☐ Accepted for publication ☐ Submitted for publication ☒ Unpublished and unsubmitted work written in manuscript style
Publication date	Not applicable

Principle Author

Name of principle author	Erinne Stirling		
Contribution to the paper	• Survey design, field work, laboratory analyses, and data analyses • Preparation of the manuscript		
Overall percentage (%)	95		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	**6/03/2019**

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of co-author	Timothy R. Cavagnaro		
Contribution to the paper	• Review and constructive comments on data analysis		
Overall percentage (%)	5		
Signature		Date	26/02/2019

Chapter 2 – Fire influences on soil nutrient cycling: a soil survey in Old Kersbrook Forest, South Australia

Written in the style of a regular paper – *Soil Biology and Biochemistry*

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2.1 Abstract

The ‘Sampson Flat Bushfire’ blackened more than 20,000 ha of rural and peri-urban land in January 2015. Substantial areas of forested land were affected by this fire, including ‘native’ forest reserves and managed *Pinus radiata* plantations. A small soil survey was conducted, seasonally, over 15 months (starting in the autumn of 2015) where soils in these reserves and plantations were analysed for total organic carbon, total organic nitrogen, microbial activity, mineral nitrogen and phosphorus, and microbial biomass phosphorus. The results of this survey were highly variable and did not show any obvious trend that could be linked universally to fire or vegetation type, neither was time since fire a significant factor. From this survey it is clear that fire affected native forest and pine plantations differently in Old Kersbrook Forest, and that these changes persist for more than a year after the event.

2.2 Introduction

Over eight days in January 2015, an uncontrolled fire burned more than 20,000 hectares of rural and peri-urban land in the Adelaide Hills, South Australia. Named the ‘Sampson Flat Bushfire’, this fire burned a wide variety of land use types including conservation and forestry reserves. This study is concerned with soils collected from ‘Old Kersbrook Forest’, a forestry reserve which is managed by a Government of South Australia owned corporation, *ForestrySA*, and which contains forest reserves for both environmental conservation and wood production. Reserves for environmental conservation (‘native’ reserves) within Old Kersbrook Forest generally consist of low open woodlands that are not intended for logging (i.e. they are maintained for environmental benefit and public recreation). Reserves for wood production (‘pine’ reserves) within Old Kersbrook Forest affected by the Sampson Flat Bushfire were managed *Pinus radiata* plantations that had been planted in the late 1980s. Managed forestry in South Australia typically occurs on land that is unsuitable for higher value uses (such as horticulture or cropping) because of limitations to soil productivity capacity, climate, topography, or distance to markets. In Old Kersbrook forest, limiting factors for productivity include shallow, sandy soils over rock and moderate slopes with rocky outcrops.

Fire can be expected to affect native and pine forests differently as each type of reserve has a different pattern of vegetation response to burning. Many native Australian trees, particularly in savanna type ecosystems such as is found in the Adelaide Hills and surrounds, have the capacity to resprout basally or epicormically after a fire (Clarke et al. 2015). Native trees in these woodlands that do not resprout may instead have fire triggered serotiny where seed release or germination is induced by events such as smoke exposure or seed heating (eg. *Acacia pycnantha*) (Brown et al. 2003). Plants that are summer dormant and present as subterranean organs only (such as tubers and rhizomes) may be largely unaffected by fire due to the insulating effect of soil particles (Badía et al. 2017). It is also probable that fires in each forest type are likely to generate different fire severities (see Keeley (2009) for definition): for example, canopy height in the native reserves of Old Kersbrook Forest was substantially lower than the pine reserves (canopy maximum heights <10 m and >30 m for native and pine, respectively), which may have increased the rate of canopy fires in the pine sites.

Pinus radiata is more sensitive to fire disturbances than the *Eucalyptus* species native to the study sites, and has characteristics of both fire evader and fire resistant plant species (Fernandes et al. 2008). These characteristics mean that mature *P. radiata* trees can withstand low severity fires but that they struggle to survive higher severity fires. Although *P. radiata* can survive low

severity fires, bole growth and litterfall may be reduced for several years after the event (Williams and Wardle 2007, Seifert et al. 2017). The reproductive cycle of *P. radiata* is a fire resistant characteristic as their cones are serotinous and both extreme summer heat and low severity fires can initiate seed release; low intensity fires do not appear to negatively affect seedling germination or emergence (Richardson et al. 1990, Reyes et al. 2015). While *P. radiata* plantations are resilient to low severity fires, high severity fires have high tree mortality with 100% mortality in forests that experience canopy fires (Ruiz-González and Álvarez-González 2011). High severity fire also negatively affects seed germination and emergence (Reyes et al. 2015).

In addition to the effects of fire on the vegetation, fire also affects the soil environment both directly and indirectly. Direct changes may include combustion of surface and soil organic matter, pyromineralisation of organic nitrogen and organic phosphorus to inorganic forms, sterilisation of surface soil, physical changes to the soil matrix, and the deposition of new forms of organic matter and ash (González-Pérez et al. 2004, Certini 2005). Combustion and pyromineralisation of organic matter have been shown to affect mineral N and mineral P contents of post fire soils, affecting microbial processing and accumulation of these elements, respectively (Wang et al. 2012, Holden and Treseder 2013); however, low intensity fires generally have only minor effects on soil (Catalanotti et al. 2018). Indirect changes include changed vegetation structure, site microclimate, organic matter inputs, and water availability (Certini et al. 2011, Certini 2014). Direct and indirect changes to the soil environment may lead to changes in microbially mediated nutrient cycling through fire induced resource or environmental limitations. Therefore, the aims of this survey were to track changes in total carbon, total nitrogen, mineral nitrogen, mineral phosphorus, and microbial nitrogen and phosphorus over five seasons after a fire to determine effects of the Sampson Flat bushfire on nutrient cycling within the soils of Old Kersbrook forest.

2.3 Methods

2.3.1 Site description

The soils used in this experiment were sourced from four study sites that were established in *Eucalyptus* woodlands ('native' hereafter) and *Pinus radiata* forests ('pine' hereafter) that were either burnt or not burnt during the Sampson Flat fire of January 2-9 2015. All sites are within the 'Old Kersbrook Forest' forest reserve of ForestrySA in the Adelaide Hills, South Australia (Figure 2.1). The region has a Mediterranean to temperate climate with average annual precipitation of 650 mm (winter dominated) and mean daily temperature ranging from 9.7°C to 19.0°C Bureau of Meteorology (2015a). Between the fire and the time of sampling, 142.6 mm of precipitation was recorded at the nearest weather station (Williamstown; 7 km away) (Bureau of Meteorology 2015b, c, d, e). Old Kersbrook Forest is located on the Barossa Complex: a highly weathered region of the Adelaide Geosyncline. The topography of the site is low, undulating hills running approximately north-south, which have been dissected on their east and west faces by small ephemeral streams. The soils are light brownish grey to reddish brown sandy clay loams with weathered rock within 15 cm of the soil surface at most sites; they are classified as Leptic Rudosols in the Australian Classification (Isbell 2002).

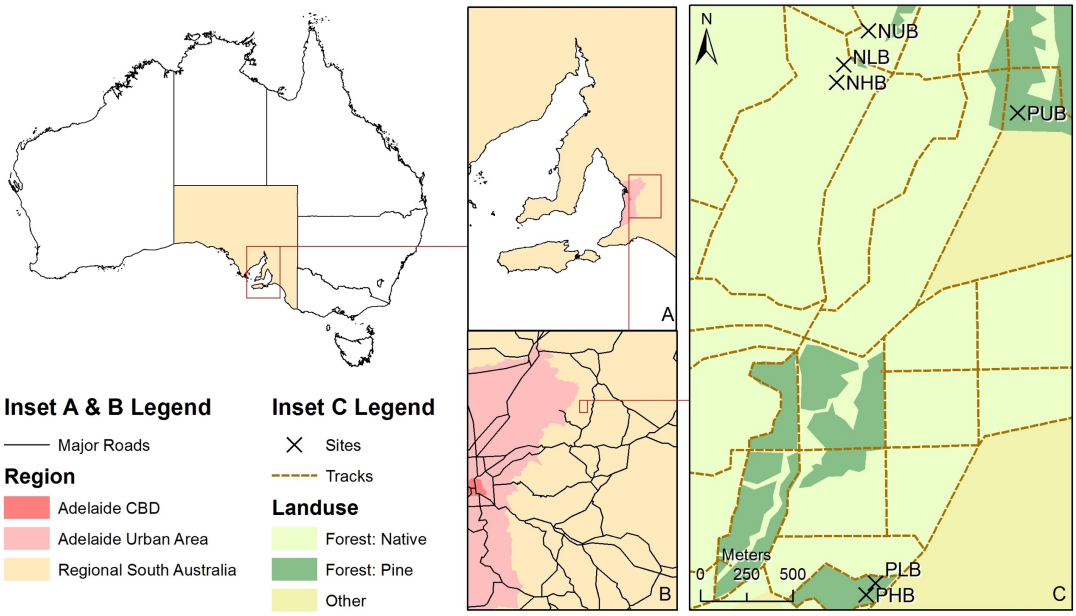

Figure 2.1 Study site locations; native site that was not burnt (NUB), native site with low severity burn (NLB), native site with high severity burn (NHB), pine site that was not burnt (PUB), pine site with low severity burn (PLB), and pine site with high severity burn (PHB).

The native sites are defined as 'Long Leaf Box Woodland' (Berkinshaw 2009) and are composed of low woodland with *Eucalyptus goniocalyx*, *E. fasciculosa*, *E. obliqua* and understorey of *Acacia pycnantha*, *Xanthorrhoea semiplana* and *Hakea rostrata*. Pine sites are managed plantations 30 years and older of *Pinus radiata*. The vegetation within burnt native sites was actively regenerating at the sampling time (four months after the Sampson Flat fire) while the vegetation within burnt pine sites was deceased.

2.3.2 Sampling

Three sites that differed in fire severity were identified in each forest type. At each of the six sites (one per vegetation type and fire severity) four 100 m^2 plots were established, and were categorised as: not burnt, low severity burnt ('low burnt', hereafter) or high severity burnt ('high burnt', hereafter) following the definitions in Keeley (2009) (Table 2.1). These sites were sampled four months after the fire and then seasonally until the following autumn; sampling ended at this point due to the risk of falling trees. Within each plot, after removal of the litter layer from the immediate coring area, soil was sampled from 0 to 10 cm depth using a 54 mm diameter auger in a haphazard pattern. Nine samples were taken from each plot, combined, sieved to <2 mm and air dried at 40°C.

Table 2.1 Fire severity classes (Keeley 2009), vegetation type, and site names and locations.

Fire severity definition	Vegetation	Site	Site location
Litter intact with plant parts green and unaltered, no direct effect from heat.	Woodland	Native not burnt	34°42'56.38'' S 138°51'17.40'' E
	Pine plantation	Pine not burnt	34°43'6.03'' S 138°51'45.05'' E
Trees with some canopy cover killed, but foliage not consumed. All understorey plants charred or consumed. Fine dead twigs on soil surface consumed and logs charred. Pre-fire soil organic layer largely consumed.	Woodland	Native low burnt	34°42'59.25'' S 138°51'13.37'' E
	Pine plantation	Pine low burnt	34°44'32.38'' S 138°51'18.88'' E
Canopy trees killed and foliage consumed. Surface litter of all sizes and soil organic layer largely consumed. White ash deposition and charred organic matter to several cm depth.	Woodland	Native high burnt	34°43'3.33'' S 138°51'12.95'' E
	Pine plantation	Pine high burnt	34°44'29.93'' S 138°51'21.08'' E

2.3.3 Soil analyses

Total organic C (TOC), total N (TN), mineral N (ammonium and nitrate/nitrite; N_{min}), resin P (resin strips; P_{res}), and microbial biomass P (MBP) were measured on all soils. Dry soil was used for TOC and TN, while 7 day incubated soils were used for all other analyses (as outlined below). Total organic C and TN were measured on dry soils using dry combustion in a Dumas furnace (Apal Agricultural Laboratory, South Australia). Mineral N, P_{res}, and MBP were measured on soils that had been incubated at 50% of WHC at 24±3°C; soil respiration (as CO_2-C flux) was measured daily (see below). Mineral N was determined after shaking soil with 2M KCl in a 1:5 ratio. Ammonium-N was measured using ammonium-salicylate oxidation (Forster 1995) and nitrate-N was measured using vanadium(III) reduction (Miranda et al. 2001). Resin P was extracted using the anion exchange resin method (Kouno et al. 1995) and the phosphorus concentration was determined colorimetrically (Murphy and Riley 1962). Soil MBP was determined as the difference between phosphorus extracted using anion exchange resins with hexanol fumigation and without hexanol fumigation (McLaughlin et al. 1986); an extraction efficiency coefficient was not used for MBP.

For cumulative respiration, soils were packed into PVC cores and then transferred to 950 mL glass jars (Ball® quart wide-mouth jars, Jarden Corporation) fitted with gas-tight lids which had stainless steel septum ports and rubber septa to allow sampling of headspace gas (Butterly et al. 2009). Soil respiration was quantified over the course of the experiment by measuring headspace CO_2 concentration using a Servomex 1450 infrared gas analyser (Servomex Group, Crowborough, England) as described in Butterly et al. (2010). Briefly, CO_2 in the incubation jars' headspace was quantified at the end of a known time interval and used to calculate respiration rate and cumulative respiration. After each measurement, the jars were opened to equilibrate CO_2 to ambient concentrations and then resealed. Detector readings were converted to mg CO_2-C by establishing a linear regression between known CO_2 concentrations and the detector readings.

Respiration was expressed per gram of organic C to take into account differences in organic C content among soils.

Two-way analysis of variance (ANOVA) and post hoc Tukey tests (Honest Significant Difference; HSD) were used to determine effects of burn type and time on measure variables (de Mendiburu 2017). Principle Component Analysis (PCA) was used to diagnose trends through time; ellipses drawn from a multivariate t-distribution and were added using the R package ggplot2 (Wickham 2009). After determining the insignificance of time, data were analysed for site differences by using collection times as replicates. Analyses were conducted using one-way ANOVA and post hoc Tukey tests (HSD). All statistical analyses were carried out using R (R Core Team 2018), and significance was set at $\alpha<0.05$.

2.4 Results

When analysed by two-way ANOVA, fire site was a significant main effect in two thirds of the analysed variables (Table 2.2); season of collection was a significant main effect in only one variable (cumulative respiration) and there was no observable interaction between site and season. The PCA to diagnose change over time indicated time was not a significant variable (Figure 2.2).

Table 2.2 Two way ANOVA results for variables measured on all soils (cumulative respiration measured for one week); n=4 for each site during each season; 6 sites and 5 seasons are included. 'ns' indicates not significant; '*' indicates significant at $p<0.05$; '**' indicates significant at $p<0.01$; '***' indicates significant at $p<0.001$.

Variable	Site	Season	Site×Season
TOC	***	ns	ns
TN	*	ns	ns
N_{min}	ns	ns	ns
P_{res}	*	ns	ns
MBP	*	ns	ns
Cumulative respiration	ns	*	ns

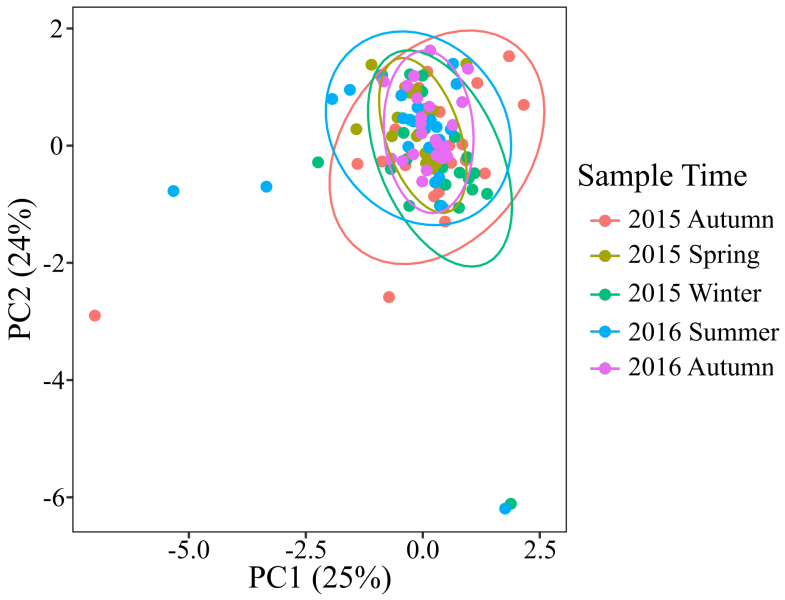

Figure 2.2 PCA for all soils at all collection times. Each point represents soil from a single plot; colours represent sample times. Ellipses are 95% confidence ellipses.

The results of this survey were highly variable and did not show any obvious trends that could be linked to fire or vegetation type (Figure 2.3). Total organic C content was highest (3.9%) in the native not burnt soil (Figure 2.3; Top left) and equally low in all other soils (range: 2.0-2.6%). Carbon:nitrogen ratio was equally high in the native not burnt and pine high burnt soils (range: 20.9-22.7%) and similarly low for all other soils (18.0-20.9%); the pine high burnt C:N was not significantly different from any other soil. No differences were observed in the cumulative respiration data.

Mineral N (Figure 2.3; bottom left) tended to increase (from 28 to 42 µg N g soil^{-1}) with fire in the native soils and decrease (from 27 to 18 µg N g soil^{-1}) in the pine soils. Resin P (Figure 2.3; bottom middle) was uniformly low in native soils (range: 0.2-0.7 µg P g soil^{-1}) and increased with fire severity in pine soils (from 1.6 to 3.3 µg P g soil^{-1}). Microbial biomass P decreased with fire in native soils (from 5.8 to 2.6 µg P g soil^{-1}) and was broadly similar in all pine soils (range: 4.5-6.9 µg P g soil^{-1}).

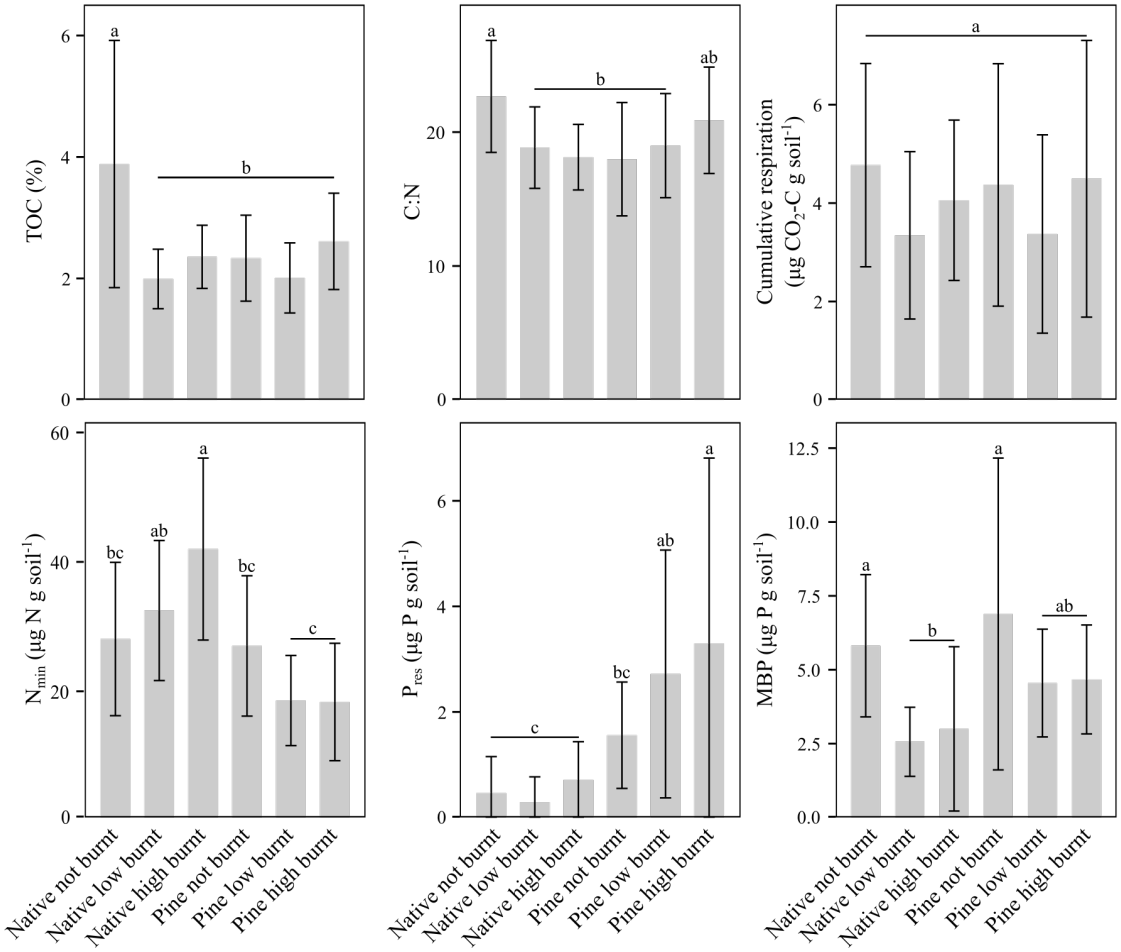

Figure 2.3 Total organic carbon (top left; TOC, %), carbon:nitrogen ratio (top middle; C:N), cumulative respiration on day 3 (top right; µg CO_2-C g soil^{-1}), mineral N (bottom left; N_{min}, µg N g soil^{-1}), resin P (bottom middle; P_{res}, µg P g soil^{-1}) and microbial biomass phosphorus (bottom right; MBP, µg P g soil^{-1}) for each soil over five seasons (n=20; values are mean ± standard deviation). Values with same letter within the same graph are not significantly different (Tukeys HSD, α=0.05).

2.5 Discussion

Although both vegetation types experienced a decrease in MBP, fire inconsistently affected the other variables measured in soil from the two sites. After burning, native sites experienced a decrease in TOC and C:N and an increase in N_{min}, while pine sites only experienced increased P_{res}. Forest fire can lead to increased or decreased soil organic carbon and nitrogen contents: fire can reduce organic carbon content if combustion is complete or increase organic carbon content via char if combustion is incomplete (Wan et al. 2001, Prendergast-Miller et al. 2017). Although C:N in boreal forests has been reported to decrease and mineral N content increase after fire (Turner et al. 2008, Hume et al. 2016), previous research in Australian woodlands and open forest sites often find that nutrient contents at these sites are not strongly affected by fire. For example, surface soil organic C (SOC) and TN was not significantly affected by recent fire in a north eastern NSW tall open eucalypt forest (Hobley et al. 2017), similarly fire was found to have no observable mid-term (<20 years) effect on SOC in open forests and woodlands of the Sydney basin (Sawyer et al. 2018). There is the possibility that the decrease in C:N in the native sites in this study was due to differences in volatilisation temperature, however the rapid recruitment of leguminous trees that established before the first sampling time should also be considered (Ma et al. 2015).

It is unclear whether changes in plant available P were due to site differences or vegetation differences or an interaction between sites and vegetation. Phosphorus content of pine soils is likely to be higher in general than in native soils as phosphorus fertiliser has historically been used in pine plantations to increase timber yields on low quality plots (Boomsma 1949). Low phosphorus content in the native soils may decrease the observable effect of fire on available P content as excess phosphorus is rapidly sorbed to the soil or assimilated into the microbial biomass (Romanyà et al. 1994). In addition, ash is highly susceptible to wind and water erosion (Giardina et al. 2000, Bodí et al. 2014) and, as the native sites were located on steeper slopes than the pine sites, ash borne P may have moved offsite before sampling. Vegetation differences that may arise after fire may be due to the higher phosphorus content of pine litter compared to *Eucalyptus* (Baker and Attiwill 1985).

Even though microbial activity (as measured by soil respiration) was not observably affected by fire, MBP was decreased in both vegetation types after fire. A similar response was observed in a meta-analysis of 139 published soil microbial responses to forest disturbances wherein microbial biomass was decreased by 49% in sites that had been disturbed by fire (Holden and Treseder 2013). This response may be due to a decrease in the quality of soil organic matter after burning where more labile components of soil organic matter are volatized at lower temperatures (González-Pérez et al. 2004). Although soil sterilisation has been posed as a reason for a decrease in microbial biomass immediately after fire (Prieto-Fernández et al. 1998), it is unlikely to be the case in this study due to the length of time since fire and the depth of the soil samples (Badía et al. 2017).

2.6 Conclusion

When considered as a whole, these results indicate that although the Sampson flat fire did not substantially affect microbial activity, microbial nutrient cycling was disturbed for the duration of sampling. In native sites, SOC and C:N ratio decreased with both low and high severity fire, and N_{min} increased with high severity fire only. In pine sites, P_{res} increased with high severity fire. Microbial biomass P was decreased by fire; however, this was only significant in the native sites.

Changes in N_{min} and MBP indicate a perturbations in nitrogen cycling mineralisation or uptake pathways and perturbations in microbial nutrient accumulation over time. From this survey it is clear that fire affected native forest and pine plantations differently in Old Kersbrook Forest, and that these changes persisted for more than a year after the event.

2.7 Acknowledgements

This work was supported by The Nature Foundation of South Australia Scholarship Grant.

Statement of Authorship

Title of paper	Soil microbial nutrient limitation in six soils with different fire histories
Publication status	☐ Published ☐ Accepted for publication ☐ Submitted for publication ☒ Unpublished and unsubmitted work written in manuscript style
Publication date	Not applicable

Principle Author

Name of principle author	Erinne Stirling		
Contribution to the paper	• Experimental design, field work, laboratory analyses, and data analyses • Preparation of the manuscript		
Overall percentage (%)	95		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	**6/03/2019**

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of co-author	Timothy R. Cavagnaro		
Contribution to the paper	• Review and constructive comments on data analysis		
Overall percentage (%)	5		
Signature		Date	26/02/2019

Chapter 3 – Soil microbial nutrient limitation in six soils with different fire histories

Written in the style of a short communication – *Nature Scientific Reports*

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3.1 Abstract

Fire changes the nature and quantity of soil organic matter during the event and affects organic matter inputs after the event. Although extensive research has been conducted on the influence of fire on soil nutrient cycling in regard to plant nutrient limitation, less is known about the effects of fire on microbial nutrient limitation (i.e. the limited availability of nutrients used for microbial growth). To address this gap, soils were collected from adjacent woodland and pine forest sites that were not burnt or experienced low or high severity fire. These soils were incubated for 50 days after being amended with carbon, nitrogen, or a combination thereof. Amendment type only had a significant impact on cumulative respiration, microbial biomass phosphorus, and mineral nitrogen. The main result from this experiment is that microbial activity was primarily increased by carbon addition, then by either nitrogen or nitrogen and phosphorus addition, and that while burning increased nitrogen limitation when pooled by vegetation type from which the soil was collected, there was not a consistent response to nutrient addition due to fire severity. Therefore, although the response to nutrient addition was different for each soil, fire severity was not an important variable for microbial nutrient limitation in soils collected from these sites.

3.2 Introduction

Globally, between 3.6 and 3.8 million square kilometres of land was burnt annually over the years 2006-2008 (Chuvieco et al. 2016); of which, fires in shrublands and forests accounted for more than 250,000 km^2. Due to different fire conditions that lead to site specific fire intensities (energy release), fires have strongly spatially variable effects (Hamman et al. 2007, Bradstock et al. 2010) that cause a patchwork of fire severities, and hence, organic matter loss (Keeley 2009). Forest fires cause an immediate loss of organic matter from vegetation and litter, and affected forests may have reduced soil organic matter inputs post-fire depending on forest resilience and fire severity (Díaz-Delgado et al. 2003, Clarke et al. 2014, Fernandez-Manso et al. 2016, Jenkins et al. 2016). These changes in litter dynamics can have significant implications for important soil processes such as nutrient cycling.

Fire can change soil nutrient cycling in forests in the period immediately after the event due to changes in the soil organic matter (SOM) and microbial biomass (González-Pérez et al. 2004, Wang et al.). Soil organic matter is thermally altered during fire and becomes char (i.e. retaining original structures) or black ash (i.e. after incomplete combustion), or white ash (i.e. after complete combustion) (Palese et al. 2004, Bodí et al. 2014). Char, black ash, and white ash have different physical and chemical properties that are relevant to soil microbial nutrient limitation (i.e. the limited availability of nutrients used for microbial growth). Whereas char and black ash have a larger particle size than white ash, and are composed of thermally altered organic compounds, white ash is rich in carbonates and oxides (Palese et al. 2004, Bodí et al. 2014). Particle size is an important factor in the decomposition of pyrogenic residues as ash can enhance microbial activity (Dooley and Treseder 2012, Bodí et al. 2014) while residues ≥1 mm are considered to be relatively resistant to microbial decomposition (Baldock and Smernik 2002, Jenkins et al. 2014). Although microbial activity can increase after fire (Palese et al. 2004), microbial biomass tends to decrease immediately following a fire (Bárcenas-Moreno and Bååth 2009, Dooley and Treseder 2012, Fontúrbel et al. 2012, Muqaddas et al. 2015). Therefore, as fire decreases the microbial biomass, and fire intensity affects the physical and chemical properties of pyro-residues, the immediate response of soil nutrient cycling to fire is expected to be strongly influenced by fire severity.

Fire affects carbon, nitrogen and phosphorus pools differently depending on fire intensity. Whereas both carbon and nitrogen volatilize at relatively low (≈200°C) temperatures (Hosking 1938, Bárcenas-Moreno and Bååth 2009), phosphorus volatilization occurs at substantially higher (≈750°C) temperatures (Raison et al. 1985). In woodlands and forests from temperate and Mediterranean climatic zones, soil organic C (SOC) increases with low severity fire and decreases with high severity fire (Heath et al. 2015, Muqaddas et al. 2015, Jenkins et al. 2016, Krishnaraj et al. 2016). In woodlands, both decreased and increased mineral N have been observed after fire (Weston and Attiwill 1990, Jones and Davidson 2014, Muqaddas et al. 2015); contrastingly, in boreal and temperature forests, mineral N pools often increase after fire (Prieto-Fernández et al. 1993, Wang et al. 2012). Nitrogen mineralisation may be lower after fire due to the overall loss of organic N or unaffected after fire (Wan et al. 2001, Durán et al. 2009, Guénon et al. 2013). Fire often increases phosphorus availability in the short-term through pyromineralisation (Bárcenas-Moreno and Bååth 2009), but may decrease both available P and total P over the long term if pyrogenic residues are not retained on site (Durán et al. 2009, Ferreira et al. 2016).

Although the effect of fire on the relationships between SOC, nutrient availability, and soil microbes has been studied extensively (Wang et al. 2012), relatively little is known about which nutrients limit microbial biomass and activity after fire. As microbially-mediated processes play an important role in the breakdown of organic matter (OM), changed soil conditions, OM, and OM inputs after a fire may affect the rate of decomposition or the fate of nutrients and therefore affect soil nutrient cycling. In this study, microbial nutrient limitation was assessed by determining microbial activity and biomass after adding carbon, nitrogen and phosphorus (alone and in combination) to soils from two vegetation types that had recently experienced different fire severities. The aim of this study was to examine the responses of soils from different forest types and fire histories on carbon, nitrogen and phosphorus limitation for microbial activity and growth. It was anticipated that the site of origin for these soils may determine the response to added nutrients.

The hypotheses were: (1) carbon is the primary limiting resource, (2) microbes will be carbon/phosphorus and carbon/nitrogen limited in unburnt and burnt soils, respectively, and (3) fire severity will increase the intensity of fire-induced nutrient limitation. The results are discussed in the context of site specific responses to the addition of simple macronutrients in order to determine differences in vegetation type response.

3.3 Methods

3.3.1 Site description and soil sampling

This study included six sites in native woodlands and pine forests from within the 'Old Kersbrook Forest' forest reserve in the Adelaide Hills, South Australia (Figure 2.1). These sites were affected by the Sampson Flat fire of January 2-9 2015 (Table 2.1), and show varying signs of fire severity. The woodland sites in this study are defined as 'Long Leaf Box Woodland' (Berkinshaw 2009) and are composed of low woodland with *Eucalyptus goniocalyx*, *E. fasciculosa*, *E. obliqua* and understorey of *Acacia myrtifolia, A. pycnantha*, *Xanthorrhoea semiplana* and *Hakea rostrata*. Pine sites were plantations of *Pinus radiata* 30 years and older. The region has a Mediterranean climate with average annual precipitation of 650 mm (winter dominated) and mean daily temperature ranging from 9.7°C to 19.0°C (Bureau of Meteorology 2015a). The soils are reddish brown to light brownish grey sandy clay loams with weathered rock within 15 cm of the soil

surface at most sites. These soils are classified as Leptic Rudosols in the Australian Soil Classification (Isbell 2002) or as Leptic Regosols in the World reference base (IUSS Working Group WRB 2015).

In each forest type three sites that differed in fire severity were identified. At each of the six sites (one per vegetation type and fire intensity) four 100 m^2 plots were established, and were categorised as: not burnt, low burnt or high burnt (fire severity estimated according to Keeley (2009)). These sites were sampled four months after the fire. The maximum distance between these sites is 3 km. During the four months after the fire, daily maximum temperature ranged from 11.4°C to 40°C and 142.6 mm of rain fell at nearby Williamstown between the fire event (January 9, 2015) and the sampling date (May 5, 2015) (Bureau of Meteorology 2015b, c, d, e).

Within each plot, after removal of the litter layer from the immediate coring area, soil was sampled from 0 to 10 cm depth using a 54 mm diameter auger. Nine samples were taken from each plot, combined, sieved to <2 mm and dried at 40 °C. Subsamples of the soils were analysed for preliminary physical and chemical properties while the remaining soil was bulked per site. These site-based samples were then subsampled to give the four replicates in the experiment.

3.3.2 Soil characterisation

After field sampling, preliminary physical and chemical properties were measured on the soils; this included bulk density, gravimetric water content, texture, water holding capacity (WHC), $pH_{1:5water}$, electrical $conductivity_{1:5water}$ (EC), total organic C (TOC), total N (TN), ammonium and nitrate (mineral N; N_{min}), and resin P (P_{res}). Bulk density of the soil ranged from 0.9 to 1.2 g cm^{-3} (Table 3.2). Maximum WHC was measured on the dried soils using thoroughly wetted soil in soil rings allowed to drain for 48 hours on a sintered glass funnel connected to a 1 m water column (ψm = -10 kPa), after which gravimetric water content was determined; maximum WHC ranged from 0.21 to 0.38 g g^{-1} (Table 3.2). Soil pH and EC were measured using a CyberScan PC 510 (Eutec Instruments) on the dried soils using a 1:5 soil:water extract after shaking for one hour; all soils were acid and non-saline (Table 3.1). Total organic C and TN content were measured on dried soils using dry combustion. Mineral N was determined for the dried soils after shaking soil with 2M KCl in a 1:5 ratio. Ammonium-N was measured using ammonium-salicylate oxidation (Forster 1995) and nitrate-N/nitrite-N was measured using vanadium(III) reduction (Miranda et al. 2001). Resin P was extracted from the dried soils using the anion exchange resin method (Kouno et al. 1995) and the phosphorus concentration determined colorimetrically (Murphy and Riley 1962).

Table 3.1 Initial properties for soil depth 0-10 cm. Values are means±SE; n=4.

	Native			Pine		
	Not burnt	**Low burnt**	**High burnt**	**Not burnt**	**Low burnt**	**High burnt**
Litter (kg m^{-2})	4.4±0.2	0.5±0.1	0.2±0.0	12.8±2.1	2.5±0.2	0.1±0.1
Bulk density (g cm^{-3})	0.93±0.02	0.81±0.01	0.84±0.04	1.17±0.05	1.21±0.10	1.07±0.07
$EC_{1:5}$ (µS cm^{-1})	20.5±4.7	39.4±1.1	52.0±4.5	21.7±5.2	26.2±4.8	41.0±2.2
$pH_{1:5}$	4.7±0.1	5.0±0.1	4.7±0.1	5.4±0.0	5.6±0.0	5.8±0.2
TOC (%)	3.9±0.4	2.6±0.3	2.7±0.3	2.4±0.2	2.2±0.4	2.4±0.5
TN (%)	0.22±0.04	0.13±0.01	0.15±0.02	0.13±0.02	0.09±0.00	0.11±0.02
NH_4^+ (mg N g $soil^{-1}$)	0.6±0.01	1.4±0.1	1.3±0.3	1.6±0.1	1.0±0.1	0.8±0.1
NO_3^- (µg N g $soil^{-1}$)	0.32±0.07	2.04±0.33	0.49±0.12	13.8±1.06	70.8±6.09	19.0±3.56
Resin P (µg P g $soil^{-1}$)	0.48±0.13	1.11±0.15	0.88±0.32	1.56±0.13	4.77±1.08	7.42±2.10

3.3.3 Experimental design

Optimal water content for soil respiration was determined in a preliminary incubation and was slightly different for each soil leading to each soil requiring a unique water content for optimal respiration in this experiment (Table 3.2) (Butterly et al. 2010). The soils were pre-incubated for 10 days in the dark at room temperature (approx. 23ºC) and 45% or 51% of WHC (depending on optimal water content) to reactivate the microbes in the previously dry soil before the beginning of the experiment.

Table 3.2 soil properties relevant to experimental water content for soil incubation and experimental water content.

Site	**Bulk density (g cm^{-3})**	**WHC (g g^{-1})**	**Optimum water content for respiration (% WHC)**	**Experimental water content (% WHC)**
Native not burnt	0.9	0.38	60-70	70
Native low burnt	0.8	0.31	ns 40-80 ns	70
Native high burnt	0.8	0.38	ns 40-80 ns	70
Pine not burnt	1.2	0.31	80	80
Pine low burnt	1.2	0.21	80	80
Pine high burnt	1.1	0.24	80	80

After pre-incubation, 20 g dry weight equivalent of soil was amended with solutions of glucose (10 g C kg $soil^{-1}$; referred to as C+ hereafter), ammonium chloride (0.5 g N kg $soil^{-1}$; referred to as N+ hereafter), monopotassium phosphate (0.05 g P kg $soil^{-1}$; referred to as P+ hereafter), or a mixture of all possible C+/N+/P+ combinations and then brought up to a water content of between 60 and 80% WHC depending on the optimal moisture content for respiration (see above and Table 3.2). Thus, there were eight treatments: an unamended control and seven amendments (C+, N+, P+, C+N+, C+P+, N+P+, C+N+P+). After amendment, the soils were packed into PVC cores and then transferred to 950 mL glass jars (Ball® quart wide-mouth jars, Jarden Corporation) fitted with gas-tight lids which had stainless steel septum ports and rubber septa to allow sampling of the headspace gas (Butterly et al. 2009). Soil respiration was quantified daily (see below). The

experiment ended once the respiration rate in all soils with carbon amendments had stabilised. Microbial biomass C, N, and P were measured at the end of the experiment (day 50). Mineral N and P_{res} were measured at the start (before pre-incubation) and at the end of the experiment.

Soil respiration was quantified over the course of the experiment by measuring headspace CO_2 concentration using a Servomex 1450 infrared gas analyser (Servomex Group, Crowborough, England) as described in Butterly et al. (2010). Briefly, CO_2 in the incubation jars' headspace was quantified at the end of a known time interval and used to calculate respiration rate and cumulative respiration. After each measurement, the jars were opened to equilibrate CO_2 to ambient concentrations and then resealed. Detector readings were converted to mg CO_2-C by establishing a linear regression between known CO_2 concentrations and the detector readings. Respiration was expressed per gram of organic C to take into account differences in organic C content among soils.

Soil microbial biomass P (MBP) was determined as the difference multiplied by 1.75 between phosphorus extracted using anion exchange resins with hexanol fumigation and without hexanol (McLaughlin et al. 1986). Soil microbial biomass C (MBC) and N (MBN) were extracted using chloroform fumigation extraction (Vance et al. 1987) with 0.5 M K_2SO_4 at a 1:5 soil:extractant ratio. The carbon concentrations in the fumigated and non-fumigated extracts were determined after Anderson and Ingram (1989). The difference in carbon concentration between fumigated and non-fumigated soil was multiplied by 2.64 to calculate MBC (Vance et al. 1987). Microbial biomass N was calculated as the difference in ammonium-N concentration between fumigated and non-fumigated samples multiplied by 1.75 (Brookes et al. 1985, Moore et al. 2000).

3.3.4 Statistical analysis

Statistical analyses were considered as a response to nutrient additions within the site level (Table 2.1) as there were no replicates at the site level. However, as multiple burnt (n=4) and not burnt (n=2) sites were sampled, responses to resource addition are analysed for burnt/not burnt sites, pooled over vegetation type (Hypothesis 2). While it is clear that differences due to fire severity cannot be statistically supported, this study explores the response of these soils to nutrient addition and provides commentary on the possible effect of severity. Analysis was by one-way ANOVA and post hoc Tukey HSD tests (at $\alpha<0.05$), using R's stats and agricolae packages (de Mendiburu 2017, R Core Team 2018).

3.4 Results and discussion

3.4.1 Post-fire soil properties

Even though the aim was not to compare between sites, some interesting patterns in initial soil properties were observed, as follows. Litter and soil organic matter were influenced by vegetation type and fire severity (Table 3.1). Litter dry mass per area was highest in the pine unburnt site than all other sites. Total organic C and TN content were highest in the native not burnt site and similar among the other sites. Less surface litter and more TOC in the native not burnt site relative to the pine not burnt site suggests either that pine litter is more slowly incorporated into the SOM than native litter, or that pine litter is being produced at a higher rate than the native litter. Slowed incorporation of pine litter into the soil may be due to greater nitrogen limitation in pine soils as pine needles have a higher C:N ratio than eucalyptus leaves (80-100 and 46-80 respectively) (Baker and Murray 2012, Martínez et al. 2013, Krishnaraj et al. 2016). Pine litter also has low soluble C concentrations and high soluble phenols which can slow decomposition and decrease

the microbial biomass (Li et al. 2014b). When considered on a per gram of TOC basis, higher cumulative respiration in the pine soils indicates that the organic C in these soils is more decomposable than the organic C in the native soils. Therefore, the accumulation of pine litter appears to be due to a slower rate of conversion from litter to soil organic C.

Basic soil physical and chemical properties differed by vegetation type and may have been affected by fire severity (Table 3.1). Soil bulk density was higher for pine site soils than native site soils, EC increased with fire severity in both vegetation types and soil pH was consistently higher in pine forest sites than native vegetation sites. While burning is expected to increase soil EC and pH in both neutral and acid soils (Ulery et al. 1993, Barreiro et al. 2016), the changes depend on soil buffering capacity and type of ash added (Khanna et al. 1994). Increased EC with fire severity in the soils at the time of collection suggests that ash had been incorporated into the surface soils in the burnt plots, while the low pH values indicates that any ash derived carbonates may have been neutralised in the four months following the fire.

Mineral N and P_{res} were inconsistently affected by vegetation type and fire severity (Table 3.1). The concentration of ammonium in the soil was highest in the burnt soils for the native sites and highest in the not burnt soils for pine sites; nitrate concentration was extremely low for all native sites and high but inconsistent in the pine sites. Resin P in pine high burnt soils was substantially higher than all other soils. Overall, the initial soil properties do not support the hypothesis that fire decreases N_{min} and increases P_{res}. For these sites, burning was associated with an increase in N_{min} in the native sites, and an increase in P_{res} in the pine sites. It is possible that heating increased mineralisation of SOM in native soils more than in pine soil because of the higher TOC concentration before the fire in native forest but that the increased plant available P was exported as result of rainfall events before sampling. Ash from native litter likely has a higher N_{min} content than ash from pine litter as nitrogen content has been found to be higher in native litter than pine litter (0.8-1.1% and 0.6-0.7%, respectively) (Baker and Murray 2012). The native burnt sites had less canopy cover and steeper slopes than the pine burnt sites, and during rain events between the fire and sampling, ash may have been lost due to run-off associated with large rainfall events (Ferreira et al. 2005, Ferreira et al. 2008, Hosseini et al. 2016) but may have been retained at the pine sites as ash washed into the soil matrix (Stoof et al. 2016). It is also possible that the overall quantity of nitrogen and phosphorus in the pine litter was higher than the native litter as dry litter mass per unit area was three times higher in the unburnt pine site than the unburnt native site.

It will be important in future work to explore the dynamics of the responses observed here over time after fire as the results discussed above may be due to the sample timing (four months after the fire event) and the initial soil properties indicate that ash had been incorporated into the burnt plots without substantial effects on the soil chemical properties. Regardless of the timing, long-term microbial nutrient limitation may be affected by the changes in the quantity and quality of the leaf litter layer. Furthermore, microbial nutrient limitation may be affected by changes in the SOM that are not captured in the properties above.

3.4.2 Microbial response to experimental nutrient addition

While the addition of carbon, nitrogen, and phosphorus affected a range of microbial properties, it is important to note that in the following section, the emphasis is on the response of individual soils to a range of different amendments rather than comparisons between soils. At the end of the experiment, soil response to nutrient addition in the incubation experiment was most significant

for cumulative respiration, MBP and N_{min}; when pooled by vegetation type and presence/absence of fire, a statistically different response was also observed in the cumulative respiration.

Only soils with carbon amendments showed differences in cumulative respiration relative to the control soils (Figure 3.1). Within the carbon amendments, two patterns of response were observed: carbon limitation, and carbon then nitrogen limitation. Carbon limitation is observed in these results as equally high cumulative respiration for the C+ amendment as the C+N+P+ amendment; this response was observed in both of the unburnt soils, and in the native low burnt soil. Carbon then nitrogen limitation is observed as equally high cumulative respiration in the C+N+ and C+N+P+ amendments; this response was observed in both of the high burnt soils, and in the pine low burnt soil. The cumulative respiration results indicate that microbes in these soils are primarily carbon limited (Hypothesis 1) and that the high burnt soils were also nitrogen limited (Hypothesis 2).

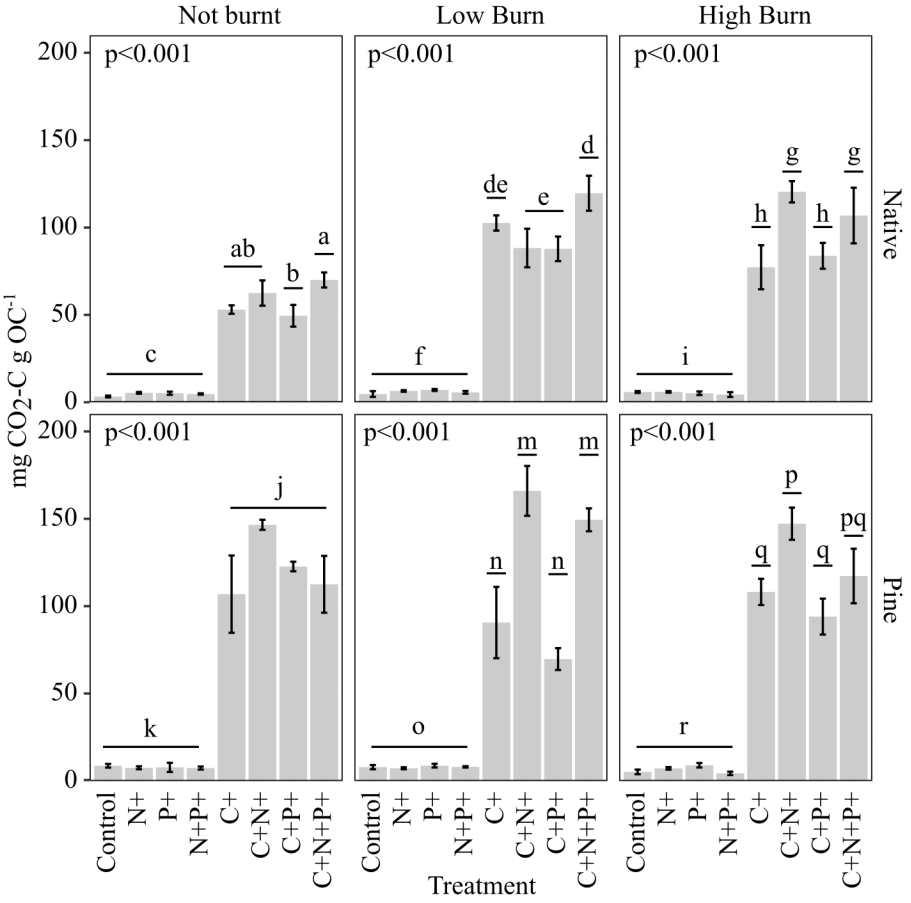

Figure 3.1 Cumulative respiration (mg CO_2-C g OC^{-1}; where 'OC' indicates organic C) on day 42 in the unamended controls and in soils amended with carbon, nitrogen, and phosphorus added separately or in mixtures (n=4, values are means±SE). Columns within the same soil type that have the same letter are not significantly different (Tukey's HSD; α=0.05).

Microbial biomass P was not consistently influenced by nutrient additions (Figure 3.2); however, three general patterns can be observed relative to the control treatment: decreased MBP in all carbon amendments, increased MBP primarily in the C+N+P+ amendment, and increased MBP with N+ (C+ or P+) with a concurrent decrease with C+, C+P+, or P+. A decrease in MBP with all carbon amendments indicates that the microbial biomass was not carbon limited and therefore

was not accumulating phosphorus storage compounds; this is observed in the pine not burnt soil. An increase in MBP with the addition of C+N+P+ indicates that the microbial biomass was actively accumulating phosphorus storage compounds, perhaps due to carbon and nitrogen co-limitation; this response is observed in all native soils. An increase in MBP with nitrogen with concurrent decreases associated with carbon or phosphorus indicate nitrogen is limiting the production of MBP in these soils if carbon or phosphorus is available; this is observed in both pine burnt soils.

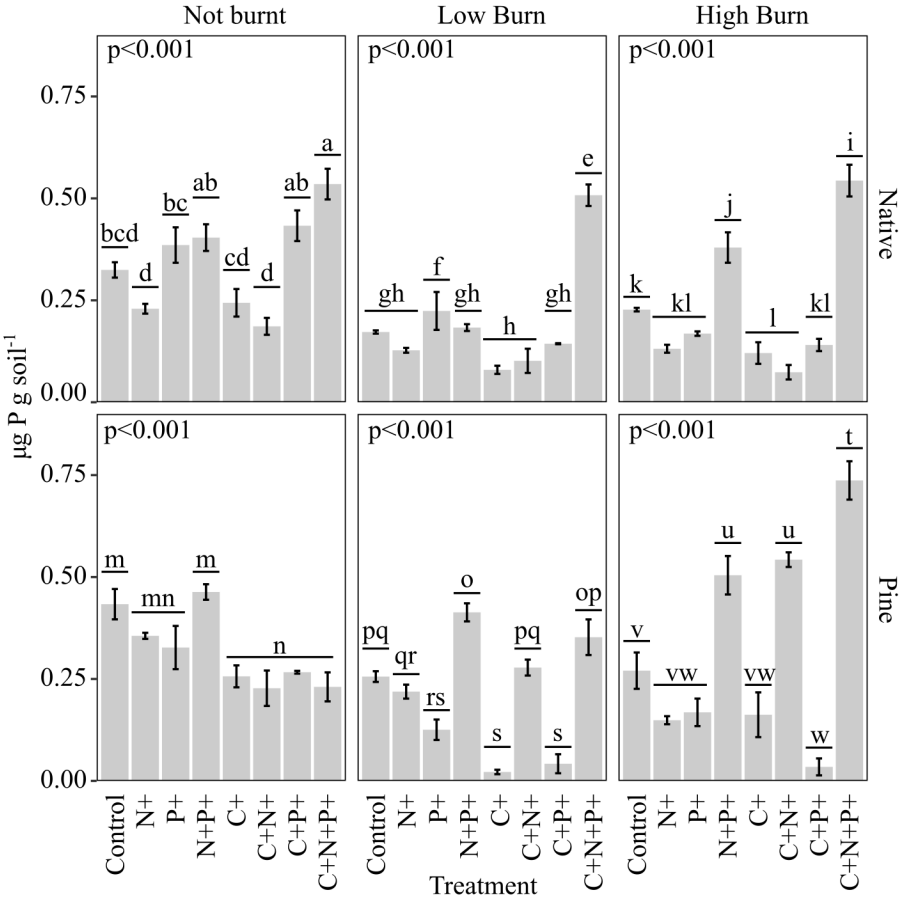

Figure 3.2 Microbial biomass P concentration (µg P g soil^{-1}) on day 50 in the unamended controls and in soils amended with carbon, nitrogen, and phosphorus added separately or in mixtures (n=4, values are means±SE). Columns within the same soil type that have the same letter are not significantly different (Tukey's HSD; α=0.05).

It is well established that whereas microbial activity typically increases after burning, microbial biomass tends to decrease (Palese et al. 2004, Liu et al. 2010, Muqaddas et al. 2015, Shen et al. 2016). In the soils studied here, MBC and MBN were highly variable and there were no significant treatment differences. It is likely that, because the amendment concentrations were so high and the soils were sampled over a month after the incubation started, the microbial biomass was more strongly influenced by treatment type than soil origin. Respiration rates in carbon amended soils were highest between days 4 and 14 indicating that the microbial biomass had probably peaked and declined well before sampling. In other soils that received similarly high carbon amendments, MBC and MBN were maximal two to three days after amendment and declined rapidly (Elmajdoub et al. 2014, Ma et al. 2016). In contrast, MBP was different between amendment types at day 50; this may be because phosphorus is stored within the microbial biomass as polyphosphate when carbon becomes limited (Doerner and Mason 2006). Although

MBP in native soils were similar regardless of fire status for the C+N+ and C+P+ amendments, MBP was much higher in the burnt soils that were amended with C+N+P+ rather than only C+N+ or C+P+. This suggests that in burnt native soils microbial phosphorus uptake was limited by phosphorus when both carbon and nitrogen were supplied - which may be due to changes in phosphorus fractions (Huang et al. 2013). In pine burnt soils, the similarity in MBP between N+P+, C+N+ and C+N+P+ amended soils indicates that microbial phosphorus uptake was limited by nitrogen only. As these soils were light textured and strongly acidic, microbial nitrogen limitation may be due to decreased denitrification and increased leaching of nitrate and nitrite (Bárta et al. 2010).

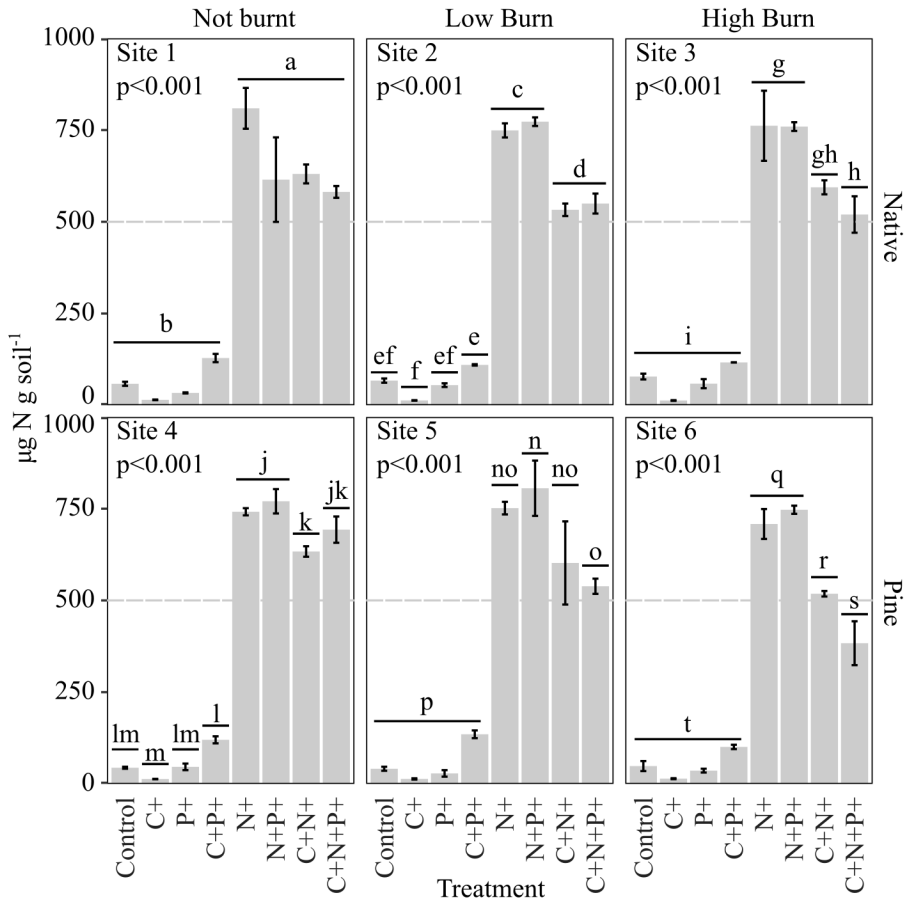

Figure 3.3 Mineral N concentration (µg N g soil^{-1}) on day 50 in the unamended controls and in soils amended with carbon, nitrogen, and phosphorus added separately or in mixtures (n=4, values are means±SE). Columns within the same soil type that have the same letter are not significantly different (Tukey's HSD; α=0.05). Dashed line indicates nitrogen addition rate.

Differences in nitrogen limitation were not observed in for N_{min} (Figure 3.3) and the effect of adding C+N+ and C+N+P+ had a similar pattern to the cumulative respiration results. Both the type of response and pattern of response were observed in the many of the same soils: both not burnt soils contain a similar amount of nitrogen for the N+ and C+N+P+ amendments, and both high burnt soils have a reduction of nitrogen in the C+N+P+ amendment relative to the N+ amendment. This indicates a change in the pattern of nutrient limitation with high severity fire (Hypothesis 3). The decrease in nitrogen associated with carbon addition is likely due to glucose increasing microbial nitrogen uptake (Dunn et al. 2006). Furthermore, burnt soils may respire more than the mass of carbon added if carbon priming increases the mineralisation of pyrogenic

OM (Hamer et al. 2004, Kuzyakov et al. 2009). However, the similar relative respiration increases between unamended and carbon amended soils in all sites suggest that neither vegetation type nor fire severity affected microbial carbon limitation.

3.4.3 Microbial response to burning

Although an increase of nitrogen limitation with burning was not observed at the site level (Hypothesis 2), when sites are pooled by presence/absence of fire, an increase in nitrogen limitation with burning is observed in the cumulative respiration results (Figure 3.4). While the not burnt soils respired an equally large amount of CO_2-C for all carbon treatments regardless of nutrient mix, the burnt soils respired more for those treatments that contained nitrogen. As previously discussed, it is possible that fire effects were undetectable at the site level because the nutrient status of these soils is already low (Li et al. 2014a). Burning may also appear to have no effect on sandy acid soils as nutrients are rapidly leached post-fire; these soils may need to be sampled immediately after a fire and to a shallower depth in order to observe any measurable differences (Dean et al. 2015).

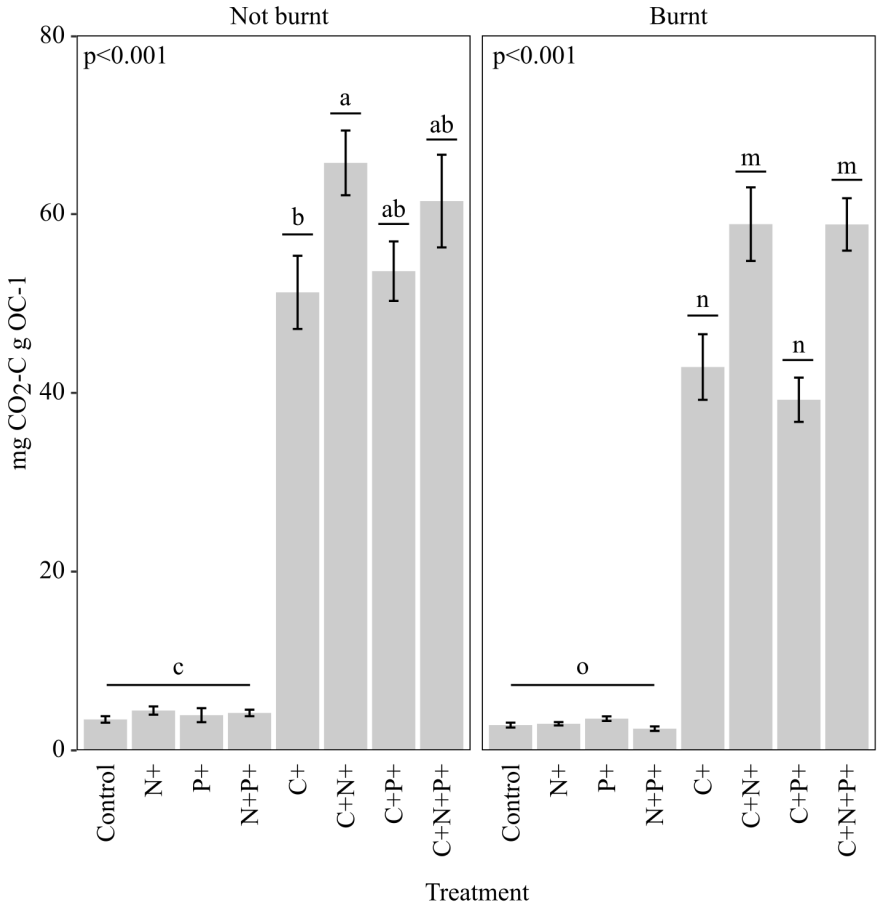

Figure 3.4 Cumulative respiration (mg CO_2-C g OC^{-1}) on day 42 in the unamended controls and in soils amended with carbon, nitrogen, and phosphorus added separately or in mixtures (unburnt n=8, burnt n=16; values are means±SE). Columns within the same burn type that have the same letter are not significantly different (Tukey's HSD; α=0.05).

Together, the results indicate the soil microbial biomass was carbon limited in all cases (Hypothesis 1), that burning introduced nitrogen limitation in microbial activity (Hypothesis 2), and that fire severity did not consistently influence microbial nutrient limitation at these sites

(Hypothesis 3). Due to the sandy, acidic, nature of the soils, it is possible that earlier sampling may have captured differences in the soil due to fire severity, and later sampling may have captured differences due to the vegetation responses. However, if the lack of a response to nutrient addition is considered, there are indications that soil nutrient cycling is more robust when disturbed by fire under native woodland than pine plantation, and that native woodland soils may return to nutrient cycling equilibrium more rapidly than pine plantation soils. The practical implication of this research is that native forest reserves and pine plantations will have different nutrient requirements to remediate fire induced microbial nutrient limitations. However, this is only relevant to the pine plantations and is likely to be addressed during normal post-fire harvest and planting activities.

3.5 Acknowledgements

Funding: This work was supported by The Holsworth Wildlife Research Endowment & The Ecological Society of Australia, the Nature Foundation of South Australia, and The University of Adelaide Australian Postgraduate Award, with further sponsorship provided by APAL Agricultural Laboratory. ES gives thanks GC Garrett for his assistance in the field and the laboratory, and to Prof. Petra Marschner for advice on the experimental design.

Statement of Authorship

Title of paper	Post fire litters are richer in water soluble carbon and lead to increased microbial activity
Publication status	☐ Published ☒ Accepted for publication ☐ Submitted for publication ☐ Unpublished and unsubmitted work written in manuscript style
Publication date	5th January, 2019

Principle Author

Name of principle author	Erinne Stirling		
Contribution to the paper	• Experimental design, field work, laboratory analyses, and data analyses Preparation of the manuscript		
Overall percentage (%)	85		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	**6/03/2019**

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

x. the candidate's stated contribution to the publication is accurate (as detailed above);
xi. permission is granted for the candidate in include the publication in the thesis; and
xii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Overall percentage (%)	5		
Signature		Date	26/02/2019

Chapter 4
Post fire litters are richer in water soluble carbon and lead to increased microbial activity

Applied Soil Ecology 136 (2019) 101–105

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Short communication

Post fire litters are richer in water soluble carbon and lead to increased microbial activity

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ARTICLE INFO

Keywords:
Labile carbon
Microbial activity
Wildfire
Post fire litter decomposition

ABSTRACT

Under conditions of increased fire season length and area affected by fire, stocks of carbon stored in forests are at increased risk of burning. While much research has investigated the immediate loss of above ground and below ground carbon stocks through combustion during a fire, there has been little research on subsequent organic matter cycling in post-fire environments. Fire can introduce new organic matter to the litter layer through the formation of a post-fire litter layer composed of debris from fire induced plant stress or death. This litter may have different chemistry and decomposition dynamics to the pre-fire litter due to the changed pathway from plant to ground and the narrow age range of the debris. In this study, litters collected from two vegetation types (*Pinus* and *Eucalyptus* dominated) and from adjacent areas either fire affected (FA) or not fire affected (NFA) were incubated as litter, or as water extracts of litter, in soils to determine fire induced changes in nutrient pools, microbial biomass and microbial activity. Post-fire litters contained more labile C (15 and 30 mg C g native/pine litter^{-1}, respectively) than litters unaffected by fire (4 mg C g litter^{-1}). Increased labile C concentration correlated ($r^2 > 0.95$) with increased microbial activity without a concurrent change in nitrogen (microbial) or phosphorus (resin and microbial) pools. Our results suggest that labile C in post-fire litter can alter microbial carbon cycling and that effects may be more pronounced under pine compared to native forest.

1. Introduction

Fire danger season and frequency of fire are increasing in many regions of the world due to the effects of climate change (Flannigan et al., 2009). In these regions, forests represent an important global carbon sink both as forest biomass and as soil organic carbon (Lal, 2005). While much research has investigated the immediate effect of fire on above ground and below ground carbon stocks (Bennett et al., 2014; Certini, 2005; Wanthongchai et al., 2008), there has been little research on organic matter cycling in post-fire environments (Butler et al., 2017a,b; Toberman et al., 2014). Forest leaf litter is a sink of nutrients from the canopy and a source of resources for the soil that can be rapidly lost in a fire; risk of fire is increased with litters of low decomposability and high flammability (Grootemaat et al., 2015). Fire changes the amounts and types of organic matter in the litter layer: organic matter is lost via combustion and may be transformed through scorching or charring (Certini, 2005). Fire can also introduce new organic matter to the litter layer through the formation of a post-fire litter layer composed of debris that falls due to fire induced plant stress or death (Alexis et al., 2010). The chemistry of this litter may be different to the litter which existed before the fire due to the changed pathway from plant to ground and the narrow age range of the debris (i.e. all formed in a single event rather than over a period of several months to years).

Tree leaves may reach the soil surface *via* two pathways: (i) a programmed loss pathway where leaves have had nutrients withdrawn before apoptosis and abscission; and (ii) an alternate pathway, where nutrients have not been withdrawn, due to physical removal (e.g. wind damage) or tree death (Cortina and Vallejo, 1994). A post fire litter layer can only form from plants that were fire resistant or were sufficiently tall to avoid full combustion of their canopy, or from nearby unaffected vegetation. Such litter layers will have low taxonomic diversity, and a tight decomposition age. Such litter has been reported to have a different chemical composition and to support a less diverse and abundant faunal community than the original leaf litter layer and this can influence litter decomposition in post-fire sites (Attiwill, 1980; Gongalsky et al., 2016). The consequences of a change in the pathway that leaves follow on their way to the litter layer on carbon and nutrient

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https://doi.org/10.1016/j.apsoil.2018.12.021
Received 28 August 2018; Received in revised form 12 December 2018; Accepted 31 December 2018
Available online 05 January 2019

cycling may be further compounded by the effect of fire at the soil surface.

Forestry in temperate regions involves a range of tree species that may be managed as a monoculture or allowed to self-manage as a multi-species woodland or forest. Both of these types of forestry are practised in south-eastern Australia, with the two most common examples being *Pinus* monoculture plantations and mixed native woodland with *Eucalyptus* dominant. Quantities and qualities of litter in *Pinus* plantations and *Eucalyptus* woodland are variable. Litterfall in close canopied *P. radiata* plantations ranges from 2.3 to 4.1 t ha^{-1} year^{-1} (Florence and Lamb, 1974; Levett et al., 1985; Theodorou and Bowen, 1990), while litterfall in *Eucalyptus* spp. woodlands from south-east Australia ranges from 1.2 to 5.5 t ha^{-1} year^{-1} (Attiwill et al., 1978; Lee and Correll, 1978; Williams and Wardle, 2007). However, although both forest types have similar rates of litterfall, the accumulation of litter under *P. radiata* plantations is usually substantially larger (up to 32 t ha^{-1}; typically between 15 and 25 t ha^{-1}) than under *Eucalyptus* forests (9.5 to 11.2 t ha^{-1}) (Baker and Attiwill, 1985; Florence and Lamb, 1974; Lee and Correll, 1978). The different litter retention times of *Pinus radiata* litter and *Eucalyptus* litter suggest different susceptibility to litter decomposition processes as was observed by Crockford and Richardson (2002).

To explore the effect of different litter origin and fire history on decomposition, we incubated *P. radiata* and *Eucalyptus* woodland litters collected from unburnt and burnt sites in four soils from the local region. As the fire that generated these materials was an unplanned bushfire, the study uses a space-for-time design rather than litters collected before and after the fire event. We hypothesised that (1) fire affected (FA) litters would contain less labile C than non fire affected (NFA) litters, (2) FA litters would affect microbial activity less than NFA litters, and (3) soil respiration would be positively correlated with labile C additions.

2. Methods

2.1. Site description and sampling

Litters and soils were collected from four sites within a forestry reserve in the Adelaide Hills, South Australia (Table 1). The region has a Mediterranean to temperate climate with average annual precipitation of 650 mm (winter dominated) and mean maximum monthly temperature ranging from 10.9 °C to 27.5 °C (Bureau of Meteorology, 2015). Soils were all oxic, non-saline ($EC_{1:5}$ < 52 µS cm^{-1}) sandy clay loams of the same soil type (Leptic Regosols; IUSS Working Group WRB, 2015) with acid pH (4.7–5.8), moderate organic carbon content (2.4–3.9%) and variable, but generally low, available P contents (0.48–7.42 µg P g soil^{-1}). These soils are formed on residuum from the Barossa Complex (Proterozoic aged; > 541 Ma). Sites were selected to span two forestry types (*Eucalyptus* woodlands and *Pinus* plantations) and two fire histories (unburnt and burnt 4 months previously).

Table 1
Fire severity classes (Keeley, 2009), vegetation type, and site names and locations.

Fire severity definition	Vegetation	Site	Site location
Litter intact with plant parts green and unaltered, no direct effect from heat	Woodland	Native NFA	34°42′56.38″S 138°51′17.40″E
	Pine plantation	Pine NFA	34°43′6.03″S 138°51′45.05″E
Canopy trees killed and foliage consumed. Surface litter of all sizes and soil organic layer largely consumed. White ash deposition and charred organic matter to several cm depth	Woodland	Native FA	34°43′3.33″S 138°51′12.95″E
	Pine plantation	Pine FA	34°44′29.93″S 138°51′21.08″E

Multiple soil samples (n = 36; 0–10 cm) were collected from sites and bulked at the site level. These soils were then treated as individual replicates in the laboratory incubation experiments (see below). *Eucalyptus* woodland sites (referred to as "native", hereafter) are defined as 'Long Leaf Box Woodland' (Berkinshaw, 2009) and "pine" sites were closed-canopy plantations 30 years and older of *Pinus radiata*. Litters were collected from multiple locations within each site and bulked per site for the laboratory incubation experiments (see below). Litters and soil were dried at 40 °C until constant mass. Litters were coarsely ground and screened (0.025–2.0 mm retained), and soils were sieved to < 2 mm.

2.2. Litter characterisation

Litters were characterized for total organic carbon (TOC), total nitrogen (TN), water extractable organic carbon (WEOC), fixed and volatile carbon, and ash content. Total organic carbon and TN content were measured by dry combustion (Dumas). Water extractable organic carbon was determined on a 1:30 litter:water extract that was shaken for 1 h and analysed for non-purgeable organic carbon using high temperature combustion in a TOC analyser (Shimadzu Corporation). Fixed and volatile carbon were determined by proximate analysis using a TGA/DSC2 sensor (Mettler Toledo) as described in Akhtar et al. (2016). Carbonate detection was conducted by mid-infrared spectroscopy using a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc.) as described in Baldock et al. (2013).

2.3. Experimental design and methodology

Experiments were conducted to determine the effect of the different litter types (Experiment 1), or water extracted litter components (Experiment 2) on microbial activity, microbial biomass and nutrient pools. In both incubations, treatments included the 'control' (water only), fire affected (FA) native litter, FA pine litter, not fire affected (NFA) native litter, and NFA pine litter.

Experiment 1: Air-dried soils were pre-incubated for 10 days at 21–23 °C at 50% of their water holding capacity to avoid the influence of the rewetting flush (Sun et al., 2015). After pre-incubation, soils were amended with litter at a rate of 34 g kg^{-1}; this litter amendment rate was chosen to reflect the average of field measurements of dry mass of litter per area (data not shown). Each treatment in this experiment used soil sites as replicates (n = 4). Soil and litter was mixed thoroughly, then readjusted to 50% of water holding capacity. After amendment, soils in Experiment 1 were packed to a bulk density of 1.2 g cm^{-3} into PVC cores with a nylon mesh bottom, following the methods of Sun et al. (2015). The cores were incubated in 174 mL glass jars (Ball® jars, Jarden Corporation) fitted with gas-tight lids with stainless steel septum ports to allow gas sampling. Soil respiration was quantified daily (see below) and soil moisture was maintained by mass. The soils were destructively sampled at two days after maximum respiration (time 1; day 3) and two days after respiration stabilization (time 2; day 6). Microbial biomass N and P (MBN and MBP), ammonia and nitrate/nitrite concentrations, and resin P were measured on all samples.

Experiment 2: Soils were amended with litter extracts at 50% of water holding capacity (sites as replicates; n = 4). Extracts were produced by shaking litters with water at a rate of 1 g ground litter to 0.65x the soil water holding capacity for each soil; liquid was extracted via centrifugation. Incubation followed the same methods as above; however, only respiration was measured on these samples as this experiment only aimed to observe the microbial activity response to water extractable litter components.

Soil respiration rates were quantified by measuring headspace CO_2 concentration using a Servomex 1450 infrared gas analyser (Servomex Group, Crowborough, England) as described in Butterly et al. (2010). Soil microbial biomass N (MBN) was determined using chloroform fumigation extraction (Vance et al., 1987) with 0.5 M K_2SO_4 at a 1:5

soil:extractant ratio; N content in the extracts was determined using ammonium-salicylate oxidation (Forster, 1995). Ammonium and nitrate/nitrite N were determined after shaking soil with 2 M KCl in a 1:5 ratio. Ammonium-N was measured using ammonium-salicylate oxidation (Forster, 1995) and nitrate-N was measured using vanadium(III) reduction (Miranda et al., 2001). Resin P was extracted using the anion exchange resin method (Kouno et al., 1995) and the P concentration was determined colorimetrically (Murphy and Riley, 1962). Soil microbial biomass P was determined as the difference between P extracted using anion exchange resins with and without hexanol fumigation (McLaughlin et al., 1986).

2.4. Data analyses

All statistical analyses were carried out using R (R Core Team, 2018) and significance was set at $p < 0.05$. Respiration decay curves were determined per treatment using stats::nsl (R Core Team, 2018) with a first order decay equation, using the 'port' algorithm until convergence. Curve fitting parameters 'C' (the asymptote of growth constant) and 'k' (rate of decay constant) were pooled by soil for further analysis. One way Analysis of Variance (ANOVA) was used to determine the effect of treatment on measured chemical variables and on the curve fitting parameters ('C' and 'k'); in the case where significance was detected, post hoc Tukey (Honest Significant Difference (HSD)) tests were used to determine significant differences.

3. Results

3.1. Litter characterisation

Litter characterisation results are detailed in Table 2. Total organic carbon was 37 ± 2% in the NFA litters and 42 ± 2% in the FA litters and the C:N ratio was similar for all litters (range: 31–33). The concentration of water extractable organic carbon was similar in both NFA litters (range: 4.0–4.1 mg C g litter^{-1}), four times larger in the FA native litter than the NFA native litter, and seven times larger in the FA pine litter than the NFA pine litter. Fixed C was similar for all litters (range: 19.5%–22.5%), and volatile matter was higher and ash content was lower in the FA litters relative to their NFA counterparts. Carbonate was not detected in any mid infrared spectra.

3.2. Microbial activity via CO_2 respiration

In both experiments, cumulative respiration (Fig. 1; Table 3) was well described by a first order decay curve. The decay rate ('k') did not vary significantly across all soils amended with litter (range: −0.29 to −0.21) or with litter-extracts (range: −0.29 to −0.22). Where soil was amended with litter (Experiment 1), the constant 'C' was highest for the pine FA amendment and equally low in the control and NFA treatments; where soils were amended with water-soluble litter extracts (Experiment 2) 'C' followed a similar pattern. Cumulative respiration in the litter extract treatments was more than half of that in soils with litter amendments (range: 56%–82%). This indicates that although much of the C respired in the litter treatments could be ascribed to decomposition of WEOC, at least 20–40% of the C respired in the litter treatments was due to the non-soluble litter component.

Table 2
Litter characterisation results for air dried litters. Definitions: non fire affected (NFA), fire affected (FA), total nitrogen (TN), total organic carbon (TOC), C:N ratio (C:N), water extractable organic carbon (WEOC).

	Native litter		Pine litter	
	NFA	FA	NFA	FA
TN (%)	1.17	1.38	1.13	1.28
TOC (%)	38.0	43.2	35.3	40.2
C:N	32.5	31.3	31.2	31.4
WEOC (mg C g litter^{-1})	4.0	15.1	4.1	30.1
Moisture (%)	5.7	5.8	7.4	3.6
Volatile matter (%)	65.7	72.3	59.5	70.7
Fixed Carbon (%)	20.3	19.6	22.2	20.9
Ash (%)	8.3	2.3	10.9	4.3

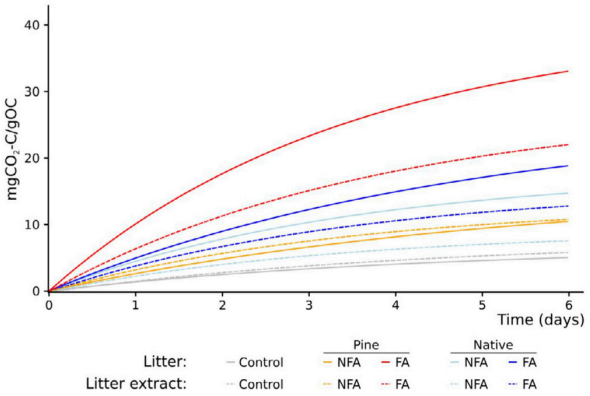

Fig. 1. C respiration (mgCO_2-C gOC^{-1}) over 6 days in soils amended with coarsely ground litter or water extracts of coarsely ground litter: water (Control), native non fire affected (NFA) litter, native fire affecter (FA) litter, pine NFA litter or pine FA litter. Days 0–3, n = 24; days 3+, n = 12; lines are decay curves (see Table 3; n = 4; $f(x) = C(1\text{-}e^{(kx)})$).

Table 3
Cumulative respiration decay curves for soils with litter amendments or with litter extract amendments (n = 4; $f(x) = C(1 - e^{(kx)})$). Definitions: non fire affected (NFA) and fire affected (FA). Values are means ± standard deviation; Columns with same letter are not significantly different (Tukeys HSD, α = 0.05).

	Treatment	'C' mg CO_2-C g OC^{-1}		k	
Ground litter or water	Control	3.7 ± 2.4	c	−0.26 ± 0.15	ns
	Pine NFA	15.9 ± 5.5	bc	−0.21 ± 0.12	ns
	Pine FA	35.7 ± 8.8	a	−0.29 ± 0.11	ns
	Native NFA	16.5 ± 6.5	bc	−0.29 ± 0.16	ns
	Native FA	22.8 ± 7.9	ab	−0.25 ± 0.09	ns
Litter water extracts or water	Control	8.1 ± 3.6	B	−0.22 ± 0.09	ns
	Pine NFA	13 ± 4.0	B	−0.28 ± 0.07	ns
	Pine FA	28 ± 11	A	−0.26 ± 0.05	ns
	Native NFA	9.3 ± 2.1	B	−0.29 ± 0.11	ns
	Native FA	16 ± 4.7	AB	−0.28 ± 0.05	ns

3.3. Nutrient pools

The mineral N pool (ammonia plus nitrate) was significantly affected by litter amendment type (Table 4). Two days after maximum respiration (time 1), the control, FA pine litter, and NFA native litter treatments had at least 3-times higher concentrations of mineral N (range: 12–20 μg N g soil^{-1}) than the pine NFA and native FA treatments (range 1.2–3.8 μg N g soil^{-1}). Two days after respiration stabilization (time 2), concentrations of mineral N were higher than at time 1 for all but the pine FA treatment and higher for the control and pine NFA litter (range: 32–34 μg N g soil^{-1}) than all other treatments (range: 15–20 μg N g soil^{-1}). Litter amendment did not have an observable effect on MBN, MBP or resin P at either sampling time.

4. Discussion

Water extractable organic carbon contents of the litters were strongly positively correlated with the asymptotes of growth for both litter and litter extract amendments (Fig. 2). The proportion of OC

Table 4
Microbial biomass N (MBN; μg N/g soil), microbial biomass P (MBP; μg P/g soil), available N (ammonium and nitrate/nitrite μg N/g soil), and available P (AP μg P/g soil) for soils incubated with water (Control) or coarsely ground litter at time 1 (two days after maximum respiration rate) and time 2 (two days after respiration rate stabilization). Definitions: non fire affected (NFA) and fire affected (FA). Values are means; Columns with same letter are not significantly different (Tukeys HSD, $\alpha = 0.05$); 'ns' indicated no significant difference and '***' indicates significance at $P < 0.001$ for each sample time.

		MBN (μg/g)		MBP (μg/g)		Ammonium and nitrate/nitrite N (μg/g)		Resin P (μg/g)	
Time 1 (day 3)	Control	2.4	ns	4.7	ns	18.4 a	***	1.8	ns
	Pine NFA	3.7		7.0		1.2 c		2.6	
	Pine FA	3.9		8.0		19.5 a		1.8	
	Native NFA	2.7		6.0		12.2 ab		1.1	
	Native FA	4.6		6.1		3.8 bc		1.4	
Time 2 (day 6)	Control	19.3	ns	5.9	ns	33.7 a	***	1.4	ns
	Pine NFA	11.7		9.2		32.0 a		2.0	
	Pine FA	16.6		9.6		15.6 b		1.9	
	Native NFA	8.6		9.0		19.5 b		1.0	
	Native FA	4.3		8.7		15.4 b		1.0	

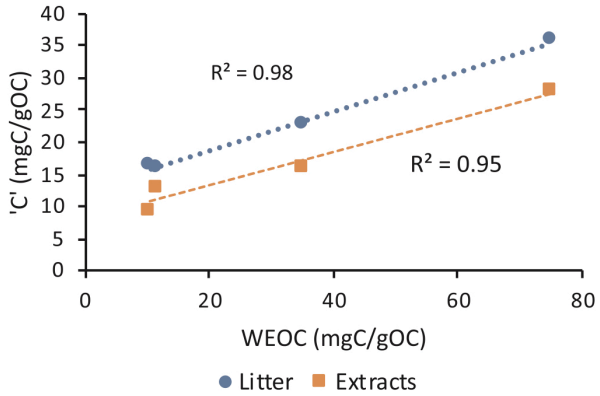

Fig. 2. Linear correlation between 'C' (mgC gOC^{-1}) and water extractable organic carbon (WEOC) (mgC gOC^{-1}) for litter incubation (blue) and litter extract incubation (orange). Each point represents the average values of 'C' (Table 3) and WEOC (Table 2).

respired in the litter treatments ranged from < 0.5% in the control to 3.6% in the pine FA litter treatment; cumulative respiration is small relative to the quantity of OC added in the litter treatments. However, as can be seen in Fig. 1, the quantity of CO_2 respired in the extract experiment is far in excess of the quantity of soluble carbon added (which was equivalent to < 0.1 mg C g OC^{-1}). These results indicate that microbial priming (Bingeman et al., 1953) is occurring in soils amended with the pine FA litter extract at a minimum. Microbial priming of SOC decomposition has been observed in response to additions of both simple sugars and litter to soil and does not appear to be directly influenced by microbial biomass quantity (Li et al., 2018; Nottingham et al., 2009). Similarly, in this study, microbial biomass N did not vary with litter amendment type. Available N was highly variable in this experiment, and although the lack of response in the microbial biomass to organic matter amendments has been linked with nitrogen limitation (Zhang et al., 2005), no conclusive evidence of N limitation was observed in our study.

Affecting pine litter with fire changed the litter from the least decomposable to the most decomposable over the first 6 days. It is possible that the NFA litters contain components with an inhibitory effect on respiration, as the modelled asymptote of growth was not significantly different between NFA litters and the control soil. Microbial inhibition may be due to compounds such as those found in essential oils; for example, essential oils derived from *Pinus radiata* and *Eucalyptus globulus* have antifungal properties (Sacchetti et al., 2005). In addition to the increased WEOC, FA litter may be relieved of this inhibitory effect by heat damage during the fire, or by the increased nutrition available in litter derived from unplanned losses (Águas et al., 2018; Attiwill et al., 1978). In either case, an increase in labile C relative to recalcitrant organic molecules promotes litter decomposition regardless of litter stoichiometry (Hättenschwiler and Jørgensen, 2010).

While the process leading to an increase in WEOC is unclear, the difference between FA pine and FA native litter is curious. It is possible that differences in macroscopic leaf/needle morphology (i.e. the cylindrical nature of pine needles vs. the lanceolate nature of *Eucalyptus* leaves), litter age or other properties led to the changes observed here, which is worthy of further investigation. Regardless of the cause of differences in the litters, the fact that they have formed in the field after the same fire shows that post-fire litters are functionally different to normally formed litters. The results herein indicate that fire has different effects on native and pine litters, and even though complex differences in litter chemistry may affect litter decomposition, water extractable organic carbon of the amendments is a simple measurement that correlates strongly with soil respiration over the first 6 days of decomposition.

5. Conclusions

From this small study, it is clear that fire has affected native *Eucalyptus* woodland litter and *Pinus radiata* plantation litter differently and that the change in labile C caused a change in microbial activity without a concurrent change in microbial biomass. The process that increased water extractable organic carbon is not clear from this study; however, the effect may be important for post fire litter decomposition and the leaching of carbon from a post fire litter layer. This study highlights some of the uncertainties that still exist on the long term effects of fire on forest ecosystems due to the unknown effect of post fire litter layers on carbon and nutrient cycling. Future research in this area would benefit from a longer incubation time and more detailed characterisation of the organic amendments.

Acknowledgements

This project was supported by The Holsworth Wildlife Research Endowment & The Ecological Society of Australia, and The University of Adelaide Research Training Program Scholarship, with further analytical assistance provided by CSIRO Agriculture. ES gives thanks to GC Garrett for his assistance in the field and the laboratory. We also thank the editor and reviewers for their constructive comments on this manuscript.

References

Águas, A., Incerti, G., Saracino, A., Lanzotti, V., Silva, J.S., Rego, F.C., Mazzoleni, S., Bonanomi, G., 2018. Fire effects on litter chemistry and early development of Eucalyptus globulus. Plant Soil 422, 495–514.

Akhtar, N., Goyal, D., Goyal, A., 2016. Physico-chemical characteristics of leaf litter biomass to delineate the chemistries involved in biofuel production. J. Taiwan Inst. Chem. Eng. 62, 239–246.
Alexis, M.A., Rumpel, C., Knicker, H., Leifeld, J., Rasse, D., Péchot, N., Bardoux, G., Mariotti, A., 2010. Thermal alteration of organic matter during a shrubland fire: a field study. Org. Geochem. 41, 690–697.
Attiwill, P.M., 1980. Nutrient cycling in a Eucalyptus obliqua (L'hérit.) Forest [in Victoria]. IV* nutrient uptake and nutrient return. Aust. J. Bot. 28, 199–222.
Attiwill, P.M., Guthrie, H.B., Leuning, R., 1978. Nutrient cycling in a Eucalyptus obliqua (L'Hérit.) Forest. I. Litter production and nutrient return. Aust. J. Bot. 26, 79–91.
Baker, T.G., Attiwill, P.M., 1985. Above-ground nutrient distribution and cycling in Pinus radiata D. Don and Eucalyptus obliqua L'Hérit. Forests in Southeastern Australia. For. Ecol. Manage. 13, 41–52.
Baldock, J.A., Hawke, B., Sanderman, J., MacDonald, L.M., 2013. Predicting contents of carbon and its component fractions in Australian soils from diffuse reflectance mid-infrared spectra. Soil Res. 51, 577–595.
Bennett, L.T., Aponte, C., Baker, T.G., Tolhurst, K.G., 2014. Evaluating long-term effects of prescribed fire regimes on carbon stocks in a temperate eucalypt forest. For. Ecol. Manage. 328, 219–228.
Berkinshaw, T., 2009. Mangroves to Mallee: The Complete Guide to the Vegetation of Temperate South Australia. Finsbury Green, Adelaide.
Bingeman, C.W., Varner, J.E., Martin, W.P., 1953. The effect of the addition of organic materials on the decomposition of an organic soil. Soil Sci. Soc. Am. J. 17, 34–38.
Bureau of Meteorology, Climate data online, 2015, < www.bom.gov.au/climate/data/ > .
Butler, O.M., Lewis, T., Chen, C., 2017a. Fire alters soil labile stoichiometry and litter nutrients in Australian eucalypt forests. Int. J. Wildland Fire 26, 783–788.
Butler, O.M., Lewis, T., Chen, C., 2017b. Prescribed fire alters foliar stoichiometry and nutrient resorption in the understorey of a subtropical eucalypt forest. Plant Soil 410, 181–191.
Butterly, C.R., Marschner, P., McNeill, A.M., Baldock, J.A., 2010. Rewetting CO2 pulses in Australian agricultural soils and the influence of soil properties. Biol. Fertil. Soils 46, 739–753.
Certini, G., 2005. Effects of fire on properties of forest soils: a review. Oecologia 143, 1–10.
Cortina, J., Vallejo, V.R., 1994. Effects of clearfelling on forest floor accumulation and litter decomposition in a radiata pine plantation. For. Ecol. Manage. 70, 299–310.
Crockford, R.H., Richardson, D.P., 2002. Decomposition of litter in a dry sclerophyll eucalypt forest and a Pinus radiata plantation in southeastern Australia. Hydrol. Process. 16, 3317–3327.
Flannigan, M.D., Krawchuk, M.A., De Groot, W.J., Wotton, B.M., Gowman, L.M., 2009. Implications of changing climate for global wildland fire. Int. J. Wildland Fire 18, 483–507.
Florence, R.G., Lamb, D., 1974. Influence of stand and site on radiata pine litter in South Australia. N. Z. J. For. Sci. 4, 205–210.
Forster, J.C., 1995. Soil nitrogen. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 79–87.
Gongalsky, K.B., Zaitsev, A.S., Korobushkin, D.I., Saifutdinov, R.A., Yazrikova, T.E., Benediktova, A.I., Gorbunova, A.Y., Gorshkova, I.A., Butenko, K.O., Kosina, N.V., Lapygina, E.V., Kuznetsova, D.M., Rakhleeva, A.A., Shakhab, S.V., 2016. Diversity of the soil biota in burned areas of southern taiga forests (Tver oblast). Eurasian Soil Sci. 49, 358–366.
Grootemaat, S., Wright, I.J., van Bodegom, P.M., Cornelissen, J.H.C., Cornwell, W.K., 2015. Burn or rot: leaf traits explain why flammability and decomposability are decoupled across species. Funct. Ecol. 29, 1486–1497.
Hättenschwiler, S., Jørgensen, H.B., 2010. Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. J. Ecol. 98, 754–763.
IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, Update 2015 International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. FAO, Rome.
Keeley, J.E., 2009. Fire intensity, fire severity and burn severity: a brief review and suggested usage. Int. J. Wildland Fire 18, 116–126.
Kouno, K., Tuchiya, Y., Ando, T., 1995. Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biol. Biochem. 27, 1353–1357.
Lal, R., 2005. Forest soils and carbon sequestration. For. Ecol. Manage. 220, 242–258.
Lee, K.E., Correll, R.L., 1978. Litter fall and its relationship to nutrient cycling in a South Australian dry sclerophyll forest. Aust. J. Ecol. 3, 243–252.
Levett, M.P., Adams, J.A., Walker, T.W., 1985. Nutrient returns in iitterfall in two indigenous and two radiata pine forests, westland, new zealand. N. Z. J. Botan. 23, 55–64.
Li, L.J., Zhu-Barker, X., Ye, R., Doane, T.A., Horwath, W.R., 2018. Soil microbial biomass size and soil carbon influence the priming effect from carbon inputs depending on nitrogen availability. Soil Biol. Biochem. 119, 41–49.
McLaughlin, M.J., Alston, A.M., Martin, J.K., 1986. Measurement of phosphorus in the soil microbial biomass: a modified procedure for field soils. Soil Biol. Biochem. 18, 437–443.
Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide – Biol. Chem. 5, 62–71.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.
Nottingham, A.T., Griffiths, H., Chamberlain, P.M., Stott, A.W., Tanner, E.V.J., 2009. Soil priming by sugar and leaf-litter substrates: a link to microbial groups. Appl. Soil Ecol. 42, 183–190.
R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., Bruni, R., 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem. 91, 621–632.
Sun, Q., Meyer, W.S., Koerber, G.R., Marschner, P., 2015. Response of respiration and nutrient availability to drying and rewetting in soil from a semi-arid woodland depends on vegetation patch and a recent wildfire. Biogeosciences 12, 5093–5101.
Theodorou, C., Bowen, G.D., 1990. Effects of fertilizer on litterfall and N and P release from decomposing litter in a Pinus radiata plantation. For. Ecol. Manage. 32, 87–102.
Toberman, H., Chen, C., Lewis, T., Elser, J.J., 2014. High-frequency fire alters C : N : P stoichiometry in forest litter. Glob. Change Biol. 20, 2321–2331.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.
Wanthongchai, K., Bauhus, J., Goldammer, J.G., 2008. Nutrient losses through prescribed burning of aboveground litter and understorey in dry dipterocarp forests of different fire history. Catena 74, 321–332.
Williams, M.C., Wardle, G.M., 2007. Pine and eucalypt litterfall in a pine-invaded eucalypt woodland: the role of fire and canopy cover. For. Ecol. Manage. 253, 1–10.
Zhang, W.J., Zhu, W., Hu, S., 2005. Soil resource availability impacts microbial response to organic carbon and inorganic nitrogen inputs. J. Environ. Sci. 17, 705–710.

Statement of Authorship

Title of paper	The effect of litter origin and charcoal on *Pinus radiata* litter decomposition
Publication status	☒ Published ☐ Accepted for publication ☐ Submitted for publication ☐ Unpublished and unsubmitted work written in manuscript style
Publication date	8th February, 2019

Principle Author

Name of principle author	Erinne Stirling		
Contribution to the paper	• Experimental design, field work, laboratory analyses, and data analyses • Preparation of the manuscript		
Overall percentage (%)	85		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	**6/03/2019**

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

iv. the candidate's stated contribution to the publication is accurate (as detailed above);
v. permission is granted for the candidate in include the publication in the thesis; and
vi. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Chapter 5

The effect of fire affected *Pinus radiata* litter and char addition on soil nitrogen cycling

Science of the Total Environment 664 (2019) 276–282

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The effect of fire affected *Pinus radiata* litter and char addition on soil nitrogen cycling

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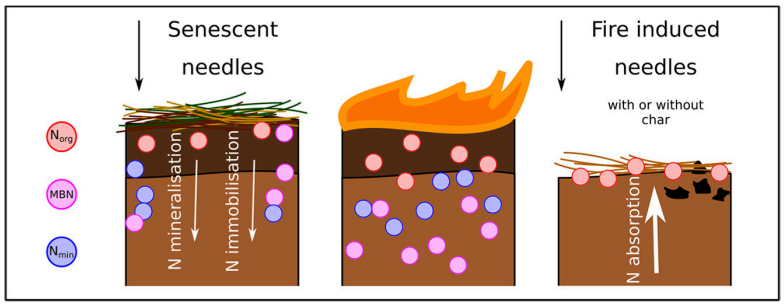
HIGHLIGHTS

- Soil respiration was enhanced after amendment with fire affected litter.
- Char incorporation did not influence microbial activity or N pools.
- Lack of detectable mineral N might be attributed to absorption by fire affected pine litter.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 November 2018
Received in revised form 8 January 2019
Accepted 24 January 2019
Available online 04 February 2019

Editor: Elena Paoletti

Keywords:
Litter decomposition
N cycling
Soil respiration
N absorption
Pinus radiata

ABSTRACT

In pine forest litters, decomposition rate is directly affected by the pathway the needle followed to the ground, whether that was via programmed apoptosis and abscission or via stress induced loss through branch damage or tree death. Stress induced losses may occur due to fire damage, which leads to a post-fire litter layer composed of non-senescent debris that fell during or after the event. This study investigates decomposition and nitrogen cycling in soils amended with two litters from *Pinus radiata* plantations that had different recent fire histories. Litters were incubated in the presence or absence of field collected char for up to 94 days. These soil treatments were analysed for microbial activity (soil respiration) and N pools (microbial, mineral, and potentially mineralisable). Soil and litter treatments were additionally incubated in the presence of ammonium nitrate solution to determine N absorption potential of the litters. Respiration was greatest in soils that received fire affected (FA) litter regardless of the presence or absence of char. Nitrogen pools were largely similar between the control (no litter) treatment and not fire affected (NFA) litter treatments. Measured N pools were exceedingly low (92% of samples <2 μg-N g soil^{-1} where detected) or not detectable (37% of samples below detection limits) in all FA litter treatments at most times. Char appeared inert throughout and had no effects on microbial activity or nitrogen cycling. This study indicates that fire affected pine litter collected four months post fire has strong N absorption properties with or without the presence of char. The presence of fire affected litter is likely to affect N availability for regeneration of forest growth.

1. Introduction

While the traditional source of income from forestry plantations is timber products, the emergence of modern carbon markets has raised

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https://doi.org/10.1016/j.scitotenv.2019.01.316
0048-9697/

interest in forestry plantations for their C sequestration potential. Part of this sequestering potential involves the litter layer, which in *Pinus radiata* plantations represent a substantial C pool. For example, pine needle accumulation has been recorded at up to 32 t ha^{-1} in south-eastern Australia (typically between 15 and 25 t ha^{-1}) (Baker and Attiwill, 1985; Florence and Lamb, 1974). An accumulation of 20 t ha^{-1} in softwood plantations in south eastern Australia (approximately 734,600 ha; (Downham and Gavran, 2018)) represents a carbon stock of approximately 14 million tonnes (Paul and Polglase, 2004). As needle deposition rates are relatively low in these systems (2.3 to 4.1 t ha^{-1} $year^{-1}$) (Florence and Lamb, 1974; Levett et al., 1985; Theodorou and Bowen, 1990), needle accumulation is largely due to a resistance to decomposition.

Quality constraints to litter decomposition include the high lignin content of pine litters leading to a period of nutrient immobilisation during early decomposition. However, this may depend on whether pine needle senescence has occurred via a programmed aging pathway (where leaves have had nutrients withdrawn before apoptosis and abscission), or from a stress induced pathway, such as fire (Cortina and Vallejo, 1994; Osono and Takeda, 2004). Foliage lost due to a programmed event has mobile nutrients withdrawn before abscission (Attiwill et al., 1978); foliage lost in response to stress events may therefore decompose faster due to the increased concentrations of nutrients (Girisha et al., 2003). It is not clear from the literature however, whether stress induced losses represent a significant proportion of normally senescent litters during fire weather; in these experiments, we have assumed that they do not. In this study, the stress induced losses of interest are those that occur during and after fire events from the death of trees whose canopies were not burnt during the fire.

In addition to C losses due to combustion of the litter layer, organic matter in soils can be expected to have different decomposition dynamics post fire. After a fire, a substantial proportion of forest litter is composed of non-senescent dead foliage that fell during or after the event (Alexis et al., 2007). While C losses during a fire are generally limited to the O horizon rather than the mineral soil horizons (Certini et al., 2011), fire can change the chemical structure of organic matter in the litter layer and in the soil (Certini et al., 2011; McBeath et al., 2014). During a fire, thermal changes of woody materials may occur at relatively low temperatures, as decarboxylation of cellulose occurs at 300–340 °C and lignin decomposition occurs at 350–430 °C (Certini et al., 2011; Lopez-Capel et al., 2005). Fire frequency, intensity, and severity can also have long term effects on litter nutrient content and therefore litter decomposition (Ficken and Wright, 2017; Holden et al., 2016; Reinhart et al., 2016). As microbial activity and organic matter decomposition are affected by substrate and decomposition environment, changes in either of these sets of variables can be expected to influence decomposition.

Fire also has multiple direct effects on organic matter in soil and litter through charring, which leads to these materials behaving differently to their pre-fire counterparts. Although the rate of char decomposition is generally slow enough that it can be considered a long-term C storage option (Lehmann et al., 2006), the net rate of organic matter decomposition may be enhanced under post fire litter layers through microbial priming (Ding et al., 2017; Farrell et al., 2013). As a component of the soil organic matter, char can be protected from microbial decomposition through physical protection within soil aggregates, through complexing with clay particles, or through the formation of chemically recalcitrant molecules (Dungait et al., 2012; Six et al., 2002). Char may also be inherently resistant to degradation as pyrogenic C exists as a spectrum of organic molecules that range from low to high reactivity depending on their size and degree of pyrolysis (degradation continuum) (Baldock and Smernik, 2002; Bird et al., 2015; Jenkins et al., 2014).

Char may also influence litter and native organic matter decomposition through negative or positive priming (Maestrini et al., 2015). In their metanalysis of 18 studies, Maestrini et al. (2015) found char tended to have a positive priming effect on native organic matter and a negative priming effect on fresh residues. During short term incubations (up to two weeks) high temperature pyrolysis chars may inhibit microbial activity (Lanza et al., 2016); however, a wide range of microbial biomass and activity responses to char have been observed over short and long-term (months – years) incubations (Gomez et al., 2014; Gul et al., 2015). While there is some evidence that char addition inhibits microbial use of glucose (Lanza et al., 2016), in most cases the addition of simple sugars (e.g. glucose) increases char degradation (Hamer et al., 2004; Nocentini et al., 2010); however, accelerated mass loss in soil organic matter (SOM)/char mixes has been attributed to SOM rather than char decomposition (Pluchon et al., 2016). Although glucose addition to the soil can stimulate char decomposition, the effect of more complex organic compounds (such as those found in leaf litters) on microbial priming remains unclear.

Here we present results of a study in which we address the following hypotheses: (1) that, due to the relative youth and lack of nutrient withdrawal prior to litterfall, addition of fire affected (FA) litter will result in higher rates of soil respiration than addition of not fire affected (NFA) litter; and (2) that, due to the age and reactive compounds in needles, FA litter will induce more nitrogen immobilisation and a larger microbial biomass than NFA litter. Char is included in this study in the interest of creating a soil habitat similar to that likely to occur under post fire conditions. These hypotheses were addressed by studying the soil responses to litters and char over shorter (weeks) and longer (months) periods; this experiment used NFA and FA pine litters and char collected from a pine forest fire ground and incubation was carried out for up to 94 days. In order to observe short and longer term effects of the amendments on microbial activity and N cycling, these soils were harvested four times in the first two weeks and three times in the final month. The overall aim of this study was to explore post fire microbial activity and nitrogen cycling using materials from a *Pinus radiata* plantation to inform forestry management following wildfire events.

2. Methods

2.1. Site description and sampling

Litter and char were collected from *Pinus radiata* plantations in Old Kersbrook Forest, South Australia four months after the "Sampson Flat" fire of January 2015. These litters and char capture a moment in time after the fire; fire as a factor could not be replicated as the fire was an uncontrolled, unplanned, event. The region has a Mediterranean to temperate climate with average annual precipitation of 650 mm (winter dominated) and monthly mean maximum temperature ranging from 10.9 °C to 27.5 °C (Bureau of Meteorology, 2015). During the four months after the fire, daily maximum temperature ranged from 11 °C to 40 °C and 135.6 mm of precipitation was recorded at the nearest weather station (approximately 7 km east of the study area; Mt. Crawford AWS 23878).

Fire affected and non-fire affected litters were collected from mature pine plantations within Old Kersbrook Forest; they were collected as pine needles from the top 15 cm of the litter layer, dried at 40 °C, then coarsely ground to between 0.025 mm and 2 mm; replicates (individual microcosms, see below) in this study use the same litter. While we acknowledge that grinding the needles reduces the experiment's representation of field conditions, grinding was necessary due to the size of the incubation chambers, which were too small to take intact needles. Charred material originating from wood was collected from the fire ground within Old Kersbrook Forest; it was visually identified as black char pieces >10 mm diameter. Charred material was dried at 40 °C, and ground to between 0.5 mm and 1.0 mm; replicates in this study use the same char. The soil used in this study was a Leptic Rudosol in the Australian Soil Classification (Isbell, 2002) or Leptic Regosol in the World reference base (IUSS Working Group WRB, 2015), collected from the 0–10 cm layer from across the Old Kersbrook Forest from

under *Pinus radiata* plantations and *Eucalyptus* woodlands; replicates in this study use the same soil. Soil texture was silty loam and soil organic carbon and nitrogen contents were 27 g kg^{-1} and 2.4 g kg^{-1}, respectively.

2.2. Litter and char characterisation

Litters and char were characterised for total organic C (TOC), total N (TN), water extractable organic C (WEOC) and pH, and were also analysed using solid state ^{13}C CP-MAS nuclear magnetic resonance (NMR) spectroscopy, mid infra-red (MIR) spectroscopy and thermal gravimetric analysis (TGA) (Table 1). Total organic carbon and TN content were measured by Dumas dry combustion; water extractable organic carbon was determined on a 1:30 litter:water extract that was shaken for 1 h and analysed for non-purgeable organic carbon using high temperature combustion in a Total Organic Carbon Analyser (Shimadzu Corporation); litter pH was measured in a 1:10 substrate: water extract after shaking for 1 h (Rayment and Lyons, 2010) using a WP-81 Cond/TDS-pH/mV-Temp meter (TPS); solid-state ^{13}C NMR analyses of plant litters were conducted on a 200 Avance spectrometer (Bruker Corporation, Billerica, MA), with a 4.7 T wide-bore superconducting magnet and operating resonance frequency of 50.33 MHz. Litter samples were packed into zirconia rotors (7 mm diameter) with Kel-F end caps and spun at the 'magic angle' (54.7°) at 5 kHz. Chemical shift values were calibrated to the methyl resonance of hexamethylbenzene at 17.36 ppm and a 50 Hz Lorentzian line broadening was applied to all spectra (Baldock et al., 2013). Proximate analysis was conducted to determine ash, fixed carbon, and volatile matter contents using a TGA/DSC2 sensor (Mettler Toledo) as described in Akhtar et al. (2016). A summary of the composition of NFA, FA, components is provided in Table 1, indicating similar C:N ratio (~31.3) between litters, but differences in carbon chemistry. FA litters had higher TOC (40.2% vs 35.3%), WEOC (30.1 vs 4.1 mg C g $litter^{-1}$), and volatile matter (70.7% vs 59.5%), and relatively higher proportions of aryl and O-aryl carbon, and lower proportions of alkyl and O-alkyl carbon, and ash content (4.3 vs 10.9%) compared to FA litters (Table 1). The pH was 0.4 units lower in the FA litter than the NFA litter.

2.3. Experimental design and methods

This study comprised two experiments. Briefly, Experiment 1 was conducted to investigate decomposition dynamics and nitrogen cycling over time and involved a 94 -day aerobic soil incubation with litters and char. The results of Experiment 1 yielded interesting responses in soil N cycling, and so a second experiment (Experiment 2) was conducted to help explain these results. Experiment 2 was conducted to investigate N absorption in the NFA and FA litters and involved a 14 day aerobic or anaerobic incubation with soil, litter, or soil and litter; the aerobic incubations involved no addition of nitrogen, the anaerobic incubations were carried out without nitrogen and also with added ammonium nitrate (2 and 4 μgNH_4-N mL^{-1}).

Table 1
Litter characterisation results for air dried pine litters; where NFA is not fire affected, FA is fire affected, TC is total carbon and WEOC is water extractable organic carbon.

	Pine litter	
	NFA	FA
TC (%)	35.3	40.2
C:N ratio	31.2	31.4
WEOC (mg C g $Litter^{-1}$)	4.1	30.1
$pH_{1:30}$	4.65	4.21
0–45 Alkyl	10.8	17.6
45–110 O-Alkyl	50.6	52.0
110–165 Aromatic	30.8	22.2
165–215 Carboxyl	7.8	8.1
Moisture (%)	7.4	3.6
Volatile matter (%)	59.5	70.7
Fixed carbon (%)	22.2	20.9
Ash (%)	10.9	4.3

2.4. Experiment 1

Experiment 1 ran for 94 days with an unamended control, two litters (NFA and FA) and two char application types (present/absent) in a fully factorial design with seven time points (0, 2, 7, 13, 65, 80 and 94 days) and three replicates. Sampling occurred in the first two weeks and last month to capture short term changes in addition to the longer term changes. Soil was pre-incubated at 0.12 g g^{-1} gravimetric water content for seven days to avoid a rewetting respiration flush. After pre-incubation, 20 g dry weight equivalent soil was unamended (control), amended with litter (10 g C kg $soil^{-1}$), char (10 g C kg $soil^{-1}$), or litter and char (5 + 5 g C g $soil^{-1}$); 0.28 g water g^{-1} soil was added to all samples, after which all soils were mixed thoroughly. After amendment, soils were packed into PVC cores (37 mm internal diameter × 50 mm height) with a nylon mesh bottom (7.5 μm, Australian Filter Specialists) (Butterly et al., 2009). Soils were packed to a bulk density of 1.2 g cm^{-3} after which the cores were transferred to 375 mL, air-tight jars fitted with rubber septa for head-space gas analysis (Butterly et al., 2009). Soil respiration was quantified regularly and soil moisture was maintained by mass; the first 6 h of respiration data were lost due to a machine malfunction. Respiration rates were quantified by measuring headspace CO_2 concentration using a LICOR (Lincoln, USA) LI-820 infrared gas analyser at the end of a known time interval and was used to calculate respiration rate and cumulative respiration. After each measurement, the jars were opened to equilibrate CO_2 to ambient concentrations and then resealed. The soils were destructively sampled on days 0, 2, 7, 13, 65, 80, and 94. Total organic C and TN were analysed on days 0, 65, 80, and 94, while mineral N (ammonium and nitrate; referred to as 'N_{min}'; hereafter), potentially mineralisable N (PMN) and microbial biomass N (MBN) were analysed on all samples; soils were mixed thoroughly before sampling and refrigerated during processing.

2.5. Experiment 2

The aim of Experiment 2 was to determine if FA litter was lacking in mineral N or if it was absorbing mineral N; absorption potential was determined by incubating litter and soil with litter anaerobically in a solution containing ammonium nitrate. Litters, soil, and soil and litter mixtures were incubated without water ('dry'), aerobically (0.25 g water g $soil^{-1}$) or anaerobically (2.5 g water g $soil^{-1}$ in an N_2 atmosphere) at 25 °C for 14 days to determine net nitrification and PMN.

2.6. Soil chemical analyses

Mineral N (ammonium and nitrate) concentration was determined after shaking soil with 2 M KCl in a 1:5 ratio. Ammonium-N concentration was measured using ammonium-salicylate oxidation (Forster, 1995) and nitrate/nitrite-N concentration was measured using vanadium(III) reduction (Miranda et al., 2001). Potentially mineralisable N concentation was analysed using ammonium-salicylate oxidation on subsamples that had been anaerobically incubated for 14 days (Smith et al., 2012). Soil microbial biomass N (MBN) concentration was determined using the chloroform fumigation extraction method of (Vance et al., 1987). Fumigated and non-fumigated samples were extracted with 0.5 M K_2SO_4 at a 1:5 soil:extractant ratio, and MBN was determined as the difference in N concentration between the two with an efficiency constant of 0.54 (Joergensen and Mueller, 1996; Moore et al., 2000). All colorimetric analyses were read using a Multiscan GO plate reader (Thermo Fisher Scientific).

2.7. Statistics

In order to compare the effects of multiple variables, three-way analysis of variance (ANOVA) and post hoc Tukey tests (R::agricolae; de Mendiburu (2017)) were used to determine the significance of interactions or main effects on the measured variables for each sampling time. All statistical analyses were conducted per sampling time using R (R Core Team, 2015), and significance was set at $\alpha < 0.05$.

3. Results

3.1. Experiment 1: litter incubation

Cumulative respiration was significantly affected by litter in the first two weeks and litter and char thereafter. Cumulative respiration was highest in the FA litter treatments and lowest in the no litter control treatments. When considered on a per gram of soil basis, there was no observable difference between char treatments when added in the absence of litter (data not shown). In the absence of char, cumulative respiration on addition of NFA litter was not significantly different to that of the control treatment. Char addition increased soil C:N ratio in all litter treatments at time 0 (Supplementary Fig. 1). There was a significant ($p < 0.01$) interaction between char and litter type for days 65–94; C:N ratio was generally highest in soils that received char only and lowest in the control soil (Fig. 1).

Whereas the N_{min} pool comprised a mixture of ammonium and nitrate at the start of the experiment, by day 65 it was completely dominated by nitrate in the control and NFA litter treatments. Litter type had a strong influence on N_{min}. After day 0, N_{min} was generally highest in the control treatment, moderately high in the NFA litter treatment, and below detection limits in the FA litter treatments. In a similar pattern to the N_{min} results, during the first 13 days, PMN (Fig. 3) was greatest in the control and NFA litter treatments and generally below detection limits in the FA litter treatments. In the treatments where PMN had been detected (i.e. control and NFA treatments), mineralisation was largely complete by day 65. Microbial biomass N concentrations (Fig. 4) were quite variable, but generally highest in the control litter with char treatment. Throughout the experiment,

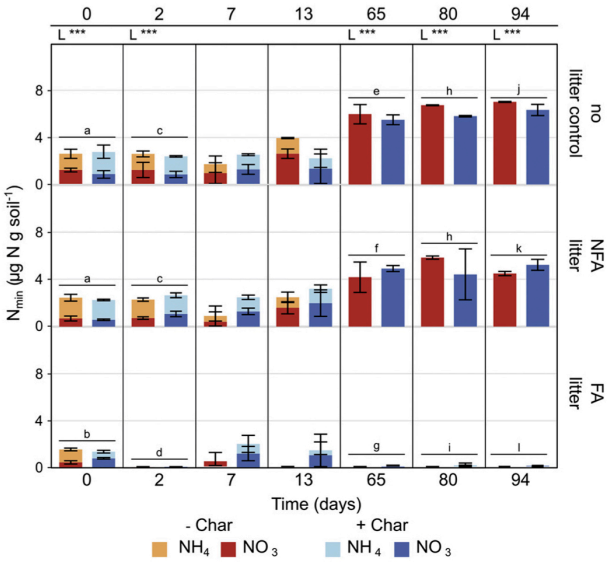

Fig. 2. Mineral nitrogen (N_{min} µg N g soil^{-1}) as measured at each sampling time point over 94 days in a 2 × 2 factorial experiment: three litter (L) treatments: no litter, not fire affected (NFA), or fire affected (FA) litter; and two char (C) treatments: absence (− Char) or presence (+ Char). Values are mean ± sd (n = 3). Highest order treatment effects as determined by a two-way ANOVA, are indicated in bold at top (*** = p < 0.001, ** = p < 0.01, * = p < 0.05). Tukey's HSD tests were performed (α = 0.05) at the level of interaction indicated at top of each column: means with same letter are not significantly different. NB: valid statistical comparisons cannot be made between different time points.

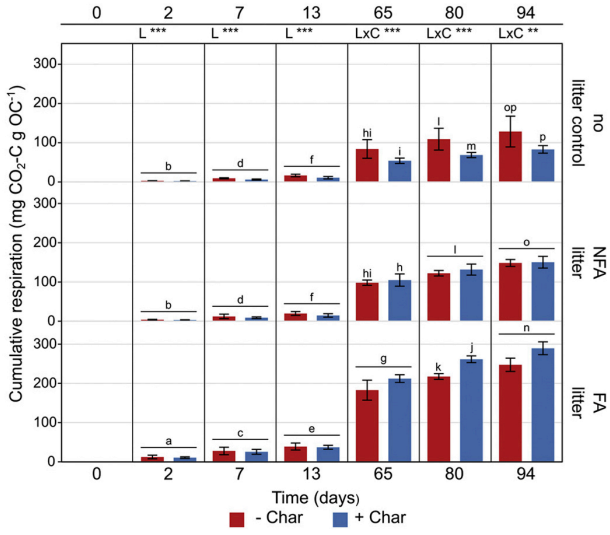

Fig. 1. Cumulative respiration (mg CO_2-C g OC^{-1}) as measured at each sampling time point over 94 days(1) in a 2 × 2 factorial experiment: three litter (L) treatments: no litter, not fire affected (NFA), or fire affected (FA) litter; and two char (C) treatments: absence (− Char) or presence (+ Char). Values are mean ± sd (days 2–13, n = 12; days 65–94, n = 3). Highest order treatment effects as determined by a two-way ANOVA, are indicated in bold at top (*** = p < 0.001, ** = p < 0.01, * = p < 0.05). Tukey's HSD tests were performed (α = 0.05) at the level of interaction indicated at top of each column: means with same letter are not significantly different. NB: valid statistical comparisons cannot be made between different time points; (1) respiration during the first 6 h of the incubation is not included due to machine malfunction.

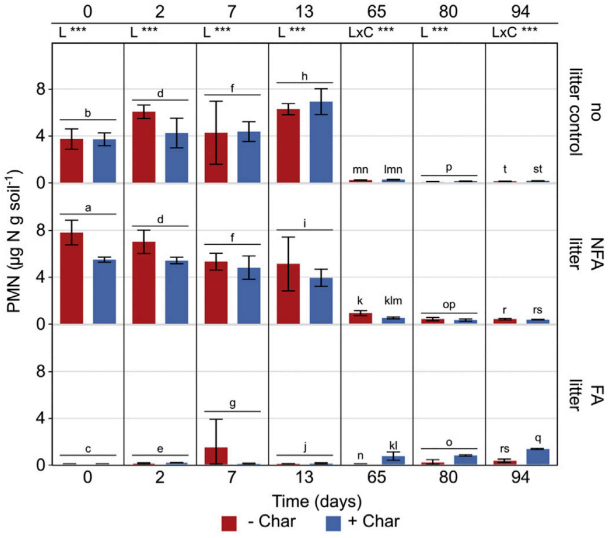

Fig. 3. Potentially mineralisable nitrogen (PMN µg N g soil^{-1}) as measured at each sampling time point over 94 days in a 2 × 2 factorial experiment: three litter (L) treatments: no litter, not fire affected (NFA), or fire affected (FA) litter; and two char (C) treatments: absence (− Char) or presence (+ Char). Values are mean ± sd (n = 3). Highest order treatment effects as determined by a two-way ANOVA, are indicated in bold at top (*** = p < 0.001, ** = p < 0.01, * = p < 0.05). Tukey's HSD tests were performed (α = 0.05) at the level of interaction indicated at top of each column: means with same letter are not significantly different. NB: valid statistical comparisons cannot be made between different time points.

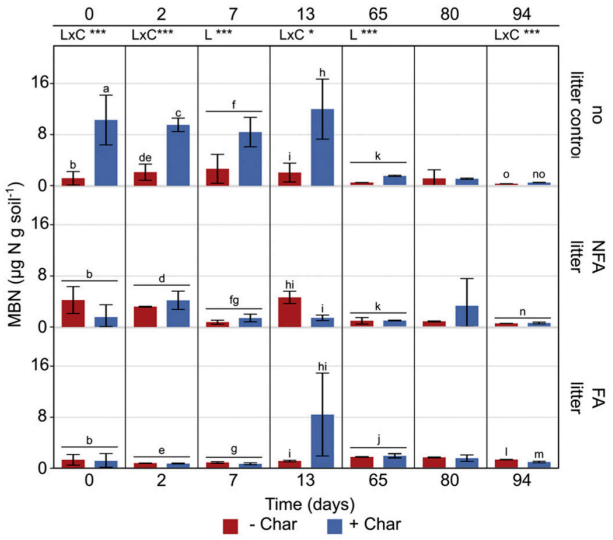

Fig. 4. Microbial biomass nitrogen (MBN µg N g soil^{-1}) as measured at each sampling time point over 94 days in a 2 × 2 factorial experiment: three litter (L) treatments: no litter, not fire affected (NFA), or fire affected (FA) litter; and two char (C) treatments: absence (− Char) or presence (+ Char). Values are mean ± sd (n = 3). Highest order treatment effects as determined by a two-way ANOVA, are indicated in bold at top (*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$). Tukey's HSD tests were performed ($\alpha = 0.05$) at the level of interaction indicated at top of each column: means with same letter are not significantly different. NB: valid statistical comparisons cannot be made between different time points.

N_{min}, PMN, and MBN values for the control litter and NFA litter treatments were broadly similar while values for the FA litter treatment were much lower; this pattern was observed in all the nitrogen analyses (Fig. 2).

3.2. Experiment 2: absorption potential

In this experiment, ammonium and nitrate/nitrite concentration varied with incubation type (aerobic or anaerobic), substrate, and ammonium nitrate solution concentration (Fig. 5; Table 2). In the dry incubation, ammonium was similar in all soil based substrate extractions, higher in the NFA litter extraction and lower in the FA litter extraction; nitrate/nitrite was uniformly at or below detection limits in the dry incubation (not shown). In the aerobic incubation, ammonium and nitrate/nitrite were detected in roughly equal proportion; ammonium concentration of the aerobic incubation decreased for all substrates relative to the dry incubation. Mineral N was not detected in the aerobic incubations of either litter, nor was it detected in the FA litter with soil treatment. In the anaerobic incubation, ammonium increased in soil, NFA litter, and soil & NFA litter treatments when incubated with increasing concentrations of ammonium nitrate solution; extracts from the substrates that included FA litter did not reflect the concentration of ammonium nitrate solution added to the soil.

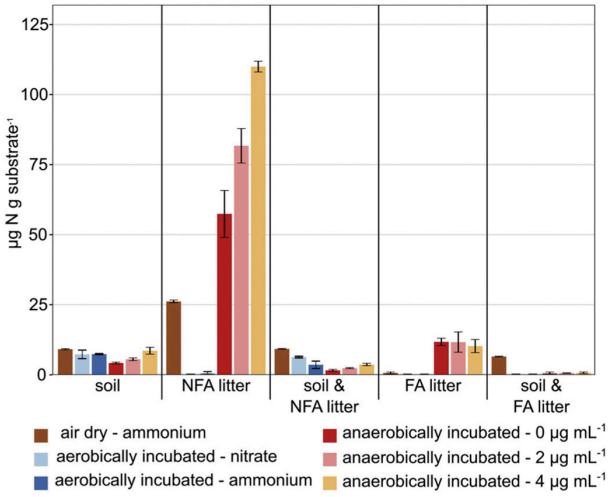

Fig. 5. Mineral nitrogen (N_{min} µg N g substrate^{-1}) as measured from extracts from soil, litter, or mixtures that were dry or after two weeks of aerobic incubation with water or anaerobic incubation (ammonium measurements only; nitrate below detection limit) with three rates of ammonium nitrate solution (0, 2, and 4 µgNH_4-N mL^{-1}). All treatments are n = 3; values are mean ± sd; NFA is not fire affected and FA is fire affected.

4. Discussion

The FA pine litter reduced mineral and microbial N pools in these experiments while increasing soil respiration during the 94 day incubation. These results support the hypothesis that FA litter would increase soil respiration (1) and partially support the hypothesis that FA litter would increase N immobilisation (2); while the N results indicate a similar nitrogen response to immobilisation, N was not elevated in the microbial biomass. We found that N was rarely detected in the FA litter treatments even though total nitrogen content was similar between the two amendments. This result, and the results of Experiment 2, indicate mineral N is being absorbed in the FA litter and indicate that N_{min} is being converted into an organic form in the treatments where FA pine litter was added. Interestingly, the presence/absence of char did not appear to enhance or repress soil respiration or nitrogen cycling. There is the potential, therefore, that fire affected pine needles may reduce the risk of nitrogen losses from leaching after a fire event, but may also constrain subsequent seedling regeneration.

When considered in the context of conventional N cycling, an increase in microbial activity suggests an increase in N cycling via immobilisation, mineralisation, or both (Cookson et al., 2005; Heijboer et al., 2016; Luo et al., 2018). However, the results of this study do not fit the conventional paradigm of N cycling; while mineral N measurements are consistent with a drawdown of N, there is no corresponding increase in microbial biomass N. Therefore, we argue that the FA litter used in this experiment has the capacity to chemically bond with nitrogen and prevent its extraction using mild potassium chloride extractant. Evidence for this capacity can be observed in both the soil incubation experiment (Experiment 1) and the litters incubated in ammonium nitrate solutions (Experiment 2). Alternative options for the unrecovered N include volatilisation, denitrification, or experimental error. Volatilisation and denitrification are unlikely in the soil and litter incubations (Experiment 1) as these processes generally require alkaline or anaerobic conditions (Christensen et al., 1990; Dendooven et al., 2010); this incubation was in acid soils under aerobic conditions. Volatilisation and denitrification also cannot explain the losses of mineral N from the FA litter but not the NFA litter in the incubations with ammonium nitrate solutions (Experiment 2). Experimental error through contamination or improper measurements is also unlikely to account for the unrecovered N as soils were randomly analysed per harvest time, and N was recovered as expected from the control and NFA soils.

The fact that added mineral N rapidly disappears over a two week timeframe suggests chemical absorption in the FA litter. Similar unaccounted nitrogen has been previously observed in organic matter (OM) isolated from soils, where soils treated with OM contained less mineral N at the end of a 28 day aerobic incubation (Whalen et al., 2000) and in a ^{15}N pool dilution study where organic matter accumulated labelled N during a 48 h incubation (Hooker and Stark, 2008). In addition, OM has been observed as a sink for mineral N in several other studies (Compton and Boone, 2002; St. Luce et al., 2014; Whalen

Table 2
Specific values and statistics results for Fig. 5: Ammonium (μg N g substrate^{-1}) as measured from extracts from soil, litter, or mixtures that were dry or after two weeks of anaerobic incubation with three rates of ammonium nitrate solution (0, 2, or 4 μg NH_4-N mL^{-1}). All treatments are n = 3; values are mean ± sd; values with same letter within the same row are not significantly different (Tukey's HSD, α = 0.05); NFA is not fire affected and FA is fire affected.

	Air dry	Aerobic	Anaerobic		
			0 μg NH_4-N mL^{-1}	5 μg NH_4-N mL^{-1}	10 μg NH_4-N mL^{-1}
Soil	9.11 ± 0.27 a	7.36 ± 0.14 b	4.19 ± 0.38 d	5.53 ± 0.55 c	8.58 ± 1.30 a
NFA Litter	26.2 ± 0.6 d	0.43 ± 0.64 e	57.4 ± 8.5 c	81.7 ± 6.2 b	110 ± 2 a
FA Litter	0.68 ± 0.41 b	nd	11.8 ± 1.4 a	11.6 ± 3.7 a	10.3 ± 2.4 a
Soil & NFA Litter	9.23 ± 0.21 a	3.62 ± 1.26 b	1.64 ± 0.40 c	2.42 ± 0.24 c	3.66 ± 0.50 b
Soil & FA Litter	6.50 ± 0.19 a	0.05 ± 0.08 c	0.52 ± 0.57 bc	0.62 ± 0.15 b	0.74 ± 0.35 b

et al., 2001). Essential oils derived from pine needles have also been shown to react with inorganic nitrogen when exposed to humid air containing 100 ppb gaseous ammonia (NH_3) to form 'brown carbon' compounds (Updyke et al., 2012). A substantial difference between the results in this study and the results in the aforementioned litter/soil studies is the scale of nitrogen loss: in this study nitrogen from the mineral and microbial pools could not be captured while in other studies these pools were still observable. However, net nitrogen movement from soil into fresh litter is commonly found during decomposition studies which may explain the lack of mineral nitrogen in the FA treatments (Xiong et al., 2014). In our study, the large increase in WEOC (4 and 30 mg C g litter^{-1} in NFA and FA, respectively) support the theory that the organic matter has absorbed the other nitrogen pools.

Chemical bonding of nitrogen in the FA litter treatments may be due to residual oxidised surfaces in the litter. Such surfaces may be formed during the breakdown of lignin as lignin decomposition, whether biotic or abiotic, produces oxygen containing functional groups (de Gonzalo et al., 2016). During biotic decomposition, depolymerisation can be achieved by extracellular enzymes such as peroxidases, which achieve lignin degradation by oxidising mediators (de Gonzalo et al., 2016; Sinsabaugh, 2010). The use of extracellular enzymes means oxygen functional groups can react with N in the soil to convert mineral N to organic N. While it is difficult to detect the presence of oxygenated surfaces in the amendments, increased alkyl-C and decreased aromaticity support the presence of such surfaces in the FA litter. Furthermore, organic matter that has experienced oxidation without increasing aromaticity may be more vulnerable to further biological and chemical oxidation, facilitating microbial access to, and dissolution of, OM into the soil solutions (Knicker et al., 2008). This phenomenon may explain the increase in WEOC and microbial respiration in the FA litter.

Finally, char did not appear to have any substantial ecological effects on soil respiration or nitrogen cycling in this study. Char is capable of inducing positive or negative priming of organic matter and fresh residues (Maestrini et al., 2015) and can improve plant uptake of N through increased mycorrhizal symbiosis (LeCroy et al., 2013). Neither of these responses were observed in this study, potentially due to the relatively large residue amendment rate or due to the lack of a host plant for mycorrhizal fungi naturally present in pine soils. As this study is inconclusive on the effects of char after fire in pine forest soils and the incidence of char is likely to increase in a warming climate (Ager et al., 2012; Hoegh-Guldberg et al., 2018), further research in this area is recommended.

5. Conclusion

Fire affected (FA) pine litter increased soil respiration, reduced mineral and microbial N pools when incubated with soil, and showed a capacity to remove ammonium and nitrate from solution when incubated anaerobically. When considered in the context of conventional N cycling, an increase in microbial activity suggests an increase in N cycling via immobilisation, mineralisation, or both; however, our results indicate mineral and microbial N is chemically captured by the FA litter. Light fraction organic matter has been shown to reduce mineral N in other studies, and the large increase in WEOC (4 and 30 mg C g litter^{-1} in NFA and FA, respectively) may be the cause of N absorption in this study. Char had no ecologically significant effects on soil respiration or N cycling. Nitrogen bonding to organic matter in fire affected areas has the potential to affect post-fire litter decomposition and nutrient cycling, which may affect post-fire ecosystem recovery. This research indicates there is an under-realised potential for fire affected litters to alter nitrogen cycling and further experimentation is required to better understand the effects of fire on nutrient cycling and nutrient budgets.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.01.316.

Acknowledgements

This project was supported by The Holsworth Wildlife Research Endowment & The Ecological Society of Australia, and The University of Adelaide Research Training Program Scholarship, with further analytical assistance provided by CSIRO Agriculture and Food. ES gives thanks to GC Garrett for his assistance in the field and the laboratory. We also thank the reviewers for their constructive comments on this manuscript.

References

Ager, A.A., Vaillant, N.M., Finney, M.A., Preisler, H.K., 2012. Analyzing wildfire exposure and source-sink relationships on a fire prone forest landscape. For. Ecol. Manag. 267, 271–283.
Akhtar, N., Goyal, D., Goyal, A., 2016. Physico-chemical characteristics of leaf litter biomass to delineate the chemistries involved in biofuel production. J. Taiwan Inst. Chem. Eng. 62, 239–246.
Alexis, M.A., Rasse, D.P., Rumpel, C., Bardoux, G., Péchot, N., Schmalzer, P., et al., 2007. Fire impact on C and N losses and charcoal production in a scrub oak ecosystem. Biogeochemistry 82, 201–216.
Attiwill, P.M., Guthrie, H.B., Leuning, R., 1978. Nutrient cycling in a Eucalyptus obliqua (L'Hérit.) Forest. I. Litter production and nutrient return. Aust. J. Bot. 26, 79–91.
Baker, T.G., Attiwill, P.M., 1985. Above-ground nutrient distribution and cycling in Pinus radiata D. Don and Eucalyptus obliqua L'Hérit. Forests in Southeastern Australia. For. Ecol. Manag. 13, 41–52.
Baldock, J.A., Smernik, R.J., 2002. Chemical composition and bioavailability of thermally altered *Pinus resinosa* (red pine) wood. Org. Geochem. 33, 1093–1109.
Baldock, J.A., Hawke, B., Sanderman, J., MacDonald, L.M., 2013. Predicting contents of carbon and its component fractions in Australian soils from diffuse reflectance mid-infrared spectra. Soil Res. 51, 577–595.
Bird, M.I., Wynn, J.G., Saiz, G., Wurster, C.M., McBeath, A., 2015. The pyrogenic carbon cycle. Annu. Rev. Earth Planet. Sci. 43, 273–298.
Bureau of Meteorology, 2015. Climate data online. 2015.
Butterly, C.R., Bünemann, E.K., McNeill, A.M., Baldock, J.A., Marschner, P., 2009. Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biol. Biochem. 41, 1406–1416.
Certini, G., Nocentini, C., Knicker, H., Arfaioli, P., Rumpel, C., 2011. Wildfire effects on soil organic matter quantity and quality in two fire-prone Mediterranean pine forests. Geoderma 167–168, 148–155.
Christensen, S., Simkins, S., Tiedje, J.M., 1990. Temporal patterns of soil denitrification. Their stability and causes. Soil Sci. Soc. Am. J. 54, 1614–1618.
Compton, J.E., Boone, R.D., 2002. Soil nitrogen transformations and the role of light fraction organic matter in forest soils. Soil Biol. Biochem. 34, 933–943.
Cookson, W.R., Abaye, D.A., Marschner, P., Murphy, D.V., Stockdale, E.A., Goulding, K.W.T., 2005. The contribution of soil organic matter fractions to carbon and nitrogen mineralization and microbial community size and structure. Soil Biol. Biochem. 37, 1726–1737.
Cortina, J., Vallejo, V.R., 1994. Effects of clearfelling on forest floor accumulation and litter decomposition in a radiata pine plantation. For. Ecol. Manag. 70, 299–310.

de Gonzalo, G., Colpa, D.I., Habib, M.H.M., Fraaije, M.W., 2016. Bacterial enzymes involved in lignin degradation. J. Biotechnol. 236, 110–119.
de Mendiburu, F., 2017. agricolae: Statistical Procedures for Agricultural Research.
Dendooven, L., Alcántara-Hernández, R.J., Valenzuela-Encinas, C., Luna-Guido, M., Perez-Guevara, F., Marsch, R., 2010. Dynamics of carbon and nitrogen in an extreme alkaline saline soil: a review. Soil Biol. Biochem. 42, 865–877.
Ding, F., van Zwieten, L., Zhang, W., Weng, Z.H., Shi, S., Wang, J., et al., 2017. A meta-analysis and critical evaluation of influencing factors on soil carbon priming following biochar amendment. J. Soils Sediments 1–11.
Downham, R., Gavran, M., 2018. Australian Plantation Statistics 2018 Update. ABARES, Canberra, p. 21.
Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Glob. Chang. Biol. 18, 1781–1796.
Farrell, M., Kuhn, T.K., Macdonald, L.M., Maddern, T.M., Murphy, D.V., Hall, P.A., et al., 2013. Microbial utilisation of biochar-derived carbon. Sci. Total Environ. 465, 288–297.
Ficken, C.D., Wright, J.P., 2017. Effects of fire frequency on litter decomposition as mediated by changes to litter chemistry and soil environmental conditions. PLoS One 12.
Florence, R.G., Lamb, D., 1974. Influence of stand and site on radiata pine litter in South Australia. N. Z. J. For. Sci. 4, 205–210.
Forster, J.C., 1995. Soil nitrogen. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 79–87.
Girisha, G.K., Condron, L.M., Clinton, P.W., Davis, M.R., 2003. Decomposition and nutrient dynamics of green and freshly fallen radiata pine (Pinus radiata) needles. For. Ecol. Manag. 179, 169–181.
Gomez, J.D., Denef, K., Stewart, C.E., Zheng, J., Cotrufo, M.F., 2014. Biochar addition rate influences soil microbial abundance and activity in temperate soils. Eur. J. Soil Sci. 65, 28–39.
Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H., 2015. Physico-chemical properties and microbial responses in biochar-amended soils: mechanisms and future directions. Agric. Ecosyst. Environ. 206, 46–59.
Hamer, U., Marschner, B., Brodowski, S., Amelung, W., 2004. Interactive priming of black carbon and glucose mineralisation. Org. Geochem. 35, 823–830.
Heijboer, A., ten Berge, H.F.M., de Ruiter, P.C., Jørgensen, H.B., Kowalchuk, G.A., Bloem, J., 2016. Plant biomass, soil microbial community structure and nitrogen cycling under different organic amendment regimes; a 15N tracer-based approach. Appl. Soil Ecol. 107, 251–260.
Hoegh-Guldberg, O., Jacob, D., Taylor, M., Bindi, M., Brown, S., Camilloni, I., et al., 2018. Impacts of 1.5 °C global warming on natural and human systems. In: Masson-Delmotte, V., Zhai, P., Pörtner, H.O., Roberts, D., Skea, J., Shukla, P.R., et al. (Eds.), Global Warming of 1.5°C. An IPCC Special Report on the Impacts of Global Warming of 1.5 °C above Pre-Industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change, Sustainable Development, and Efforts to Eradicate Poverty.
Holden, S.R., Rogers, B.M., Treseder, K.K., Randerson, J.T., 2016. Fire severity influences the response of soil microbes to a boreal forest fire. Environ. Res. Lett. 11.
Hooker, T.D., Stark, J.M., 2008. Soil C and N cycling in three semiarid vegetation types: response to an in situ pulse of plant detritus. Soil Biol. Biochem. 40, 2678–2685.
Isbell, R., 2002. The Australian Soil Classification. CSIRO Publishing, Melbourne.
IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, Update 2015 International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. FAO, Rome.
Jenkins, M.E., Bell, T.L., Norris, J., Adams, M.A., 2014. Pyrogenic carbon: the influence of particle size and chemical composition on soil carbon release. Int. J. Wildland Fire 23, 1027–1033.
Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the kEN value. Soil Biol. Biochem. 28, 33–37.
Knicker, H., Hilscher, A., González-Vila, F.J., Almendros, G., 2008. A new conceptual model for the structural properties of char produced during vegetation fires. Org. Geochem. 39, 935–939.
Lanza, G., Rebensburg, P., Kern, J., Lentzsch, P., Wirth, S., 2016. Impact of chars and readily available carbon on soil microbial respiration and microbial community composition in a dynamic incubation experiment. Soil Tillage Res. 164, 18–24.
LeCroy, C., Masiello, C.A., Rudgers, J.A., Hockaday, W.C., Silberg, J.J., 2013. Nitrogen, biochar, and mycorrhizae: alteration of the symbiosis and oxidation of the char surface. Soil Biol. Biochem. 58, 248–254.
Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems - a review. Mitig. Adapt. Strateg. Glob. Chang. 11, 403–427.
Levett, M.P., Adams, J.A., Walker, T.W., 1985. Nutrient returns in iitterfall in two indigenous and two radiata pine forests, Westland, New Zealand. N. Z. J. Bot. 23, 55–64.
Lopez-Capel, E., Sohi, S.P., Gaunt, J.L., Manning, D.A.C., 2005. Use of thermogravimetry-differential scanning calorimetry to characterize modelable soil organic matter fractions. Soil Sci. Soc. Am. J. 69, 136–140.
Luo, G., Li, L., Friman, V.P., Guo, J., Guo, S., Shen, Q., et al., 2018. Organic amendments increase crop yields by improving microbe-mediated soil functioning of agroecosystems: a meta-analysis. Soil Biol. Biochem. 124, 105–115.
Maestrini, B., Nannipieri, P., Abiven, S., 2015. A meta-analysis on pyrogenic organic matter induced priming effect. GCB Bioenergy 7, 577–590.
McBeath, A.V., Smernik, R.J., Krull, E.S., Lehmann, J., 2014. The influence of feedstock and production temperature on biochar carbon chemistry: a solid-state 13C NMR study. Biomass Bioenergy 60, 121–129.
Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide Biol. Chem. 5, 62–71.
Moore, J.M., Klose, S., Tabatabai, M.A., 2000. Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biol. Fertil. Soils 31, 200–210.
Nocentini, C., Guenet, B., Di Mattia, E., Certini, G., Bardoux, G., Rumpel, C., 2010. Charcoal mineralisation potential of microbial inocula from burned and unburned forest soil with and without substrate addition. Soil Biol. Biochem. 42, 1472–1478.
Osono, T., Takeda, H., 2004. Accumulation and release of nitrogen and phosphorus in relation to lignin decomposition in leaf litter of 14 tree species. Ecol. Res. 19, 593–602.
Paul, K.I., Polglase, P.J., 2004. Prediction of decomposition of litter under eucalypts and pines using the FullCAM model. For. Ecol. Manag. 191, 73–92.
Pluchon, N., Vincent, A.G., Gundale, M.J., Nilsson, M.C., Kardol, P., Wardle, D.A., 2016. The impact of charcoal and soil mixtures on decomposition and soil microbial communities in boreal forest. Appl. Soil Ecol. 99, 40–50.
R Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rayment, G.E., Lyons, D.J., 2010. Soil pH. In: Walker, J. (Ed.), Soil Chemical Methods - Australasia. CSIRO Publishing, Victoria, Australia, pp. 33–48.
Reinhart, K.O., Dangi, S.R., Vermeire, L.T., 2016. The effect of fire intensity, nutrients, soil microbes, and spatial distance on grassland productivity. Plant Soil 409, 203–216.
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–404.
Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241, 155–176.
Smith, M., Conte, P., Berns, A.E., Thomson, J.R., Cavagnaro, T.R., 2012. Spatial patterns of, and environmental controls on, soil properties at a riparian-paddock interface. Soil Biol. Biochem. 49, 38–45.
St. Luce, M., Whalen, J.K., Ziadi, N., Zebarth, B.J., Chantigny, M.H., 2014. Labile organic nitrogen transformations in clay and sandy-loam soils amended with 15N-labelled faba bean and wheat residues. Soil Biol. Biochem. 68, 208–218.
Theodorou, C., Bowen, G.D., 1990. Effects of fertilizer on litterfall and N and P release from decomposing litter in a Pinus radiata plantation. For. Ecol. Manag. 32, 87–102.
Updyke, K.M., Nguyen, T.B., Nizkorodov, S.A., 2012. Formation of brown carbon via reactions of ammonia with secondary organic aerosols from biogenic and anthropogenic precursors. Atmos. Environ. 63, 22–31.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.
Whalen, J.K., Bottomley, P.J., Myrold, D.D., 2000. Carbon and nitrogen mineralization from light- and heavy-fraction additions to soil. Soil Biol. Biochem. 32, 1345–1352.
Whalen, J.K., Bottomley, P.J., Myrold, D.D., 2001. Short-term nitrogen transformations in bulk and root-associated soils under ryegrass. Soil Biol. Biochem. 33, 1937–1945.
Xiong, Y., Zeng, H., Xia, H., Guo, D., 2014. Interactions between leaf litter and soil organic matter on carbon and nitrogen mineralization in six forest litter-soil systems. Plant Soil 379, 217–229.

Supplementary data

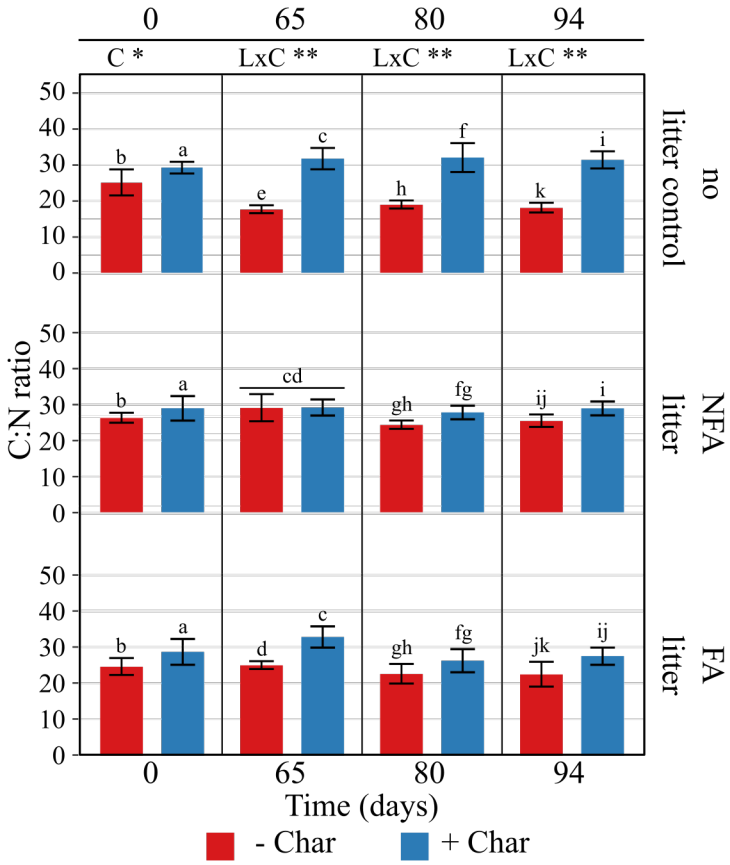

Fig. 6 soil C:N ratio as measured at each sampling time point over 94 days in a 2 factor experiment: three litter (L) treatments: no litter, not fire affected (NFA), or fire affected (FA) litter; and two char (C) treatments: absence (- Char) or presence (+ Char). Values are mean±sd (n=3). Highest order treatment effects as determined by a two-way ANOVA, are indicated in bold at top (*** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$). Tukey's HSD tests were performed ($\alpha=0.05$) at the level of interaction indicated at top of each column: means with same letter are not significantly different. NB: valid statistical comparisons cannot be made between different time points.

Statement of Authorship

Title of paper	Fire influences needle decomposition: tipping point in *Pinus radiata* carbon chemistry and soil nitrogen transformations
Publication status	☐ Published ☐ Accepted for publication ☒ Submitted for publication ☐ Unpublished and unsubmitted work written in manuscript style
Publication date	

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Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	**6/03/2019**

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

vii. the candidate's stated contribution to the publication is accurate (as detailed above);
viii. permission is granted for the candidate in include the publication in the thesis; and
ix. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Chapter 6 – Fire influences needle decomposition: tipping point in *Pinus radiata* carbon chemistry and soil nitrogen transformations

Under review – *Soil Biology and Biochemistry*

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6.1 Abstract

As climate change proceeds, a change in the frequency and intensity of fire events is expected to affect soil organic matter (SOM) transformations within forestry systems. A likely consequence is the development of post-fire litter layers composed of thermally altered non-senescent materials that have fallen during a fire event. In this study, *Pinus radiata* needles were thermally altered to determine the effect of changes in carbon chemistry on needle decomposition and nitrogen cycling. Live needles were collected and dried at 40°C before being further heated for 1 hour in a muffle furnace at a range of temperatures >40°C (max. = 320°C) to simulate a range of canopy temperatures that can occur during a fire, and then coarsely ground and screened (0.5-1.0 mm fraction retained). These needles were characterized for carbon and nitrogen content, and carbon chemistry (solid-state ^{13}C NMR spectroscopy); they were also used in an incubation experiment (14 days) which was performed to assess the impact of heating on nitrogen transformations. Soil respiration and extractable nitrogen pools (mineral, potentially mineralizable, and microbial biomass) were measured throughout the incubation. During the incubation, cumulative respiration and nitrogen absorption capacity decreased with increasing thermal alteration. The results indicate a step change in the response of nitrogen pools to thermal alteration of pine needles, with a critical change occurring at or before 200°C. This step change in nitrogen response may be due to the thermal degradation of light fraction organic matter, simple polysaccharides, or both. From this experiment it is clear that a post fire litter layer can have distinctly different effects on the soil environment depending on canopy temperature conditions during the fire with post fire litters composed of low temperature needles absorbing most mineral nitrogen that they contact. This will in turn affect post-fire plant recovery and therefore ecological succession.

6.1 Introduction

Fire danger season and frequency of fire are continuing to increase in many regions of the world due to the effects of climate change (Flannigan et al. 2009, IPCC 2014). This increase is expected to disrupt forest feedback mechanisms in fire prone regions and increase the losses of carbon from forest biomass and soil organic carbon (Lal 2005, Serra-Diaz et al. 2018). While much research has investigated the immediate effect of fire on above ground and below ground carbon and nitrogen stocks (Certini 2005, Wanthongchai et al. 2008, Bennett et al. 2014), there has been little research on the contributions of fire affected litters to soil nutrient cycling after a fire event. These litters are largely composed of non-senescent leaves that fall during a fire and may have substantially different decomposition properties to leaves that fall during normal senescence (Attiwill et al. 1978, Alexis et al. 2007, Alexis et al. 2010). In addition to increased nutrient contents in leaves that have fallen due to unplanned losses, the chemistry of this litter may be changed due to heat effects (i.e. thermal alteration) from the fire.

Fire induced thermal alteration of leaf organs and woody materials generates a continuum of altered materials with varying degrees of chemical and structural alteration (Schmidt and Noack 2000, Baldock and Smernik 2002). Composition of materials in this continuum may be highly variable depending on initial composition of the material, and conditions before, during, and after the heating event (González-Pérez et al. 2004, Merino et al. 2015). Carbon chemistry is frequently analysed on fire affected materials and, taking the overall variability into account, heating above 200°C typically decreases alkyl carbon, O-alkyl carbon, and N-alkyl carbon and increases aromatic carbon (Baldock and Smernik 2002, Águas et al. 2018, Bonanomi et al. 2018).

These changes in carbon chemistry are associated with the degradation of paraffinic structures (alkyl C), and polysaccharides such as cellulose and hemicelluloses (O-alkyl C) and their partial conversion into aromatic structures (Kögel-Knabner 2002, Águas et al. 2018). The transformation of these relatively simple organic resources during heating, means that charred biomass is less available to microbial decomposers (Baldock and Smernik 2002, Farrell et al. 2013).

Two forest types likely to be affected by increasing fire frequency are *Pinus radiata* (pine) forests and pine plantations. However, even without fire induced losses of easily decomposed resources, pine litter is typically slow to decompose due to its physical and chemical properties. Litter accumulation in *P. radiata* forests in situations with relatively low litter inputs indicates *P. radiata* needles are resistant to decomposition (Florence and Lamb 1974, Lee and Correll 1978, Baker and Attiwill 1985); indeed, mass losses over 10 weeks of incubation has been recorded at <4% while *Eucalyptus* leaves at the same site lost >10% (Crockford and Richardson 2002). Physical properties that may contribute to this resistance include having a low specific leaf area (Portillo-Estrada et al. 2016, Liu et al. 2018) and the presence of hydrophobic coatings on needle surfaces (Li et al. 2015). Chemical properties restricting decomposition of pine needles include a relatively low concentration of labile carbon relative to recalcitrant carbon (8% and 92%, respectively) (Carrasco et al. 2017) and the presence of anti-oxidant and anti-microbial compounds (Chao et al. 2000, Sacchetti et al. 2005, Guri et al. 2006). Although these properties may slow decomposition of senesced litter, during a fire there is the potential for unplanned needle losses which may increase decomposition potential; green needles typically decompose faster than senesced needles due to their greater nutrient (particularly N) concentration and significantly lower lignin content (Attiwill et al. 1978, Girisha et al. 2003).

During decomposition of high C:N materials (i.e C:N >30-35), nitrogen is typically drawn from the soil and immobilised in the microbial biomass until a time where carbon becomes limiting, after which nitrogen is mineralised. However, abiotic absorption and net transfer from soil to litter has also been observed (Compton and Boone 2002, Li and Fahey 2013) and may be influenced by chemical changes wrought by heating needles in the canopy. Although nitrogen cycling has been the focus of many post fire soil studies (see Wan et al. (2001) for review) and biochar studies (see Nguyen et al. (2017) for review), the decomposition of post-fire litter layers and their effects on post fire nitrogen cycling are largely unknown. In particular, as much of the post-fire litter in a pine plantation affected by moderately severe fire damage may be composed of non-senescent needles that fell during the fire (Alexis et al. 2007), there is a knowledge gap on the effect of heat exposure in the canopy on the subsequent decomposition of non-senescent needles (Stirling et al. 2019b). Therefore, this study addresses the following research questions: firstly, will heating pine needles increase the aromatic carbon content of the needles and decrease decomposition rate as measured by soil respiration; and secondly, will heating pine needles affect nitrogen pool distributions after incorporation with soil. These questions are addressed by the generation of a gradient of thermally altered *P. radiata* needles which were subsequently incubated for 14 days in a mineral soil where soil respiration, microbial biomass nitrogen, potentially mineralizable nitrogen, and mineral nitrogen were monitored over time.

6.3 Materials and methods

6.3.1 Pine needle collection, heat treatment and characterisation

Pinus radiata needles were sourced during June 2017 from a managed forestry plantation in South Australia, Australia. Live needles (<1 year old) were collected as fascicles from immature trees (height: 2-5 m); the needles were stripped from their scale leaves and dried in a convection oven at 40°C until a constant mass was achieved. These dry needles were then mixed and divided for further heating in a muffle furnace for 1 hour at 40, 85, 100, 135, 150, 180, 200, 225, 260 or 320°C. Needles were then ground to between 0.5 and 1.0 mm for use in the incubation experiment; needles used for carbon chemistry characterization were further ground to <0.15 mm, with care taken to prevent friction heating.

Needles were characterised for total organic carbon (TOC), total nitrogen (TN) and carbon chemistry was determined using solid state ^{13}C CP-MAS nuclear magnetic resonance (NMR) spectroscopy. Total organic carbon and TN were measured by dry combustion using a LECO CNS200 Analyser. Moisture accounted for 62% of fresh needle weight when dried to 40°C; further mass loss of needles and of carbon and nitrogen content due to thermal alteration (Table 6.1) was minimal between 40°C and 180°C; C:N ratio was also stable over this temperature range. From 180°C to 320°C, needle mass loss was steep and linear (4% loss per 10°C increase); C:N ratio also decreased over this temperature range, as carbon loss occurred at approximately 1.5 × the rate of nitrogen loss.

Table 6.1 Chemical data for the ten heating treatments: Mass loss (past initial loss from fresh to 40°C) total organic C (TOC), total organic N (TON) and C:N ratio. Values are single measurements.

Temperature (°C)	Mass loss (%)	TOC (%)	C loss (%)	TON (%)	N loss (%)	C:N
40	0.0	48.2	0	1.3	0	36.2
85	5.6	50.3	1	1.3	8	38.8
100	5.7	49.7	3	1.3	5	37.1
135	7.9	50.6	3	1.4	2	35.6
150	8.3	50.3	4	1.4	4	36.2
180	10.4	52.2	3	1.4	4	36.7
200	16.6	53.8	7	1.5	6	35.9
225	25.7	56.5	13	1.7	4	33.0
260	43.3	61.3	28	2.0	16	31.0
320	66.8	64.4	56	2.8	30	22.9

Nuclear magnetic resonance spectra were detected at a ^{13}C frequency of 50.33 MHz on a Bruker 200 Avance spectrometer. Needle samples were packed into zirconia rotors (7 mm diameter) with Kel-F end caps and spun at the 'magic angle' (54.7°) at 5 kHz. Chemical shift values were calibrated to the methyl resonance of hexamethylbenzene at 17.36 ppm and a 50 Hz Lorentzian line broadening was applied to all spectra (Baldock et al. 2013). Spectra were integrated across the following chemical shift limits to provide estimates of broad carbon types: 0-45 ppm (alkyl C), 45-60 ppm (N-alkyl C), 60-95 ppm (O-alkyl C), 95-110 ppm (di-O-alkyl C), 110-145 ppm (aryl C), 145-165 ppm (O-aryl C), and 165-190 (amide/carboxyl C) 190-215 ppm (keytone C) (Baldock and Smernik 2002, Kögel-Knabner 2002). While there are subtle changes at the cooler temperatures, the needles heated up to 180°C (inclusive) were broadly similar as revealed by ^{13}C NMR spectroscopy (Figure 6.1). An exception was the alkyl and N-alkyl/methoxyl groups, the

proportions of which increased from 100°C through to 200°C, after which they decreased to less than a third of the proportions present at 40°C. At temperatures greater than 180°C, O-alkyl C and di-O-alkyl C decreased, and aryl C, O aryl C and ketones increased. Over the full temperature range, O-alkyl C comprised the largest proportion of NMR signal up to, and including, needles heated to 225°C and aryl C comprised the largest proportion of NMR signal thereafter. Needles from 40°C to 225°C existed in a similar plane on the first principle component in the NMR PCA. Needles heated to the final three temperatures were increasingly different to the low temperature samples; the NMR spectra for the 320°C amendment more strongly resembled char than plant tissue (Figure 6.6).

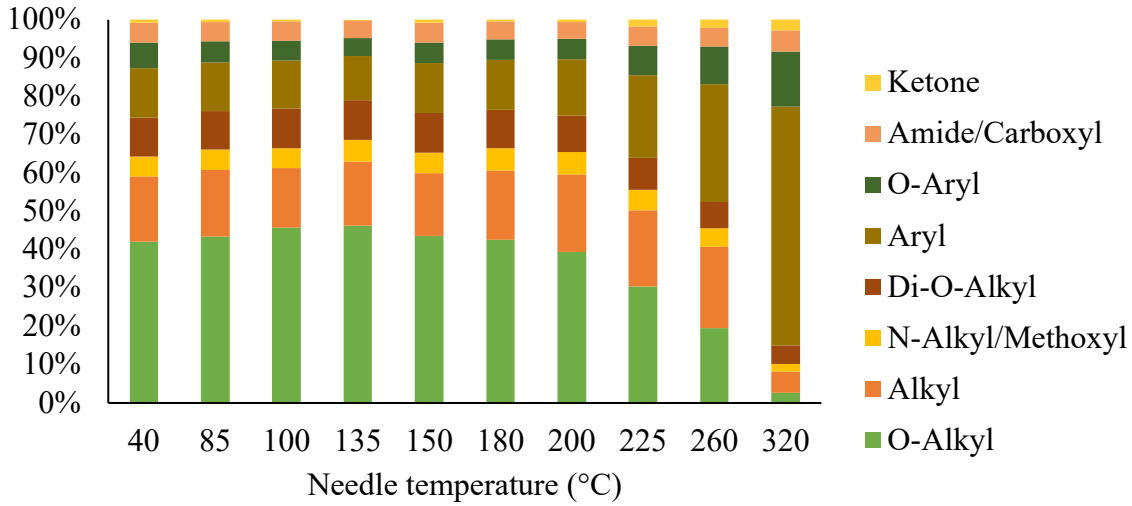

Figure 6.1 Proportional content of carbon chemical shift regions using ^{13}C CP-MAS nuclear magnetic resonance (NMR) spectra for *Pinus radiata* needles heated to 10 temperatures (n=1). See Figure 6.6 for individual spectra.

6.3.2 Nitrogen transformations and cumulative respiration over 14 days

Milled needles (0.5 – 1 mm particle size) were incubated (20 g kg soil^{-1}) for 14 days with an acidic (pH 5.6; depth 0-10 cm) fine sandy clay loam soil collected from *P. radiata* plantations in Old Kersbrook Forest, South Australia, Australia; soil respiration was measured over the course of the experiment, and microbial biomass nitrogen (MBN), potentially mineralizable nitrogen (PMN), and mineral nitrogen (N_{min}) forms (ammonium and nitrate/nitrite) were measured on destructively harvested samples at 12 time points over 14 days. The laboratory based incubation was conducted in the dark and under moisture conditions for optimal CO_2 efflux (0.25 g water g soil^{-1}) with a bulk density of 1.2 g cm^{-3} achieved by tapping incubation containers until the reaching the required soil depth; samples were incubated at 24±1°C. Soil respiration (as CO_2 flux) was measured using an infrared gas analyser (IRGA, Model 6262, Li-Cor, Lincoln, NE, USA) throughout the 14 days (37 measuring events) on the samples that were harvested on day 14 (n=3).

Soils were destructively sampled every 6 hours for the first day (0, 6, 12, 18, and 24 hrs), then on the completion of days 2, 3, 4, 6, 8, 11 and 14. This schedule was chosen to capture the large nitrogen variations that can occur in the first three days of incubation while also providing further data on nitrogen dynamics in the first two weeks of incubation (Elmajdoub et al. 2014, Ma et al. 2016). Soil MBN was extracted using chloroform fumigation extraction (Vance et al. 1987) with 0.5 M K_2SO_4 at a 1:5 soil:extractant ratio, and was determined as the difference in nitrogen

concentration between fumigated and non-fumigated soil with an extraction coefficient of 0.54 (Joergensen and Mueller 1996, Moore et al. 2000). Potentially mineralizable nitrogen was analysed using ammonium-salicylate oxidation on a subset of each sample that had been anaerobically incubated for 14 days and then extracted with 2 M KCl (Smith et al. 2012). Mineral nitrogen (ammonium and nitrate) was determined after shaking soil with 2 M KCl at a 1:5 ratio. Ammonium-N was measured using ammonium-salicylate oxidation (Forster 1995) and nitrate/nitrite-N was measured using vanadium(III) reduction (Miranda et al. 2001). All colorimetric analyses were read using a Multiscan GO plate reader (Thermo Fisher Scientific).

6.3.3 Data analyses

Modelled cumulative respiration curves were based on a three parameter logistic growth function (Table 6.2) modified from Paine et al. (2012) using the R non-linear mixed effects model package nls2::nls2 with 'algorithm' set to "port" (Pinheiro et al. 2018). In this model, '*M*' is cumulative respiration ($mgCO_2$-C gOC^{-1}) as a function of '*t*' (time), '*K*' is the upper asymptote of growth, '*r*' is the decay constant, and '*b*' accounts for linear growth past '*K*'. Parameters were tested for significance using one-way Analysis of Variance (ANOVA) and post hoc Tukey tests (n=3, $\alpha<0.05$). Average parameter values were used as the final model and tested against the measured respiration data using the R linear model package stats::lm (R Core Team 2017). Alternative models that were tested included first order decay, first order decay with linear alteration, and three parameter logistic growth without linear alteration (Table 2).

Table 6.2 equations tested for cumulative respiration model. Where '*M*' is cumulative respiration (mg CO_2-C g OC^{-1}; 'OC' is organic C) as a function of '*t*' (time), '*K*' is the upper asymptote of growth, '*r*' is the decay constant, and '*b*' accounts for linear growth past '*K*'. Equations 1 and 2, three parameter logistic growth are modified from Paine et al. (2012). Equation 2 was used in the cumulative respiration model.

Number	Equation	Description
1	$M_t = \frac{M_0 K}{M_0 + (K - M_0)e^{-rt}}$	three parameter logistic growth
2	$M_t = \frac{M_0 K}{M_0 + (K - M_0)e^{-rt}} + bt$	three parameter logistic growth with linear alteration
3	$M_t = K(1 - e^{rt})$	first order decay
4	$M_t = K(1 - e^{rt}) + bt$	first order decay with linear alteration

Statistical analyses for the nitrogen response data were analysed per harvest time using one-way Analysis of Variance (ANOVA) and post hoc Tukey tests (n=3, $\alpha<0.05$). All graphs were generated using the R package ggplot2 with smoothing via Loess regression and post production cosmetic alterations in Inkscape (version 0.92).

6.4 Results

6.4.1 Modelled respiration response indicates labile components are equally decomposable

A modified three parametric growth model (see equation number 2 in Table 6.2) was the best fit for the cumulative respiration data over the first 14 days (Figure 6.2; Table 6.3). All parameters except the decay rate 'r' and linear growth rate 'b' significantly decreased in soils amended with needles that had experienced increasing thermal alteration temperature; the control soil had equally low parameters as soils amended with the highest needle temperatures. Cumulative respiration broadly split into three groups: 1) 40°C-180°C, 2) 200°C, and 3) 225°C-320°C; group 3 also included the control. Resources available in the first 3 days ('*K*') significantly decreased

with increasing thermal alteration up to 225°C, after which it stabilized as equivalent to the control soil. The rate of consumption of less labile resources ('*b*') tended to decrease with increasing thermal alteration, however this was not significant.

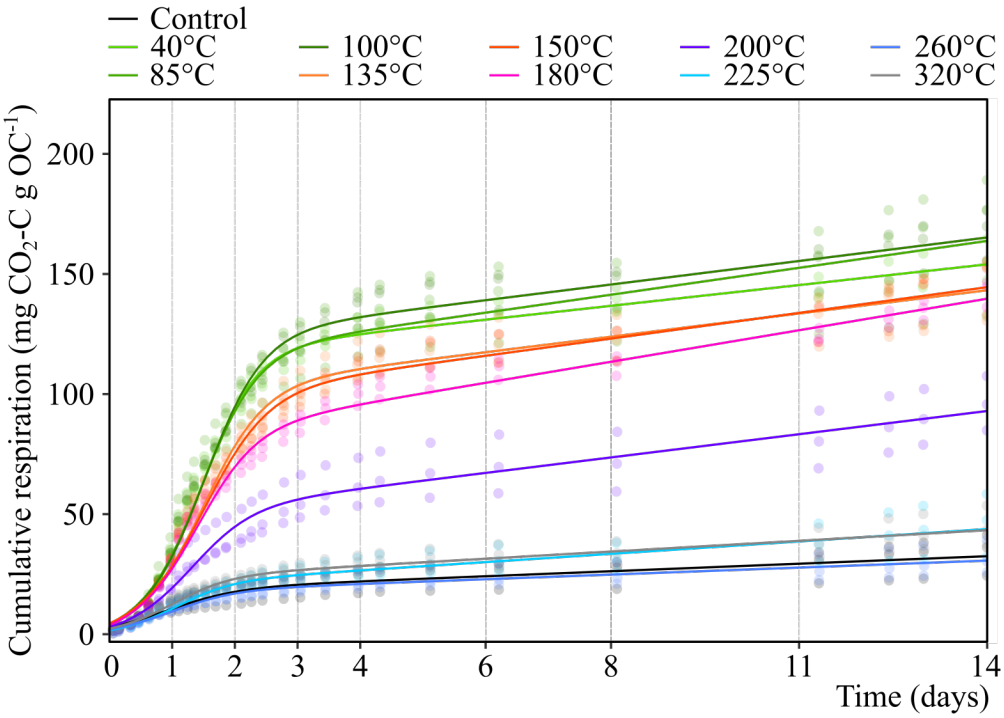

Figure 6.2 Measured and modelled (see text) cumulative respiration (mg CO_2-C g OC^{-1}; 'OC' is organic C) in soils amended with thermally altered *P. radiata* needles (temperature in legend) or left unamended ("Control") and aerobically incubated for 14 days (points indicate measured variables; lines are average modelled response, n=3). Refer to Table 6.2 for parameter values and fit measure.

Table 6.3 Parameter value and significance table for Figure 6.2. Three parameter logistic growth modified (see text) from Paine et al. (2012). Where '*M*' is cumulative respiration (mgCO_2-C gOC^{-1}; 'OC' is organic C) as a function of '*t*' (time), '*K*' is the upper asymptote of growth, '*r*' is the decay constant (%M_0 day^{-1}), and '*b*' accounts for linear growth past '*K*' (mgCO_2-C gOC^{-1} day^{-1}). All treatments are n=3; values are mean±sd; values with same letter within the same column are not significantly different (Tukeys HSD, α=0.05).

	***K* (p<0.001)**	***r* (ns)**	***M₀* (p<0.001)**	***b* (ns)**	**fit (r^2)**
40°C	114 ab	2.31 -	3.68 abc	2.89 -	0.99
85°C	111 ab	2.21 -	4.12 ab	3.74 -	0.95
100°C	119 a	2.18 -	4.10 ab	3.27 -	0.99
135°C	98.0 bc	2.08 -	4.01 ab	3.23 -	0.98
150°C	94.5 cd	1.98 -	4.33 a	3.57 -	0.98
180°C	78.4 d	2.14 -	3.55 abc	4.38 -	0.99
200°C	47.8 e	2.08 -	2.77 bcd	3.23 -	0.93
225°C	19.7 f	2.24 -	1.55 d	1.72 -	0.90
260°C	17.1 f	1.99 -	2.00 d	0.97 -	0.88
320°C	22.5 f	2.15 -	2.23 cd	1.49 -	0.94
Control	17.9 f	1.79 -	2.82 bcd	1.05 -	0.82

6.4.2 Nitrogen pools are strongly affected by thermal alteration

Two patterns of response were observed in the MBN (Figure 6.3; Table 6.4). The response observed in the control and the soils that received needles heated above 200°C was a high MBN content (average 26 µgN gSoil^{-1}) throughout the first 8 days followed by a sharp decline over the last 4 days to 7 – 11 µgN gSoil^{-1}. In contrast, the response in the soils that received needles heated between 40°C and 200°C was more variable over time: these soils had initially high MBN (28 – 41 µgN gSoil^{-1}), followed by a trough (8 – 13 µgN gSoil^{-1}), partial recovery (10 – 19 µgN gSoil^{-1}), and then decline to a similar content as the control on day 14 (7 – 9 µgN gSoil^{-1}).

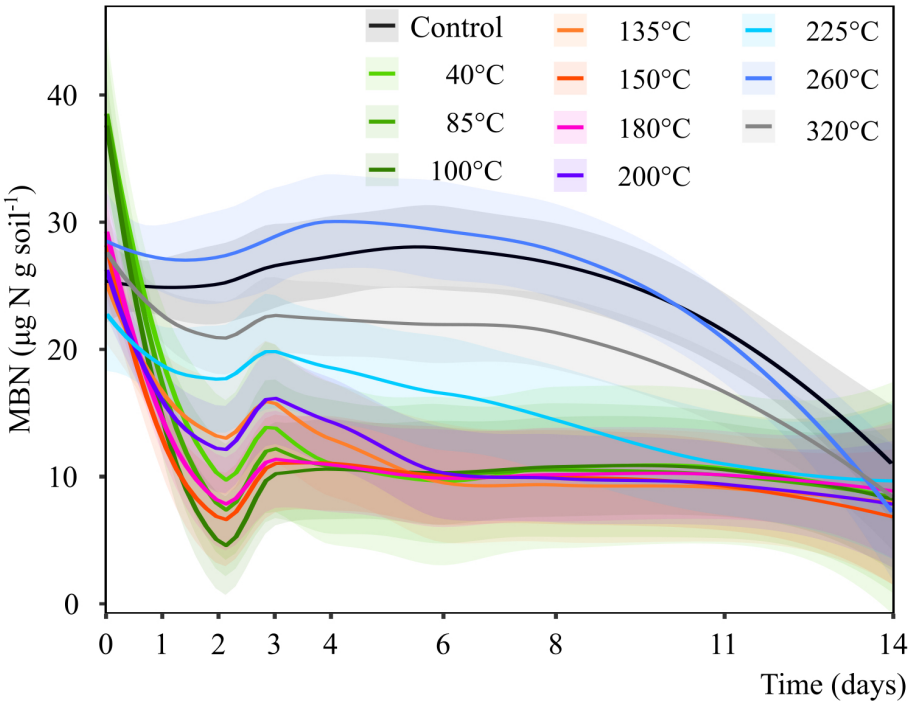

Figure 6.3 Microbial biomass nitrogen (MBN, µg N g soil^{-1}) in soils amended with thermally altered *P. radiata* needles (temperature in legend) or left unamended ("Control") and aerobically incubated for up to 14 days (lines are means smoothed using Loess regression; shaded areas indicate confidence interval, $\alpha = 0.05$; $n = 3$).

Potentially mineralizable nitrogen (Figure 6.4; Table 6.5) was significantly affected by temperature; differences were largest at time 0 (t_0 range: -22 to 18 µgN gSoil^{-1}), and decreased over the 14 day incubation (t_{14} range: 0.1 to 5.5 µgN gSoil^{-1}). During the first 24 hours, soils that received needles heated between 40°C and 150°C had PMN < 0; PMN for the 180°C treatment was negative up to 18 hours (inclusive). In needle treatments up to 200°C, PMN increased to a maximum at day 3; the control soil and needle treatments that were heated to >200°C had maximum PMN at 6 hours, after which there was a trough and a secondary peak at 2 or 4 (260°C only) days.

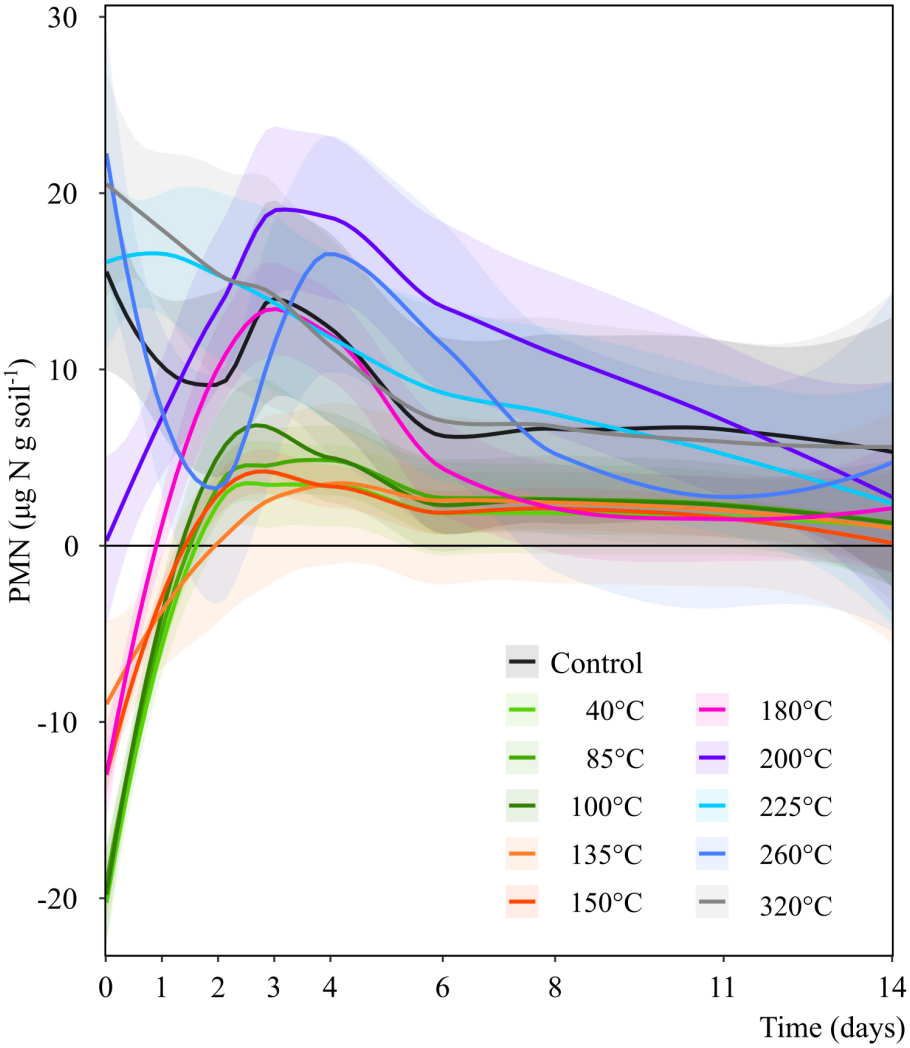

Figure 6.4 Potentially mineralisable nitrogen (PMN, µg N g soil^{-1}) in soils amended with thermally altered *P. radiata* needles (temperature in legend) or left unamended ("Control") and aerobically incubated for up to 14 days (lines are means smoothed using Loess; shaded areas indicate confidence interval, $\alpha = 0.05$; $n = 3$). Note negative values are due to decreased ammonium content of samples after anaerobic incubation.

Mineral nitrogen (Figure 6.5; Table 6.6) was significantly affected by temperature; nitrate was only observed in the control and needle treatments heated to >200°C after day 4 (data not shown); the effect of nitrate can be observed in Figure 3 as near constant N_{min} content over the 14 day incubation for these treatments. Three patterns of response were observed in the N_{min} results: firstly, 1) 40°C-100°C: initially high N_{min} (t_0 range: 22 to 25 µgN gSoil^{-1}) that rapidly decreased over the first day (t_1 range: 4.2 to 6.5 µgN gSoil^{-1}), and low N_{min} on the final day (t_{14} range: 1.3 to 1.4 µgN gSoil^{-1}). Secondly, 2) 135°C- 200°C: initially moderate N_{min} (t_0 range: 14 to 15 µgN gSoil^{-1}) that followed a similar pattern to the 40°C-100°C treatments thereafter. Finally, 3) 225°C-320°C and the control: initially moderate N_{min} (t_0 range: 13 to 15 µgN gSoil^{-1}) followed by a sustained, elevated N_{min} throughout the incubation.

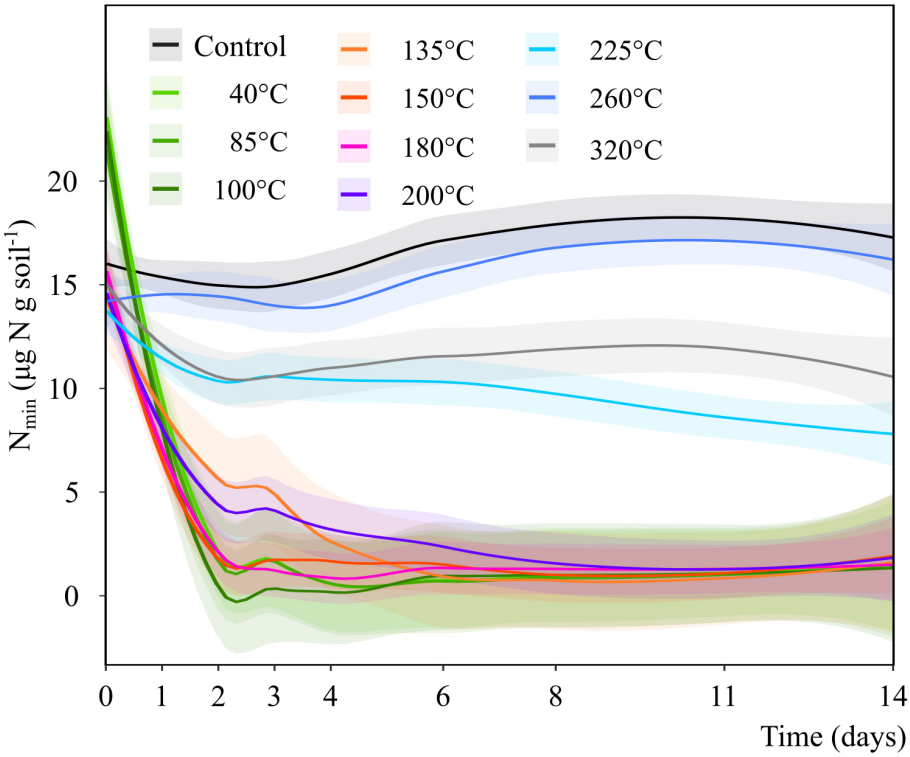

Figure 6.5 Mineral nitrogen (N_{min}, µg N g soil^{-1}) in soils amended with thermally altered *P. radiata* needles (temperature in legend) or left unamended ("Control") and aerobically incubated for up to 14 days (lines are means smoothed using Loess; shaded areas indicate confidence interval, $\alpha = 0.05$; $n = 3$).

6.5 Discussion

6.5.1 Needle characterisation indicates carbon chemistry is relatively stable when heated to ≤180°C

Thermally driven changes in the needles were dominated by water loss in needles heated to ≤180°C and by aromatization of organic carbon in needles heated to ≥200°C. Mass loss in the needles heated to 180°C was an additional 10% on the mass lost during drying to 40°C; C:N ratio and carbon chemistry did not change significantly over this thermal range indicating that the dominant response to heating was water loss. The C:N ratio was expected to change at about 200°C during thermal alteration as organic nitrogen and organic carbon have relatively low volatilisation temperatures (Hosking 1938, Bárcenas-Moreno and Bååth 2009); however, even before alteration the C:N ratio of these needles is unusually low for conifers. When compared to fresh foliage in temperate coniferous forest (global average of 60), the unaltered foliage in this study (C:N of 36) is low and instead similar to the average global values for temperate broadleaf forest (C:N of 35) (McGroddy et al. 2004). The relatively low C:N (between 25 and 75) of the needles means nitrogen limitation should not pose a significant barrier to decomposition over the timeframe of the experiment (Heal et al. 1997). Although the total carbon content did not vary substantially over the 40-180°C temperature range, striking changes in the needle colour (ranging from green to dark brown; data not shown) and subtle changes in the NMR spectra indicate changes occurring in the needles that are not solely due to water loss. In addition, the N_{min} results during the instantaneous extractions (i.e. at 0 days) also indicate a change in nitrogen availability in needles heated above 100°C. In a similar study by Bonanomi et al. (2018), heating litter up to

200°C caused a decrease in heterotrophic soil respiration even though there was no significant change in carbon chemistry.

Changes in carbon chemistry were more pronounced in needles heated to ≥200°C. Mass loss in these needles ranged from a further 17% (200°C) to a further 67% (320°C); these temperature treatments also caused a decrease in C:N ratio from 36 (200°C) to 23 (320°C), indicating carbon was lost preferentially to nitrogen under these heating conditions. Structural changes in carbon chemistry become apparent at approximately 200°C when materials are heated in the presence of air. Degradation of lignin and hemicellulose begins before 190°C while cellulose degradation begins at approximately 210°C (González-Pérez et al. 2004, Trehan et al. 2004). Charring also begins at approximately 200°C as organic matter structures are dehydrated to form more aromatic compounds (Baldock and Smernik 2002, González-Pérez et al. 2004). The loss of alkyl C, O-alkyl C and di-O-alkyl C suggests the degradation of polysaccharides such as cellulose and hemicelluloses (Fernandez et al. 2001, Águas et al. 2018) with some of the carbon associated with those structures becoming aryl C, O-aryl C and ketone structures. Although carbonisation of litters during fire is low compared to woody materials (Knicker et al. 2008, McBeath et al. 2013), the carbon chemistry of SOM heated to ≥200°C is progressively altered as heating increases leading to O-alkyl structures being converted to aromatic structures (Baldock and Smernik 2002, Certini 2005). The high temperature needles (≥200°C) may therefore represent a relatively small pool of post-fire litter that has experienced carbonisation.

6.5.2 Modelled respiration response indicates a thermal tipping point

The overall proportion of labile resources in the needles rather than the decomposability of those resources limited soil respiration over the first 14 days of exposure to soil and water. This restriction in the proportion of labile resources is observed in the decreasing asymptote of growth parameter ('K') as amendment temperature increased. The fastest rate of decrease in this parameter with temperature is between 180°C and 225°C, which correlates with the initial degradation of polysaccharides as determined by solid state ^{13}C CP-MAS NMR spectroscopy. The importance of 'K' also indicates that the changes in the needles caused by heating are more strongly expressed in the initial days of exposure than over the full two weeks of incubation, therefore indicating that the most labile resource pool is strongly affected by heating. The results here show the same pattern to those observed in a similar incubation study by Bonanomi et al. (2018), where litters were heated for 30 minutes at larger temperature intervals and a decrease in cumulative respiration was observed starting at an amendment heating temperature of 200-300°C depending on litter species (Bonanomi et al. 2018).

Although the high temperature needles in our study did not respire more than the control treatment, microbes are capable of mineralising char over longer periods ranging from months to years. For example, in a study over 48 days soil microbes caused structural changes to chars and also mineralised <0.3% of wood char-C and <3% of grass char-C (Hilscher et al. 2009). In a longer example, up to 22% of carbon was mineralised in a low temperature (300°C) wood char exposed to a humid tropical rainforest soil over three years (Bird et al. 2017). Decreases in microbial activity due to thermal alteration have been inversely correlated with alkyl C to O-alkyl C ratio (Abraham and Chudek 2008); while this relationship holds true for cumulative respiration and needles heated to up to 225°C (linear; $R^2 = 0.86$), the relationship breaks down for needles above 225°C. A relationship between carbonyl-C and microbial activity has also been observed, but this relationship was not observed in this study (Ng et al. 2014). Finally, while

needles appear to have decreasing quantities of labile carbon as temperature increases, the decomposition rate of those resources did not change over either phase of respiration captured in this experiment: i.e. '*r*' and '*b*' were not significantly different for any treatment.

6.5.3 Nitrogen pools indicate a thermally altered absorption potential

The similarities in nitrogen pools between the high temperature needles (i.e. ≥200ºC) and the control soil indicate that these needles are not decomposing at a sufficient rate over the first 14 days of exposure to influence nitrogen availability. This is not an unexpected result as this incubation only investigated the first 14 days of decomposition, while many studies concerned with biochar decomposition have found that high temperature chars do not affect microbial activity or community structure when considered over several years (Kuzyakov et al. 2009, Quilliam et al. 2013). In studies where the labile fraction of char was considered, this fraction was generally less than 1% of char-C and had a mean residence time of <3 days to >100 days depending on the study (Hamer et al. 2004, Farrell et al. 2013, Wang et al. 2016). It is likely, however, that the CO_2 that is released in <1 day is due to the abiotic reaction of carbonates in the char rather than microbial mineralisation (Farrell et al. 2013). Although char can inhibit SOM mineralisation through adsorption to mineral fractions (Lu et al. 2014, Han et al. 2016), it has also been shown to increase native SOM mineralisation without necessarily affecting soil respiration (Pluchon et al. 2016, Zheng et al. 2016). In this study, the lack of decomposition in the high temperature needles was most likely due to the decrease in simple polysaccharides and increase in aromatic carbon. This can be observed as a step change in soil respiration and nitrogen pools where needles heated to 200°C were the mid-point of the step change.

For the needles before the step change in respiration and nitrogen pools, the low detection of MBN, PMN, and N_{min} indicates the low temperature needles are chemically absorbing a substantial quantity of mineral nitrogen. Microbial biomass nitrogen was generally lower in the low temperature needles than the high temperature needles even though respiration was higher for these treatments. While an increase in carbon use efficiency is possible, when considered against the PMN and N_{min} results, it appears that nitrogen from the MBN extraction is being rapidly bound by the needles during fumigation and extraction in the low temperature treatments. This rapid binding in the low temperature treatments can also be seen in the PMN and N_{min} results, where mineral nitrogen is unable to be detected during both anaerobic (PMN) and aerobic (N_{min}) incubations. The pattern of nitrogen loss is different for each type of incubation however: absorption potential decreases with time for PMN and increases with time for N_{min}.

The potential for pyrogenic organic matter to absorb substantial amounts of mineral nitrogen via chemisorption has been recently observed in pyrolyzed maple (*Acer rebrum*) wood and ammonia gas wherein 0.18 g N g OC^{-1} was absorbed by pyrolyzed wood chips under ambient conditions (Hestrin et al. 2019). Nitrogen retention in organic matter has also been observed in urea treated humified brown coal (Saha et al. 2017). In addition to these observations, absorption of nitrogen within litter is not unusual as fresh litter tends to be nitrogen limited whereas soil tends to be carbon limited (Heal et al. 1997, Demoling et al. 2007). As such, net nitrogen transfer is possible, and has been observed, from soil to litter leading to enhanced decomposition rates (Li and Fahey 2013, Xiong et al. 2014); carbon transfer from soil to litter and *vice versa* has also been observed (Frey et al. 2003, Bird et al. 2017). Rapid non-biotic nitrogen losses have previously been connected to light fraction organic matter (Compton and Boone 2002). In the study of Compton and Boone (2002), this fraction was capable of incorporating an average of 39% of added ammonium and 17% of added nitrate after an 18 hour incubation; absorption occurred as quickly

as 5 minutes after nitrogen addition in this study. The decreasing capacity for thermally altered needles to capture nitrogen in our study may therefore be connected with the thermal degradation of light fraction organic matter.

6.6 Conclusions

From the respiration response and nitrogen pools it is clear that heat damage sustained by needles during a fire will affect post-fire nitrogen cycling in *Pinus radiata* plantations. In particular, it appears that heating above 100°C decreases the mineral nitrogen available in pine needles over the first day of exposure, and that heating needles to above 180°C reduces microbial decomposition, potentially due to changes in litter carbon chemistry. The capacity for needles to capture mineral nitrogen compounds is also reduced by heating. This capacity to absorb nitrogen could be both beneficial and detrimental to plantation management after a fire event – beneficial in that it could prevent nitrogen losses, such as leaching, and detrimental in that it could prevent the successful establishment of plants through nitrogen limitation. Therefore, there is an obvious need for further research on the role of fire affected pine litters in the field.

6.7 Acknowledgements

This project was supported by The Holsworth Wildlife Research Endowment & The Ecological Society of Australia, and The University of Adelaide Research Training Program Scholarship, with further analytical assistance provided by CSIRO Agriculture & Food. Janine McGowan is thanked for acquisition and data processing of NMR spectra, and for the acquisition of the total carbon and total nitrogen data. ES gives thanks to GC Garrett for his assistance in the field and the laboratory.

6.8 Supplementary data

Table 6.4 Significance table for microbial biomass nitrogen (MBN, μg N g soil^{-1}) in soils amended with thermally altered *P. radiata* needles (temperature in legend) or left unamended ("Control") and aerobically incubated for up to 14 days (values are means; n = 3). Values with same letter within the same column are not significantly different (Tukeys HSD, α=0.05). N.B. statistical analyses are only valid within table columns.

Time (days)	0.00	0.25	0.5	0.75	1	2	3	4	6	8	11	14
40°C	38 a	47 a	26 a	8.9 d	11 cd	19 abc	12 c	9.2 e	11 cd	10 d	13 bc	7.7 a
85°C	41 a	40 ab	29 a	8.0 d	8.5 d	16 bc	11 c	9.8 e	11 cdc	10 d	11 cd	8.1 a
100°C	40 a	36 ab	24 a	10 cd	6.4 d	9.5 c	12 c	10 de	10 d	11 d	12 bcd	7.8 a
135°C	28 bc	27 b	18 a	13 bcd	10 cd	18 abc	16 bc	10 de	10 cd	9.0 d	11 cd	7.3 a
150°C	29 bc	30 b	20 a	7.7 d	7.4 d	10 c	12 c	10 de	9.8 d	9.8 d	11 cd	6.4 a
178°C	31 b	27 b	26 a	9.6 d	7.6 d	11 bc	13 c	9.6 e	9.8 d	10 d	11 cd	8.5 a
200°C	28 bc	29 b	19 a	13 bcd	8.3 d	16 bc	18 bc	12 de	10 d	10 d	11 cd	7.1 a
225°C	28 bc	27 b	14 a	16 abcd	19 bc	20 abc	20 abc	17 cd	16 bc	16 c	7.8 d	11 a
260°C	28 bc	34 b	21 a	27 a	29 a	28 a	28 a	32 a	28 a	28 a	23 a	6.6 a
320°C	28 bc	33 b	18 a	23 ab	22 ab	21 ab	24 ab	21 bc	22 b	23 b	16 b	8.5 a
Control	23 c	33 b	19 a	23 ab	25 ab	28 a	25 ab	28 ab	29 a	25 ab	23 a	11 a

Table 6.5 Significance table for potentially mineralizable nitrogen (PMN, μg N g soil^{-1}) in soils amended with thermally altered *P. radiata* needles (temperature in legend) or left unamended ("Control") and aerobically incubated for up to 14 days (values are means; n = 3). Values with same letter within the same column are not significantly different (Tukeys HSD, α=0.05). Note negative values are due to decreased ammonium content of samples after anaerobic incubation. N.B. statistical analyses are only valid within table columns.

Time (days)	0.00	0.25	0.50	0.75	1	2	3	4	6	8	11	14
40°C	-22 c	-16 c	-12 cd	-5.9 d	-3.6 c	-0.3 f	4.7 b	1.3 c	1.2 d	2.4 b	1.7 c	1.0 d
85°C	-20 c	-17 c	-16 d	-4.3 cd	-2.2 c	1.1 ef	5.1 b	3.7 bc	1.3 d	3.6 b	2.6 c	1.2 d
100°C	-19 c	-15 c	-12 cd	-7.1 d	-1.8 c	4.2 cdef	7.0 b	1.6 bc	2.1 d	3.7 b	2.3 c	1.2 cd
135°C	-13 bc	-0.8 bc	-8.8 cd	-4.7 cd	-3.1 c	-2.9 f	5.8 b	1.5 c	1.7 d	3.2 b	1.7 c	1.1 d
150°C	-13 bc	-10 bc	-8.0 cd	-3.7 cd	-2.8 c	2.6 def	4.3 b	1.3 c	1.6 d	2.8 b	1.6 c	0.1 d
178°C	-12 bc	-8.9 bc	-8.0 cd	-0.5 cd	1.0 bc	10 bcde	13 ab	9.9 bc	2.1 d	2.7 b	3.3 bc	1.5 bcd
200°C	0.6 abc	9.0 ab	-1.7 c	-0.2 cd	9.5 ab	13 bc	21 a	16 ab	12 a	12 a	7.4 ab	2.6 abcd
225°C	8.1 ab	28 a	17 ab	17 ab	11 a	18 ab	11 ab	12 abc	8.3 b	7.9 ab	5.9 abc	2.1 bcd
260°C	18 a	30 a	14 ab	4.4 bcd	-3.1 c	12 bcd	6.7 b	25 a	6.3 b	4.1 ab	3.5 bc	4.5 abc
320°C	19 a	23 a	21 a	19 a	9.6 ab	14 a	8.1 b	13 abc	5.6 bc	8.7 ab	6.0 abc	5.5 a
Control	15 a	22 a	9.1 b	8.5 abc	0.5 bc	16 ab	12 ab	12 abc	2.9 cd	9.5 ab	7.9 a	4.8 ab

Table 6.6 Significance table for mineral nitrogen (N_{min}, μg N g $soil^{-1}$) in soils amended with thermally altered *P. radiata* needles (temperature in legend) or left unamended ("Control") and aerobically incubated for up to 14 days (values are means; n = 3). Values with same letter within the same column are not significantly different (Tukeys HSD, α=0.05). N.B. statistical analyses are only valid within table columns.

Time (days)	0.00	0.25	0.50	0.75	1	2	3	4	6	8	11	14
40°C	25 a	21 a	16 abc	7.9 a	6.5 bc	4.9 de	1.4 c	0.8 c	1.6 c	0.7 c	1.3 b	1.4 c
85°C	22 ab	21 a	16 ab	6.6 a	4.2 c	4.9 de	1.2 c	0.7 c	1.4 c	0.7 c	1.4 b	1.4 c
100°C	23 a	20 a	15 abcd	9.1 a	3.8 c	1.8 e	1.2 c	0.6 c	1.5 c	0.8 c	1.3 b	1.3 c
135°C	15 bc	14 b	12 cd	8.5 a	5.3 c	7.1 bcd	5.3 bc	0.9 c	1.9 c	0.6 c	1.5 b	1.5 c
150°C	15 bc	15 b	12 d	5.7 a	4.6 c	2.8 de	2.0 c	2.3 c	1.6 c	0.6 c	1.9 b	1.9 c
178°C	15 bc	15 b	13 bcd	7.3 a	4.7 c	2.7 de	1.7 c	1.2 c	1.9 c	1.0 c	1.5 b	1.5 c
200°C	14 c	15 b	12 cd	8.5 a	4.4 c	5.3 cde	4.7 bc	2.6 c	3.1 c	0.9 c	1.7 b	1.7 c
225°C	14 c	14 b	12 d	11 a	12 a	10 abc	11 ab	10 b	10 b	10 b	11 a	8.1 b
260°C	13 c	15 b	15 abcd	14 a	14 a	14 a	14 a	14 ab	16 a	17 a	8.9 a	16 a
320°C	14 bc	16 b	13 abcd	13 a	11 ab	10 ab	11 ab	11 b	12 b	11 b	9.1 a	10 b
Control	15 bc	16 b	17 a	16 a	15 a	14 a	16 a	15 a	18 a	18 a	12 a	17 a

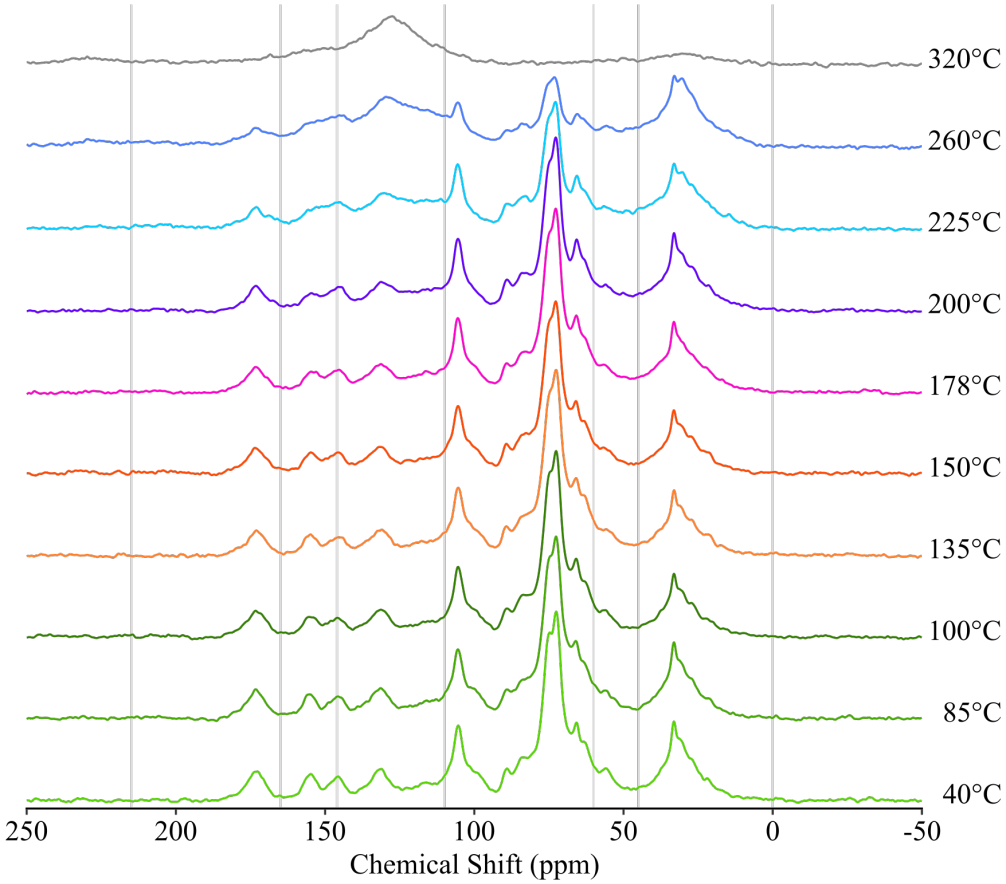

Figure 6.6 Solid state ^{13}C CP-MAS NMR spectra for pine needles hated to 10 temperatures. Values are single measurements. Vertical grey lines indicate the boundaries of chemical shift regions.

Statement of Authorship

Title of paper	Divergent responses of soil microbial community after amendment with thermally altered *Pinus radiata* needles
Publication status	☐ Published ☐ Accepted for publication ☒ Submitted for publication ☐ Unpublished and unsubmitted work written in manuscript style
Publication date	

Principle Author

Name of principle author	Erinne Stirling		
Contribution to the paper	• Experimental design, field work, laboratory analyses, and data analyses Preparation of the manuscript		
Overall percentage (%)	85		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	**6/03/2019**

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

x. the candidate's stated contribution to the publication is accurate (as detailed above);
xi. permission is granted for the candidate in include the publication in the thesis; and
xii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the paper	• Experimental design • Critical review of manuscript		
Overall percentage (%)	5		
Signature		Date	26/02/2019

Chapter 7 – Soil microbial community responses after amendment with thermally altered *Pinus radiata* needles

Under review – *Microbial Ecology*

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7.1 Abstract

Post-fire litter layers are composed of leaves and woody debris that predominantly fall during or soon after the fire event. These layers are distinctly different to pre-fire litters due to their common origin and deposition time. However, heterogeneity can arise from the variable thermal conditions in the canopy during fire. Therefore, in this study we used thermally altered pine needles (heated to 40, 150, 260, and 320°C for 1 hour) in a laboratory incubation study for 43 days. These samples were measured for respiration throughout and extracted for DNA at the experiment's end; soil rRNA was analysed using Illumina Sequencing (16S and ITS amplicons). Addition of pine needles heated to 40 or 150°C caused a substantial shift in community structure, decreased alpha diversity, and significantly increased soil respiration relative to the control treatment. In contrast, pine needles heated to 260 or 320°C had little effect on microbial community structure or soil respiration. These results indicate that highly thermally altered needles are not microbially decomposed during the first 43 days of exposure and therefore that biomass temperature may have significant effects on post-fire litter decomposition and carbon flux. This research outlines an important knowledge gap in forest fire responses that may affect post-fire carbon emissions estimates.

7.2 Introduction

In fire affected forests, post-fire litter layers are composed of leaves and woody debris that fell during or soon after the fire event (Alexis et al. 2007). These layers are distinctly different to non-fire affected litters due to the origin of the debris (i.e. from plants not consumed by a canopy fire) and their truncated deposition time (i.e. during or soon after the fire) (Alexis et al. 2010). Post-fire litter layers may experience thermal alteration while in the canopy or after falling to the forest floor; thermal alteration of organic matter generates a continuum of materials with varying degrees of chemical and structural alteration (Schmidt and Noack 2000, Baldock and Smernik 2002). These materials may only be slightly or partially charred, increasing the heterogeneity of post-fire litter chemistry (Merino et al. 2015). While much research has investigated the overall effects of fire on the soil microbial biomass and microbial community structures (Bååth et al. 1995, Certini 2005, Holden and Treseder 2013), little is known about the specific effects of thermal alteration of litters on soil microbes.

Thermal alteration of biomass can increase resistance to microbial decomposition through volatilisation of simple organic molecules and aromatisation of organic carbon. Volatilisation of simple organic molecules starts when organic matter temperatures exceed 100°C while the formation of aromatic structures starts at approximately 200°C (Fernandez et al. 2001, González-Pérez et al. 2004). In woody materials, exposure to temperatures >200°C leads to dehydration followed by a conversion of O-alkyl C to aryl and O-aryl furan-like structures with increasing temperature (Baldock and Smernik 2002). In contrast, leaf organs typically char at lower temperatures than woody materials due to their higher cellulose and lower lignin contents (Keiluweit et al. 2010, Merino et al. 2015). The higher sensitivity of non-woody materials to relatively low temperatures may lead to a greater incidence of combustion during fire; therefore, deposition of smaller amounts of charcoal residues after a fire may be more susceptible to microbial degradation (Alexis et al. 2010). Microbial degradation of organic matter not strongly modified by fire may be significant at the decadal timescale as microbial access and decomposition of thermally altered organic matter has been shown to be affected by organic matter origin (such as leaves vs. wood) and degree of thermal alteration (Knicker et al. 2008, Alexis et al. 2012).

Forest fires pose a substantial risk to pine plantation production systems. Within *Pinus* plantations, low intensity fires can consume the O horizons and cause stress induced litterfall without seriously harming the trees. However, moderate intensity fires (with canopy scorching) can have high mortality rates which lead to most of the remaining needles in the canopy falling as litter during or after the fire (Fernandes et al. 2008, Alexis et al. 2010). Overall mortality is strongly affected by pine species (Fernandes et al. 2008). Fire has variable effects on soil microbial biomass; both positive and negative responses have been reported when biomass is measured as biomass C, N or viable organisms (Mabuhay et al. 2006, Muqaddas et al. 2015, Rodríguez et al. 2017). Although heat doesn't penetrate far into the soil profile (Badía et al. 2017), the loss of the O horizon and creation of a new post-fire litter layer is likely to affect soil microbial activity after the event through changes in microbial habitat and available resources. Similarly to microbial biomass response, no consistent trend in microbial activity (as respiration) has been observed after fire or in studies using fire affected litters (González-Pérez et al. 2004, Wang et al. 2012, Stirling et al. 2019a). However, carbon mineralisation rate has been observed to decrease when woody and non-woody materials are heated to >200°C (Baldock and Smernik 2002, Bonanomi et al. 2018).

Thermal alteration of soil organic matter and the development of a post-fire litter layer are likely to affect microbial diversity and community structure (Bååth et al. 1995, Certini 2005). Changes in soil microclimate, physical and chemical properties, and the addition of new organic resources from charred materials allows an increase in phylogenetic diversity by changing the dominance of competing microbes (Pérez-Valera et al. 2017). This has previously been observed in fungal biomass where it is theorised that post-fire litter pulses supported the proliferation of fungi specialised in early stage substrate decomposition (Sun et al. 2015a, Rodríguez et al. 2018); in one case, this larger fungal biomass declined after two years (Rodríguez et al. 2018). In similar studies, however, fire did not appear to substantially affect bacterial diversity or biomass (Liu et al. 2015, Sun et al. 2016).

More frequent fire conditions due to a warming climate is expected to increase the incidence of catastrophic fire events (Ager et al. 2012, Hoegh-Guldberg et al. 2018); forests have been increasingly affected by fire in a warming climate (Stevens-Rumann et al. 2018). An initial forest response to fire includes the loss of substantial quantities of litter. In pine forests, litter mass has been estimated as high as 128 t ha^{-1} (Old Kersbrook Forest, South Australia) (Stirling 2019); however, litter mass is more typically between 15 and 32 t ha^{-1} (Florence and Lamb 1974, Baker and Attiwill 1985). Although litter losses from combustion are easily calculated from field data, further losses from enhanced mineralisation of the post-fire litter layer have not yet been quantified. Due to the vulnerability of litter to fire, there is a need to quantify this pool and understand how it behaves after fire.

From the information presented above, it is not clear how a post-fire litter layer will affect microbial community structure and function and thus it is not clear how ecosystem function will recover after a forest fire. Therefore, we aimed to investigate the response of the microbial community to thermally altered non-woody plant materials (i.e. needles) without the confounding effects of fire by incubating soil with thermally altered pine needles. The hypotheses tested here are as follows: firstly, that all needle additions will cause a change in microbial structure to a community best able to take advantage of the newly available resources; and secondly, needles exposed to low temperatures will have higher microbial activity due to the availability of

cellulose and other easily degraded leaf structures. Understanding the soil ecological response to post-fire litters is important because of the increasing risk of fire in a warming climate as forecasted in global change scenarios (IPCC 2014).

7.3 Materials and methods

7.3.1 Needle alteration and characterisation

Pinus radiata needles were sourced during June 2017 from a managed forestry plantation in South Australia, Australia. Sample trees were from a wilding population within a mature stand; needles were collected from more than 20 trees. Live needles (<1 year old) were collected as fascicles from immature trees (height: 2-5 m); the needles were stripped from their scale leaves and dried in a convection oven at 40ºC until a constant mass was achieved. Dry needles were then mixed and heated in a muffle furnace for 1 hour at 40, 150, 260 or 320°C. Approximately 300 g of needles were used in each thermal treatment. Needles were ground to between 0.5 and 1.0 mm for use in the incubation experiment; needles used for carbon chemistry characterization were further ground to <0.15 mm, with care taken to prevent friction heating.

Thermally altered needles were characterised for total organic carbon (TOC), total nitrogen (TN) and their carbon chemistry determined using solid-state ^{13}C CP-MAS nuclear magnetic resonance (NMR) spectroscopy. Total organic carbon and TN were measured by dry combustion using a LECO CNS200 Analyser on thermally altered needles without replication. Carbon content of the needles appeared to increase from 48% (40°C) to 64% (320°C) while C:N appeared to decrease from 36 (40°C and 150°C) to 23 (320°C) with thermal alteration of the pine needles (Table 7.1; Figure 6.6). Solid-state ^{13}C NMR analyses of thermally altered needles were conducted on a 200 Avance spectrometer (Bruker Corporation, Billerica, MA), with a 4.7 T wide-bore superconducting magnet and operating resonance frequency of 50.33 MHz. Needle samples were packed into zirconia rotors (7 mm diameter) with Kel-F end caps and spun at the 'magic angle' (54.7°) at 5 kHz. Chemical shift values were calibrated to the methyl resonance of hexamethylbenzene at 17.36 ppm and a 50 Hz Lorentzian line broadening was applied to all spectra (Baldock et al. 2013). Spectra were integrated across the following chemical shift limits to provide estimates of broad carbon types: 0-45 ppm (alkyl C), 45-60 ppm (N-alkyl C), 60-110 ppm (O-alkyl C), 110-145 ppm (aryl C), 145-165 ppm (O-aryl-C), and 165-215 ppm (carbonyl C) (Baldock and Smernik 2002). Heating the pine needles caused changes in the carbon chemistry (Table 7.1; Figure 6.6), with alkyl C compounds (alkyl, N-alkyl, O-alkyl, and di-O-alkyl) decreasing with thermal alteration and aromatic compounds (aryl, O-aryl) and ketones increasing with thermal alteration.

Table 7.1 Chemical data for the four thermally altered needle treatments: total organic carbon (TOC), total organic nitrogen (TON), C:N ratio, and carbon chemistry as detected by solid-state ^{13}C CP-MAS nuclear magnetic resonance. Values are single measurements.

Temp	TOC (%)	TON (%)	C:N
40	48	1.3	36
150	50	1.4	36
260	61	2.0	31
320	64	2.8	23

Temp	Alkyl (%)	N-Alkyl/ Methoxyl (%)	O-Alkyl (%)	Di-O-Alkyl (%)	Aryl (%)	O-Aryl (%)	Amide/ Carboxyl (%)	Ketone (%)
40	17	5.1	42	10	13	6.7	5.2	0.7
150	16	5.4	44	10	13	5.4	5.1	0.8
260	21	4.9	20	6.9	31	9.7	4.9	2.1
320	5.4	1.9	2.7	5.0	62	14	5.4	2.8

7.3.2 Experimental setup

Thermally altered needles were incubated for 43 days with an acidic (pH 5.6; depth 0-10 cm) fine sandy clay loam soil collected from *P. radiata* plantations in Old Kersbrook Forest, South Australia, Australia. Incubation ended approximately one month after soil respiration stabilisation. Soils were randomly sampled from 12 plots on five dates equally spanning 16 months; these soils were air dried and stored in a cool and dark location for up to 3 years prior to use. On the one occasion where soils were moist at sampling, they were returned under cool conditions to the lab wherein they were dried at 40ºC.

Soil respiration was measured over the course of the experiment, and soil was destructively sampled for DNA extraction at the conclusion of the experiment. In all treatments except the control, needles were added to the soil at a rate of 2% w/w and mixed thoroughly with the soil; five 50 g replicates of each soil and needle mixture were incubated in 250 mL airtight bottles with rubber seals at a gravimetric water content of 0.17 g g^{-1}. The control treatment was maintained in the same manner as litter treatments. Bottles were spatially randomised in three dimensions during incubation. The laboratory based incubation was conducted in the dark and under optimal moisture conditions for CO_2 efflux (gravimetric water content 0.17 g g^{-1}); samples were incubated at 27±2°C. Soil respiration (as CO_2 flux) was measured using a non-dispersive infrared gas analyser (IRGA, LI-820, Li-Cor, USA) throughout the 43 days on all replicates (n=5). Respiration measurements were collected manually via a gas syringe (5 ml) and analysed using one machine throughout the experiment; incubation chambers were vented after measurement. Gas CO_2 content per second was interpolated and converted to g CO_2 using R (version 3.5.1 "Feather Spray") (R Core Team 2018).

7.3.3 DNA extraction and rRNA analyses

Soils were destructively sampled at 43 days and frozen (-4ºC) prior to DNA extraction and Illumina Sequencing; an incubation period of 43 days was considered long enough to induce changes in microbial responses to needle addition. DNA extractions, PCR amplification and sequencing were conducted on whole soil samples by an external laboratory (Australian Genome Research Facility (AGRF)) as described previously by Smith et al. (Smith et al. 2018). In brief, DNA was extracted from 0.250 g of soil using a PowerSoil Soil DNA Isolation Kit (MoBio

Laboratories, Solana Beach, CA, USA). Ribosomal RNA was analysed for bacterial and fungal communities using the forward and reverse primers 27F-519R (AGAGTTTGATCMTGGCT CAG and GWATTACCGCGGCKGCTG; 16S) and ITS1F-ITS2 (CTTGGTCATTTAGAGGAA GTAA and GCTGCGTTCTTCATCGATGC; ITS). The following PCR conditions were used: an initial denaturation at 95 °C for 7 minutes, followed by 35 cycles of 94ºC for 30 seconds, 55ºC for 45 seconds and 72ºC for 60 seconds. A final extension was carried out at 72 °C for 7 minutes. Image analysis was performed in real time using MiSeq Control Software v2.6.2.1 and Real Time Analysis v1.18.54. Sequence data were generated with the Illumina bcl2fastq 2.20.0.422 pipeline. Data was received as FastQ formatted sequence files containing paired end sequences of untrimmed 300 bp reads with phred-like quality score +33.

7.3.4 Data analysis

Bioinformatic analyses were conducted using Qiime2 (version 2018.6) while respiration modelling and statistical analyses were conducted using R (version 3.5.1 "Feather Spray") [35, 37-39]; data was lodged with the European Nucleotide Archive (ENA; accession number ERP113345). Taxonomic diversity, alpha diversity and beta diversity were determined using Qiime2 with metadata validated with Keemei [37, 40]. Deblur and DADA2 pipelines were used for the 16S and ITS data, respectively [41, 42]; in both cases bp reads were truncated (or trimmed) during denoising to 250 and no trimming occurred on the left end of reads. Quality filtering based on phred scores (median of 37 for both amplicons) and the removal of chimeric sequences were also conducted during denoising with Deblur and DADA2 [41, 42]. Total retained reads per sample ranged from 35,973 to 120,632 for 16S and from 30,046 to 121,582 for ITS. Classification of 16S data used a trained classifier with the Greengenes 99% alignment taxonomy [43, 44]; classification of ITS data used the UNITE database for molecular identification of fungi [45, 46]. Phylogenetic trees were constructed using 'MAFFT' for alignment, 'mask' to remove high variability regions, and 'FastTree' to build the phylogeny [47-49]. Taxonomic diversity is displayed at the level where 10 classifications are equal to approximately 90% of organisms by abundance; taxonomic Principle Coordinate Analyses (PCoA) plots (Bray-Curtis dissimilarity) were generated using Qiime2's phylogenetic core metrics. Data was rarefied during the diversity analyses; sample depth (p) equalled 5,900 for both sets of data. Alpha diversity is reported using Shannon's diversity index and Beta diversity is reported using weighted unnormalized UniFrac [50, 51]. Alpha and Beta diversity treatments were tested for differences using Analysis of Variance (ANOVA) and post hoc Tukey tests ($n=5$, $\alpha<0.05$) using the R package agricolae::hsd [52]. Statistical differences in abundance as a proportion of amplicon sequence variants (ASV) were tested using ANCOM at level 6 (genus) for both datasets and at levels 3 (class) and 4 (order) for the 16S and ITS datasets, respectively [53]. Total number of ASV was 4,854 for the 16S data and 1,355 for the ITS data.

Modelled cumulative respiration curves are based on a three parameter logistic growth function (Equation 7.1) modified from Paine et al. [54] using the R non-linear mixed effects model package nls2::nls2 with 'algorithm' set to "port" [55]. In this function, 'M' is cumulative respiration ($mgCO_2$-C gOC^{-1}) as a function of 't' (time), 'K' is the upper asymptote of growth, 'r' is the decay constant, and 'b' accounts for linear growth past 'K'. Parameters were tested for significance using one-way ANOVA and post hoc Tukey tests ($n=5$, $\alpha<0.05$). Average parameter values were used as the final model and tested against the data using the R linear model package stats::lm [35].

Equation 7.1 three parameter logistic growth modified from Paine et al. (2012).

$$M_t = \frac{M_0 K}{M_0 + (K - M_0)e^{-rt}} + bt$$

Where '*M*' is cumulative respiration ($mgCO_2$-C gOC^{-1}) as a function of '*t*' (time), '*K*' is the upper asymptote of growth, '*r*' is the decay constant, and '*b*' accounts for linear growth past '*K*'.

7.4 Results

7.4.1 Low temperature needles changed microbial community structure

Bacterial taxonomy (synonymous with the 16S amplicon data; Figure 7.1a) was dominated by Proteobacteria in all soil samples; this phylum accounted for 50-63% of bacteria by abundance. Differences in abundance within the ten most abundant classes were not detected using ANCOM. Of the organisms classified to genus, thermally altered needles significantly affected the abundances of *Herbiconiux* ssp., *Telmatospirillum* ssp., *Sphingomonas* ssp., and *Dyella* ssp.. Fungal taxonomy (as determined by the ITS amplicon data; Figure 7.1b) was dominated by Ascomycota; this phylum accounted for 58-77% of fungi by abundance. From the ANCOM analyses, significant differences in abundance were detected in the orders *Venturiales* (W = 50), *Coniochaetales* (W = 48), *Sordariales* (W = 49), and *Sebacinales* (W = 49). In all cases, abundance was higher in low temperature needle treatments than the high temperature needle treatments or the control. Of the organisms classified to genus, thermal alteration of needles significantly affected the abundances of *Aureobasidium* spp., *Kabatiella* spp., *Venturia* spp., *Pustularia* spp., *Wilcoxina* spp., and *Coniochaeta* spp.. *Pustularia* spp. and *Wilcoxina* spp. were more abundant in the high temperature treatments and the control while all other genera were less abundant in the high temperature treatments and the control. When analysed with Bray-Curtis distance matrices (Figure 7.1c-d), both 16S and ITS data group high temperature (260, 320°C) needle treatments with the control and group low temperature (40, 150°C) needle treatments separately.

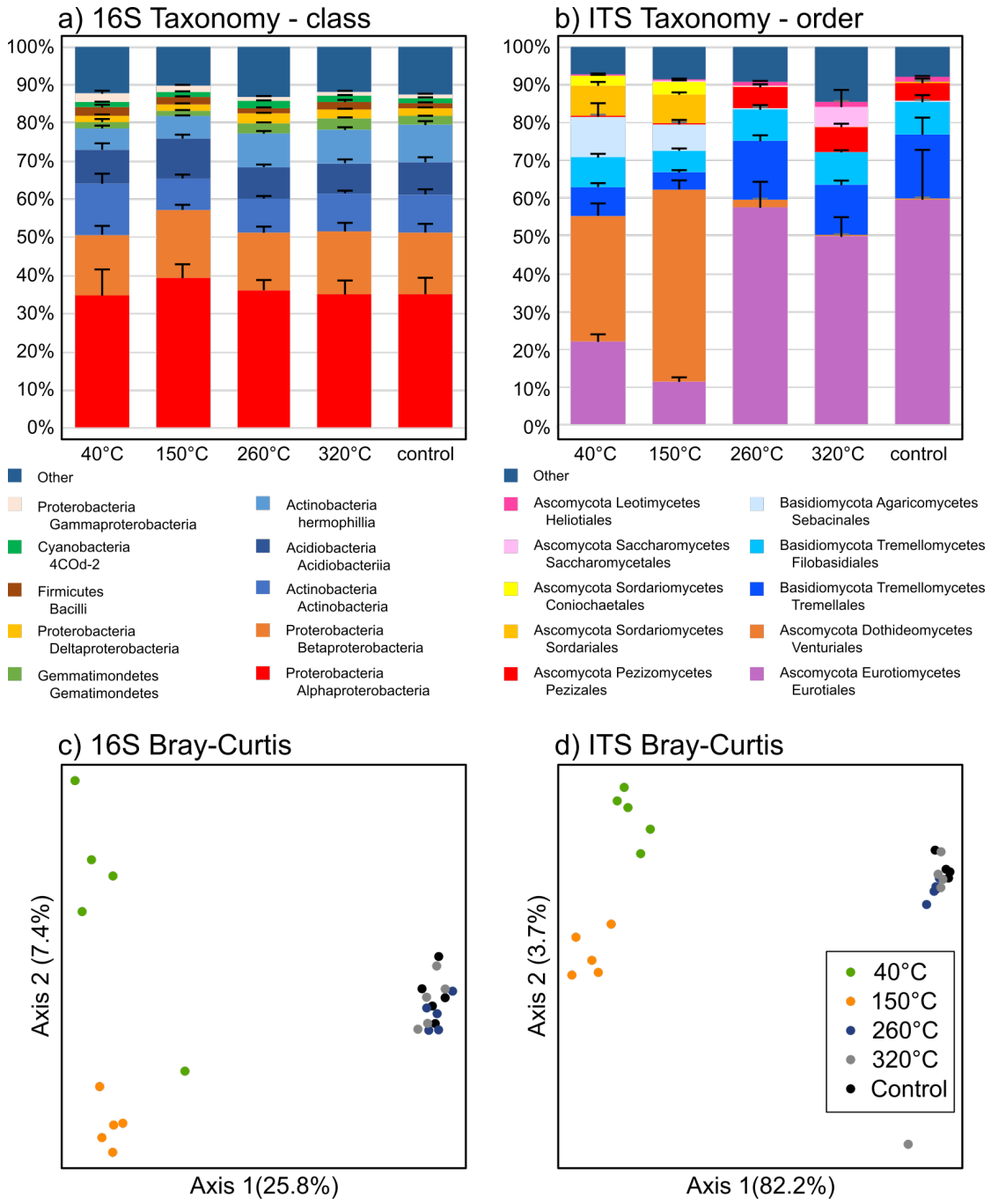

Figure 7.1 microbial community structure presented as composition (a, b) and Principle Coordinate Analysis (PCoA) plots (c, d) for 16S (taxonomic class, left panels) and ITS (taxonomic order, right panels). Columns are average + standard error (n=5).

7.4.2 Microbial diversity is decreased by low temperature needle treatments

Whereas 16S alpha diversity was lower in low temperature needle treatments than high temperature treatments (Figure 7.2a), ITS alpha diversity was only lower in the 150ºC treatment relative to the high temperature treatments (Figure 7.2b). Beta diversity followed a similar pattern to cumulative respiration; low temperature needle treatments grouped strongly in both 16S and ITS while high temperature treatments grouped with the control treatment (Figure 7.3). Beta diversity accounted for more variability for the ITS data than the 16S data (first two PC axis captured 87% and 66%, respectively). In all cases, low temperature needle treatments were significantly different to the control treatment; high temperature needle treatments were similar to the control in all but one case – 16S was significantly different to the control in the 320ºC treatment.

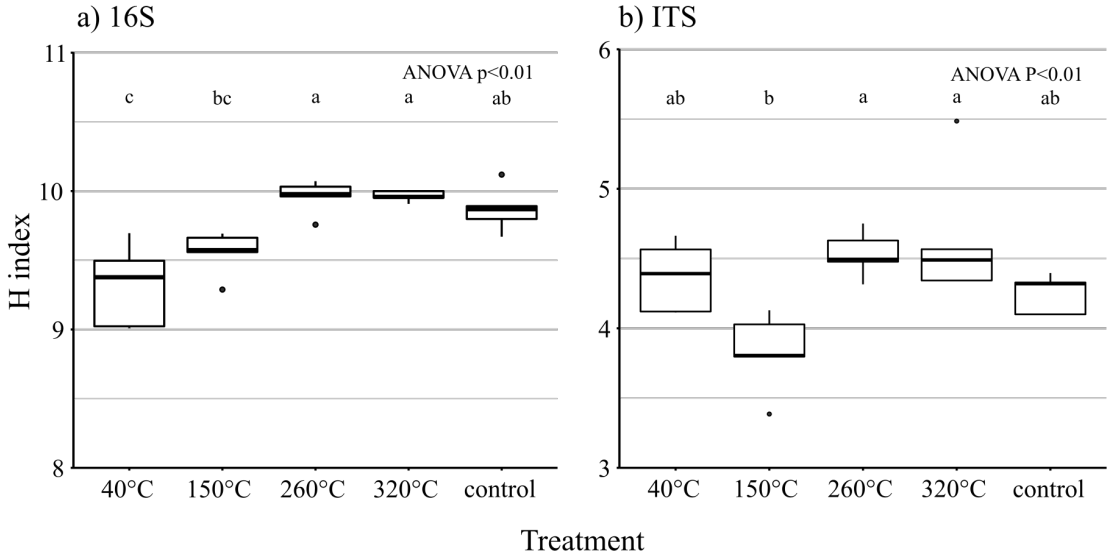

Figure 7.2 Alpha diversity (Shannon index; 'H') for 16S (a) and ITS (b) Illumina Sequencing data. Overall treatment differences determined using one-way ANOVA. Individual differences determined using Tukeys HSD (α=0.05); boxes with same letter are not significantly different.

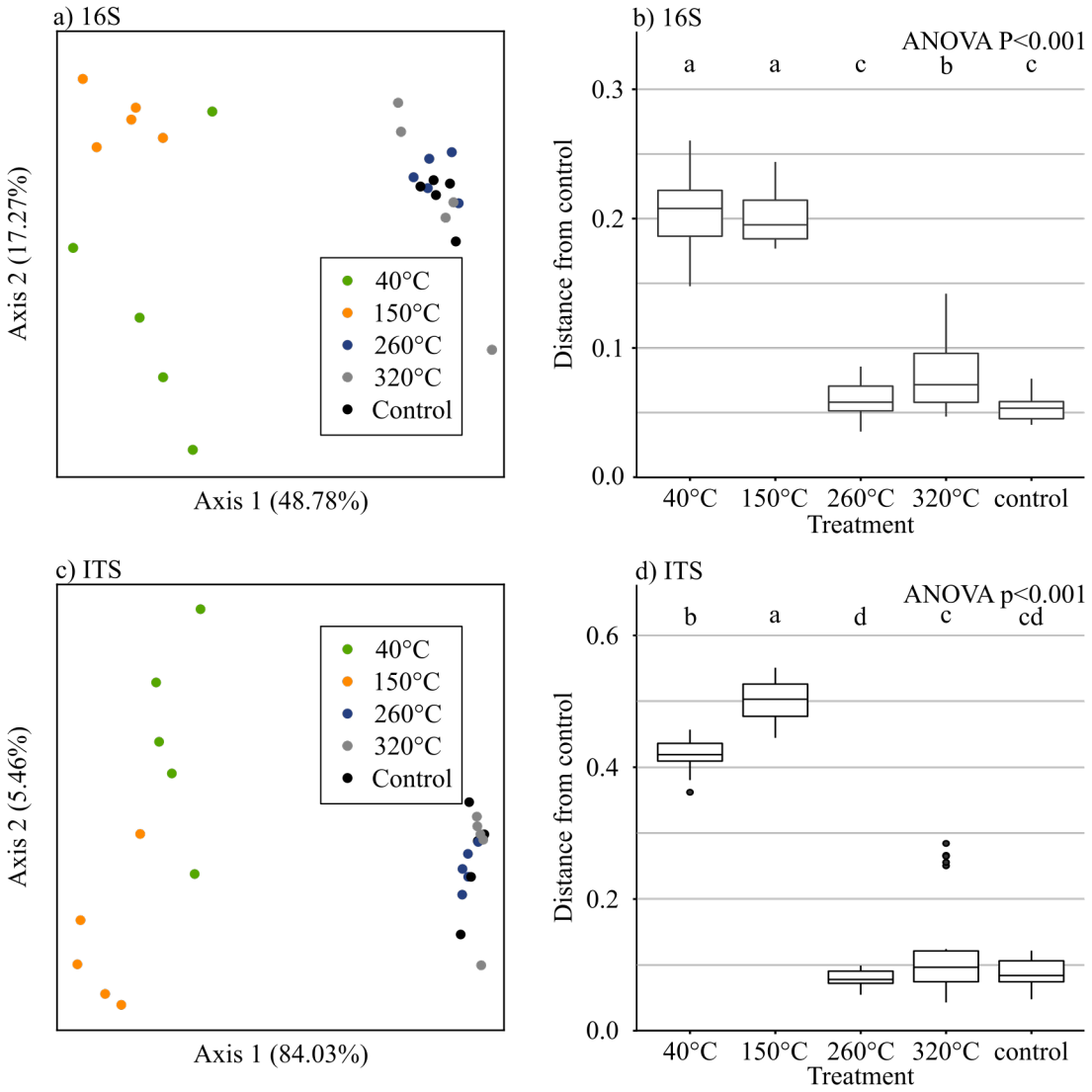

Figure 7.3 Beta diversity (weighted unnormalized UniFrac) for 16S (a, b) and ITS (c, d) displayed as PCoA (a, c) and as distance from control (pairwise analyses; b, d). For plots 16S B and ITS B, overall treatment differences determined using one-way ANOVA and individual differences determined using Tukeys HSD (α=0.05; 25 pairs per treatment); boxes with same letter are not significantly different.

7.4.3 Soil respiration is increased after amendment with low temperature needles

Modelled cumulative respiration for low temperature needles (40ºC and 150ºC) was significantly higher than for the high temperature needles (260ºC and 320ºC) and the control (Figure 7.4). This significant increase occurred in the asymptote of growth '*K*', decay constant '*r*', and the linear rate of growth '*b*' (Table 2). Model fit (r^2) was above 0.95 for all treatments.

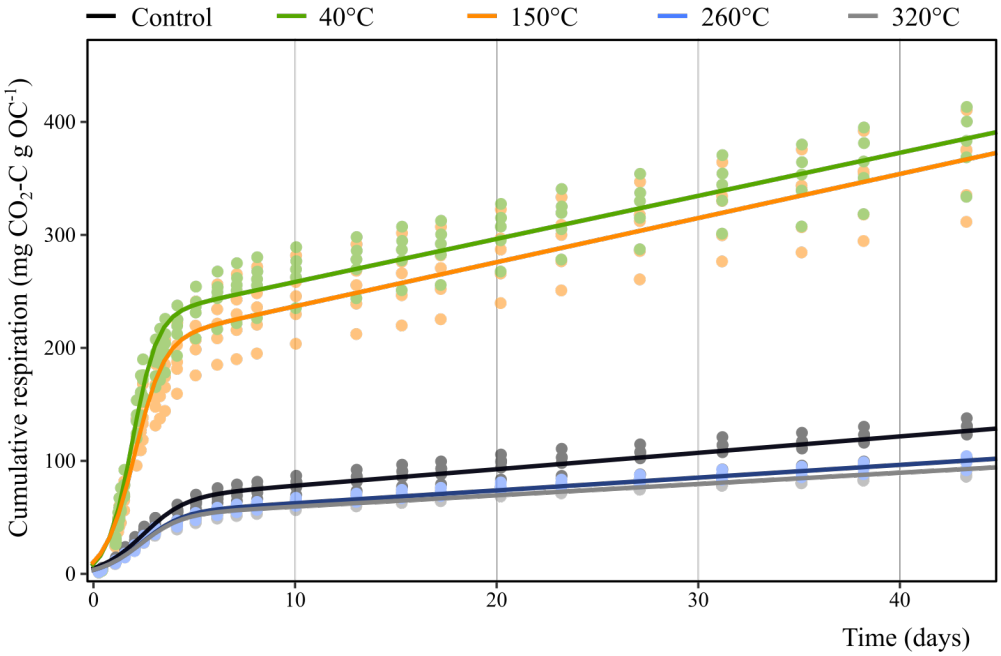

Figure 7.4 Measured and modelled (see text) cumulative respiration ($mgCO_2$-C gOC^{-1}) in soil amended with thermally altered *P. radiata* needles (temperature in legend) or left unamended ("Control") and aerobically incubated for 43 days (points indicate measured variables; lines are average modelled response, n=5). Refer to Table 2 for parameter values and fit measure.

Table 7.2 Parameter value and significance table for Figure 3. Three parameter logistic growth modified (see text) from Paine et al. (2012). Where '*M*' is cumulative respiration (mg CO_2-C g OC^{-1}; 'OC' is organic C) as a function of '*t*' (time), '*K*' is the upper asymptote of growth, '*r*' is the decay constant, and '*b*' accounts for linear growth past '*K*'. All treatments are n=5; values are mean±sd; values with same letter within the same column are not significantly different (Tukeys HSD, α=0.05).

	***K* (p<0.001)**	***r* (P<0.001)**	***M₀* (p<0.001)**	***b* (p<0.001)**	**fit (r^2)**
40°C	220 a	1.64 a	7.5 b	3.8 a	0.97
150°C	198 a	1.39 b	9.3 a	3.9 a	0.95
260°C	51 b	1.01 c	3.7 c	1.1 b	0.99
320°C	50 b	1.01 c	3.8 d	1.0 b	0.99
Control	64 b	0.95 c	5.0 d	1.4 b	0.97

7.5 Discussion

Microbial responses to post-fire litters will depend on biomass temperatures reached during the fire: litters formed from needles that have experienced high temperatures in the canopy will resist decomposition after litterfall. In contrast, needles that experience low temperatures (such as might be found in a moderately severe fire (Keeley 2009)) may experience enhanced mineralisation after litterfall. This relationship with temperature can be seen in the beta diversity and cumulative respiration results, where needles exposed to high temperatures caused only minor changes in microbial diversity or activity compared to the control soil. This resistance to microbial decomposition means that litterfall following high temperature thermal alteration may lead to an increase in recalcitrant soil organic carbon, indicating that a blackened (high temperature thermally altered) post-fire litter layer may be an effective carbon sink when

compared to a low temperature thermally altered litter layer. The quantity of this pool after an actual fire (4-6% of pre-fire leaf C stocks), however, may mean that it is an overall insignificant C sink (Alexis et al. 2007, Alexis et al. 2010). Nevertheless, this study clearly demonstrates that it is not appropriate to consider all post-fire litters as the same in terms of their impact on belowground processes.

Post-fire pine litters with a low degree of thermal alteration will affect microbial community structure to a greater degree than litters with a high degree of thermal alteration. Soil microbial responses are likely to be more defined in the fungal biomass as microbial community changes were observed to a greater degree in the ITS data than the 16S data. In our study, low temperature needle treatments tended to increase the proportion of saprotrophic fungi (such as *Venturia* spp.) while decreasing the proportion of some ectomycorrhizal fungi (such as *Wilcoxina* spp.) and increasing the proportion of other mycorrhizal fungi, including *Sebacinales*, an order known for its diverse orchid mycorrhizal abilities (Selosse et al. 2009). While mycorrhizal fungi are generally unable to thrive in the absence of their host plants, the absence of their rRNA in the low temperature treatments indicates degradation of residual genetic organic matter by the expanded presence of saprotrophic organisms. The expansion of saprotrophic fungi is a logical response to the addition of new organic resources; in field situations where fire increases soil organic matter availability, the microbial community responds with increased activity and biomass production (Pérez-Valera et al. 2018, Rodríguez et al. 2018). However, as the test soil was not recently burnt, fungal response in the field may be significantly different to that which was observed under test conditions as fire generally has a negative effect on fungal abundance (Dooley and Treseder 2012). The extent to which this is the case in Australian woodlands, however, is unclear due to the incidence of pyrophillia in Australian fungi (McMullan-Fisher et al. 2011).

The rapid recovery of bacteria after soil heating events allows them to take advantage of new resources available after fire (Rodríguez et al. 2017, Pingree and Kobziar 2019). In studies where soil bacterial communities were analysed after fire, fire resistant bacteria are present directly after the fire, and are succeeded by competitively superior bacteria that are able to take advantage of fire affected soil organic carbon resources (Pérez-Valera et al. 2019). When ecosystem function was considered, enzyme activities (β-glucosidase, phosphatase, and urease) had largely returned to pre-fire levels 12 months post-fire, however bacterial community structure remained different, indicating functional redundancy (Pérez-Valera et al. 2018, Pérez-Valera et al. 2019). Functional redundancies within the microbial community may have a mediating effect on the decomposition of thermally altered needles leading to increased decomposition of otherwise 'inert' materials if sufficient time is considered.

Microbial function needs to be considered in parallel to microbial diversity when investigating the soil microbiome (Wagg et al. 2014). In this study, post-fire litters composed of needles that experienced a low degree of thermal alteration generated increased microbial activity relative to soils with no or highly thermally altered litter. Thermal alteration of pine needles decreased microbial activity by decreasing the proportion of resources available in the first week ('K', Table 2) and by decreasing the organic matter decay rate during both the logistic ('r', Table 2) and linear ('b', Table 2) phases of decomposition. This decrease in resource availability is associated with a degradation of polysaccharides as determined by solid-state ^{13}C CP-MAS NMR spectroscopy. Similar changes have been observed by Bonanomi et al. (Bonanomi et al. 2018), where a sharp increase of aromatic C and a decrease of other fractions was observed when heating

litters to 200°C. The results here show the same pattern to those observed in a similar incubation study by Bonanomi et al. (Bonanomi et al. 2018), where litters were heated for 30 minutes at larger temperature intervals and a decrease in cumulative respiration was observed starting at a litter heating temperature of 200-300°C depending on litter species (Bonanomi et al. 2018). The differences in 'K' between respiration responses to different litters indicate that the labile resource pool is important in mediating the microbial responses during the early phases of exposure. This indicates that the most labile resource pool is strongly affected by heating, possibly due to the release of simple organic compounds from larger or protected structures during thermal alteration (De la Rosa et al. 2008, Stirling et al. 2019a). The importance of litter chemistry as a driver of microbial community succession has also been shown for non fire affected litters with lignin:N and labile C strongly influencing species present over decomposition time (maximum 180 days) (Bonanomi et al. 2019). Although not measured here, litter labile C content of litters from the same research area was positively correlated with microbial activity when measured during the first 6 days of exposure (Stirling et al. 2019a). It is unsurprising, then, that high temperature thermally altered needles have limited effects on the microbial biomass as simple carbon structures such as O-alkyl C and di-O-alkyl C were lost during the thermal alteration process.

Forest fires are at an increasing risk under the warming conditions of climate change scenarios (IPCC 2014); forest soil respiration is an important terrestrial flux of carbon to the atmosphere and therefore poses a significant emissions risk (Dixon et al. 1994, Huffman and Madritch 2018). Where there is incomplete combustion of the canopy and tree or branch death, leaves that fall during or soon after a fire may add to the overall emissions risk. Moreover, thermally altered needles can strongly affect microbial communities, including causing significant shifts in the fungal community, possibly depending on biomass temperatures reached during the fire. The results of this work may have significant consequences for interpreting post-fire impacts as simply classifying sites by the presence/absence of fire will be insufficient for predicting soil ecological responses to the event. In addition, it is clear from this study that thermal alteration of pine needles leads to increased decomposition at low temperatures (<150°C) and decreased decomposition at high temperatures (>260°C) during the initial stages of needle decomposition; the effects of temperature on long term decomposition cannot be extrapolated from this experiment. As hypothesised, the decrease in microbial activity at higher temperatures was associated with the degradation of polysaccharides. These changes in activity were also associated with community structure shifts towards saprotrophic organisms in the low temperature treatments. However, it is important to note that this study focussed on one species typically grown as a monoculture and it is probable that forests containing more diverse assemblages or trees with different fire strategies may have litter that responds differently to the results here.

7.7 Acknowledgements

This project was supported by The Holsworth Wildlife Research Endowment & The Ecological Society of Australia, and The University of Adelaide Research Training Program Scholarship, with further analytical assistance provided by CSIRO Agriculture & Food. We thank the editor and reviewers for their constructive comments on our original submission.

Chapter 8 – Conclusions

The body of work presented in this thesis explored the effect of fire on soils and nutrient cycling in two Australian forestry systems to reduce the knowledge gap identified in Chapter 1. Fire had limited effects on soil nutrient contents and microbial activity in the sites investigated at Old Kersbrook Forest (Chapter 2). When investigating soil microbial activity however, fire was found to increase nitrogen limitation in soil when pooled by fire history (Chapter 3). Fire affected litters were found to induce distinctly different soil responses than litters not affected by fire; pine litter changed from the least decomposable to the most decomposable when affected by fire (Chapters 4 and 5). Native litter responded similarly throughout (Chapter 4). Through investigating thermally altered pine needles, this research identified a thermal tipping point at approximately 200ºC. Needles heated to ≤200ºC caused: the abiotic absorption of mineral nitrogen (Chapter 6), a microbial community shift to decomposer organisms (Chapter 7), and an increase in microbial activity (Chapters 6 and 7). Soils incubated with needles heated to >200ºC acted similarly to the control soil. The results from Chapter 6 have implications for the current understanding of nitrogen cycling that may support a paradigm shift on the importance of abiotic reactions in a nutrient cycle that is currently thought to be dominated by biotic processes. These results are supported and extended by the microbial community responses in Chapter 7, indicating that soil microbial communities are not perturbed by the addition of highly thermally altered organic materials. Taken together, this body of work highlights the capability of organic matter in post-fire ecosystems to strongly influence nitrogen cycling and microbial ecology and shows that there are subtle properties in post-fire litters that need to be considered when studying these systems (Figure 8.1).

8.1 The soils of Old Kersbrook Forest are resilient to the effects of fire

The soils of Old Kersbrook forest proved resilient to changes in soil organic matter and nutrient content over time. It was expected, during the survey (Chapter 2), to see strong fire effects during the first sampling with diminished effects over time. This was not the case at the Sampson Flat fire ground as time was not a significant variable and the fire effects on soil organic matter and nutrient contents were inconsistent across the two forestry sites. Inconsistent changes due to fire may be due to the methods used to collect and analyse these soils, the time elapsed between the event and sampling (4 months), the impoverished nature of the original soils, or a combination of multiple issues.

While the issue of pseudoreplication is discussed below (Section 8.4), the high degree of variability in nutrients and microbial activity in the soil survey suggests that the number of samples was insufficient to capture the spatial heterogeneity of the sites. In addition, analytical methods used during the soil survey and nutrient limitation study were relatively rudimentary which may have had negative effects on analysis accuracy. Some of these analytical methods (such as the use of Walkley and Black (1934) wet digestion for microbial biomass C; data not reported in this thesis) were not optimal for the nutrient poor soils of Old Kersbrook Forest.

Forestry sites in the Adelaide Hills occur in areas unsuitable for more intensive production systems. In the case of Old Kersbrook Forest, this limitation appears to be due to the shallow, sandy, skeletal, soils that have developed on rocks from the Gawler Complex (minimum age >1 000 Ma; Figure 8.2) (Meaney 2017). Phosphorus limitation has long been an issue in plantation operations (Boomsma 1949)and phosphorus concentration as observed using anion exchange resins were typically very low (typically <2 μg resin extractable P g soil^{-1}). This naturally low

phosphorus content may have decreased the observable effect of fire on available phosphorus content if excess phosphorus was rapidly sorbed to the soil or assimilated into the microbial biomass (Romanyà et al. 1994). In contrast, the effect of fire on nitrogen contents should have been easier to determine as the ecosystem nitrogen content is not an inherent property resulting from the parent geology. It should be noted however, that the increase in nitrogen in the soils from the native reserve may be due to the recruitment of leguminous trees (*Acacia longifolia*) that germinated soon after the event rather than via mineral N in ash from the fire (Ma et al. 2015).

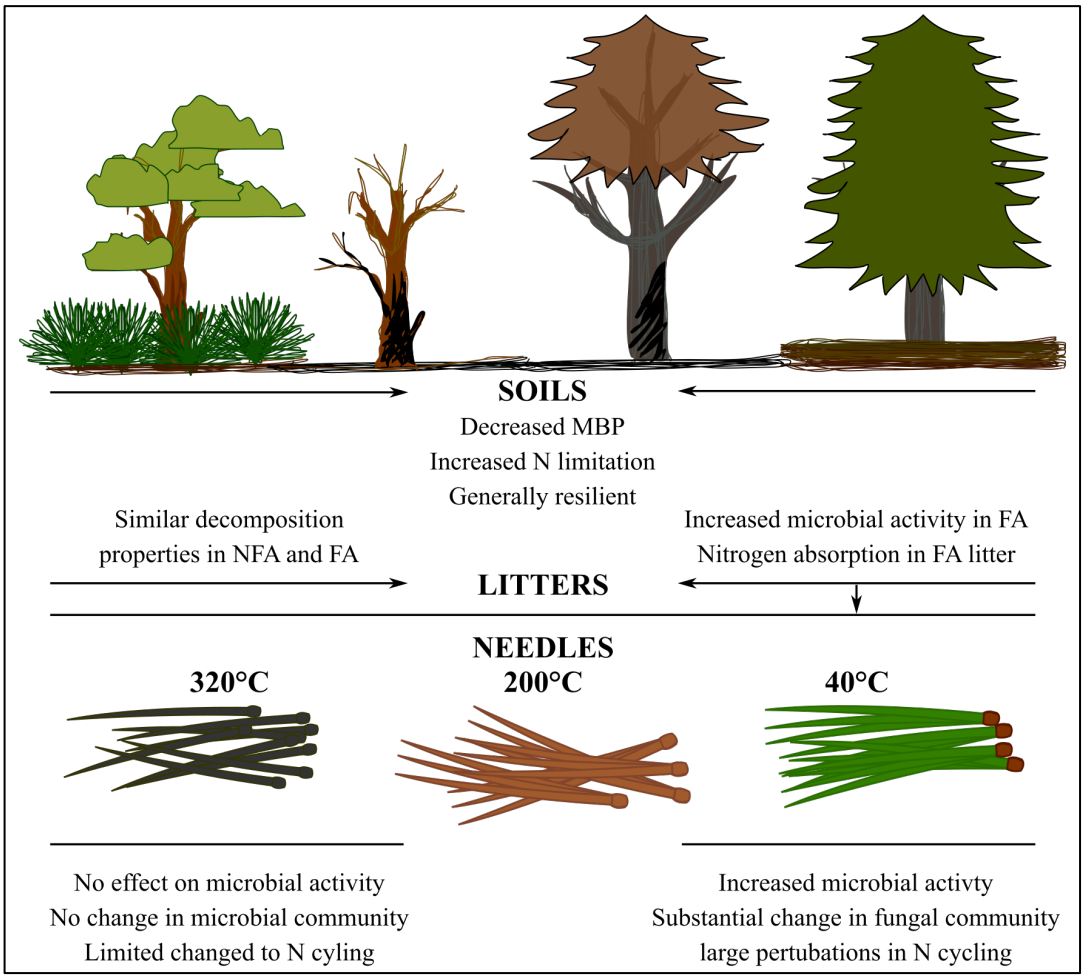

Figure 8.1 Conceptual overview of the conclusions achieved by investigating native and pine forest soils, litters, and thermally altered needles. Where MBP is microbial biomass phosphorus, N is nitrogen, NFA is not fire affected native or pine litter and FA is fire affected native or pine litter. Note the tipping point of 200ºC in the needles.

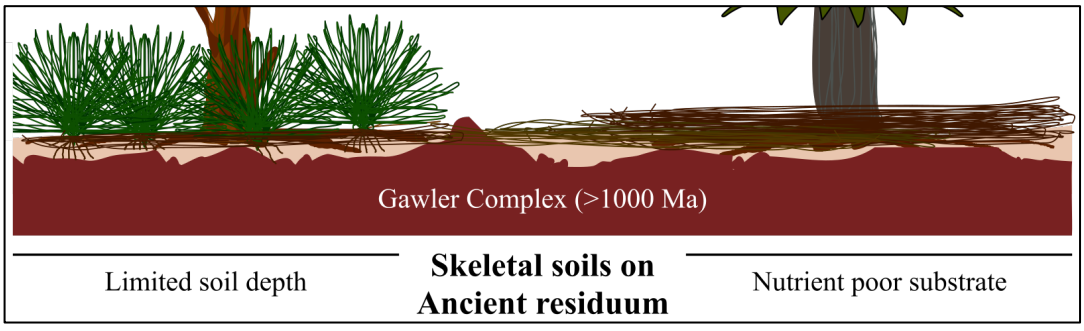

Figure 8.2 soil limitations in Old Kersbrook Forest.

The one variable that was consistently affected by fire status was microbial biomass P (MBP; Chapter 3). A decrease in MBP has been observed in many soils affected by fire and is suspected to be a response to changes in organic matter quality (González-Pérez et al. 2004, Holden and Treseder 2013). As microbial activity was not significantly affected by fire, however, it may be that the quality of organic matter containing phosphorus was more strongly affected by the fires, or that ash formed during the fire reduced ability of the microbial biomass to access P. From this dataset, however, there is insufficient information to speculate on the processes leading to decreased MBP at the burnt sites.

Even though soil nutrient contents were resilient to changes induced by fire (Chapter 2), an increase in nitrogen limitation for microbial activity was observed in the cumulative respiration results when sites were pooled by presence/absence of fire (Chapter 3). Although the time variable (i.e. season of collection) was not a significant variable in the soil survey, it is possible that soil nutrient limitations (nitrogen or phosphorus) may have been affected by sampling time and that earlier sampling may have captured differences in the soil due to fire severity while later sampling may have captured differences due to the vegetation responses. However, if the lack of a response to nitrogen and phosphorus addition is considered, there are indications that soil nutrient cycling is more robust when disturbed by fire under native woodlands than pine plantations, and that native woodland soils may return to nutrient cycling equilibrium more rapidly than pine plantation soils.

In conclusion on the resilience of Old Kersbrook Forest soils to fire, resilience in Australian woodlands and forestry systems appears to be due to the low nutrient contents that these landscapes typically contain. The Old Kersbrook Forest site is severely limited both by depth of soil and by phosphorus content. Other forests in similarly nutrient limited positions also appear strongly resilient to the effects of fire on organic matter and nutrient availability (Hobley et al. 2017, Sawyer et al. 2018). In addition to bulk nutrient contents, burning may also appear to have no effect on sandy acid soils as nutrients are rapidly leached post-fire; these soils may need to be sampled immediately after a fire and to a shallower depth in order to observe any measurable differences (Dean et al. 2015).

8.2 Fire affected litters have changed decomposition properties

Fire affected litters induced limited microbial activity responses when native litter was added and significant microbial activity responses when pine litter was added (Chapter 4). In particular, microbial activity was increased in fire affected (FA) pine treatments and the detectability of microbial biomass N and N_{min} was substantially reduced. Microbial activity indicated that fire caused pine needles to go from the least decomposable to the most decomposable organic matter over the first 6 days, most likely due to a significant increase in water extractable organic C. In addition to the increased water extractable organic C, FA litter may have been relieved of the inhibitory effects of essential oils by heat damage during the fire, or by the increased nutrition available in litter derived from unplanned losses (Attiwill et al. 1978, Águas et al. 2018). As an example of the effects of essential oils, oils derived from *Pinus radiata* and *Eucalyptus globulus* have antimicrobial properties (Sacchetti et al. 2005). In either case, an increase in labile C relative to recalcitrant organic molecules promotes litter decomposition regardless of litter stoichiometry (Hättenschwiler and Jørgensen 2010).

The incubation of FA pine needles also caused significantly different nitrogen pool composition to not fire affected (NFA) pine needles (Chapter 5). Nitrogen was rarely detectable in FA litter

treatments in mineral (ammonium or nitrate/nitrite) or microbial forms (microbial biomass N); the total nitrogen results, however, indicate that there was no significant difference in total nitrogen content between samples that received NFA or FA litter. Mineral N of extracts taken of FA litter or soil amended with FA litter did not increase in concentration even when the samples were incubated in increasing concentrations of ammonium nitrate solution. These results indicate that mineral N is being converted into an organic form in the treatments where FA pine litter was added. There is the potential, therefore, that fire affected pine needles may reduce the risk of nitrogen losses from leaching after a fire event and that these needles may also reduce seedling success by inducing nitrogen limitation.

Net nitrogen movement from soil into fresh litter is commonly found during decomposition studies which may explain the lack of mineral nitrogen in the FA treatments observed here (Xiong et al. 2014). two Essential oils derived from pine needles have been shown to react with inorganic nitrogen when exposed to humid air containing 100 ppb gaseous ammonia (NH_3) to form 'brown carbon' compounds (Updyke et al. 2012). A substantial difference between the results in this study and the results in other studies is the scale of nitrogen loss: in this study nitrogen from the mineral and microbial pools could not be captured while in other studies these pools were still observable.

Chemical bonding of nitrogen in the FA litter treatments may be due to residual oxidised surfaces in the litter. Such surfaces may be formed during the breakdown of lignin as lignin decomposition, whether biotic or abiotic, produces oxygen containing functional groups (de Gonzalo et al. 2016). During biotic decomposition, depolymerisation can be achieved by extracellular enzymes such as peroxidases, which facilitate lignin degradation by oxidising mediators (Sinsabaugh 2010, de Gonzalo et al. 2016). The use of extracellular enzymes means oxygen functional groups can react with nitrogen in the soil to convert mineral N to organic N. While it is difficult to detect the presence of oxygenated surfaces in the needles, increased alkyl C and decreased aromaticity support the presence of such surfaces in the FA litter. Furthermore, organic matter that has experienced oxidation without increasing aromaticity may be more vulnerable to further biological and chemical oxidation, facilitating microbial access to, and dissolution of, organic matter into the soil solution (Knicker et al. 2008). This phenomenon may explain the increase in water extractable organic C and soil respiration in the FA litter.

The results described in this thesis (specifically in Chapters 5 and 6) indicate a significant and novel relationship between organic matter and mineral nitrogen. Biological processes are frequently used to explain nutrient cycling in Australian soils (Wang et al. 2014); however, the experiments used to show these relationships occur under conditions optimal for biological processes (Shi and Marschner (2014) and Cookson et al. (2005) for example). These experimental conditions (optimal moisture and optimal temperature) are not representative of natural conditions. While the incubations conducted herein also occur under biologically optimal conditions, the rapidity (<24 hours) of reactions may indicate chemical processes rather than biological processes are responsible for the results observed.

Due to their skeletal nature, position in the landscape, and relatively large plant biomass demands, the soils of Old Kersbrook Forest are unlikely to be at optimal moisture and temperature for microbial growth and microbially mediated nutrient cycling during much of the year. Therefore, these soils are likely to be both drier and hotter than the optimal conditions for microbial growth and reproduction interspersed with periods of increased microbial activity after precipitation or

other moisture/nutrient delivering events (Blagodatskaya et al. 2010, Sun et al. 2015b). In the absence of a biological driver of nutrient cycling during hot and dry periods, chemical reactions may be the main driver in this system. This phenomenon has been recently observed in phosphorus cycling where warm to hot, dry, conditions causes the degradation of organic phosphorus storage compounds (namely phytate), a process not observed under cool and wet conditions (Doolette et al. 2017, Doolette and Smernik 2018). It is possible, therefore, that chemical reactions occuring under hot and dry conditions may have an inportant role in soil nutrient cycling at the study site.

8.3 Needle decomposition potential is controlled by alteration temperature

Post-fire litters composed of needles that have experienced a low degree of thermal alteration will generate increased microbial activity relative to soils with no or highly thermally altered litter layers. This was observed in both the 14 day and 43 day incubations of thermally altered pine needles with soil (Chapters 6 and 7, respectively). Changes in microbial activity were due to the availability of resources in the first week of incubation and due to the decomposability of resources thereafter. This decrease in resource availability is associated with a degradation of polysaccharides as determined by solid state ^{13}C CP-MAS NMR spectroscopy. Similar changes have been observed by Bonanomi et al. (2018), where a sharp increase of aromatic C and a decrease of other fractions above was observed when heating litters to 200°C. The results here also show the same pattern to those observed in Bonanomi's study where a decrease in cumulative respiration was observed starting at an litter heating temperature of 200-300°C depending on litter species (Bonanomi et al. 2018). The changes in the litters caused by heating are strongly expressed in the initial days of exposure, indicating that the most labile resource pool is strongly affected by heating.

Similarly to the FA pine needles, needles heated to ≤200ºC absorbed a large proportion of mineral nitrogen, probably via a chemical process (Chapter 6). This rapid binding in the low temperature treatments can be seen in all nitrogen pools that were measured on the thermally altered needles. Absorption of nitrogen within litter is not unusual as fresh litter tends to be nitrogen limited whereas soil tends to be carbon limited (Heal et al. 1997, Demoling et al. 2007). As such, net nitrogen transfer has been observed from soil to litter, leading to enhanced decomposition rates (Li and Fahey 2013, Xiong et al. 2014); carbon transfer from soil to litter and *vice versa* has also been observed (Frey et al. 2003, Bird et al. 2017). Rapid non-biotic nitrogen losses have previously been connected to light fraction organic matter (Compton and Boone 2002). In the study of Compton and Boone (2002), this fraction was capable of incorporating an average of 39% of added ammonium and 17% of added nitrate after an 18 hour incubation; absorption occurred as quickly as 5 minutes after nitrogen addition in their study. The decreasing capacity for thermally altered needles to capture nitrogen in our study may therefore be connected with the thermal degradation of light fraction organic matter.

The relationship between nitrogen cycling, heating temperature, and time further supports the claim that abiotic reactions are driving nitrogen transformations in the post-fire system. The consumption of ammonium during the potentially mineralisable N analyses indicates a non-microbially mediated reaction as nitrification requires oxygen. The effect of temperature in the early stages of the incubation (i.e. that ammonium consumption decreases with increasing temperature until approximately 200ºC after which ammonium consumption ceases) indicates that this reaction involves compounds or surfaces that are highly temperature sensitive. Observations from both the two week incubation and the 94 day incubation indicate that this

effect is sustained over a long period. Therefore, there are three important findings from these experiments: firstly, that complex abiotic relationship can form between litters and soil; secondly, that needle nitrogen absorption could outweigh an ash bed effect; and finally, that post-fire litter decomposition dynamics will be affected by canopy temperature during the fire event.

In addition to the nitrogen responses, post-fire pine litters with a low degree of thermal alteration affected microbial community structure to a greater degree than litters with a high degree of thermal alteration (Chapter 7). In field situations where fire increases soil organic matter availability, the microbial community responds with increased activity and biomass production (Pérez-Valera et al. 2018, Rodríguez et al. 2018). Forest soil respiration is an important terrestrial flux of carbon to the atmosphere (Dixon et al. 1994, Huffman and Madritch 2018) and forest fires pose a significant carbon emissions risk. However, it is probable that this risk continues during fire recovery as post-fire litter layers composed of dead but not charred needles can potentially undergo enhanced microbial decomposition. While the expansion of decomposer organisms may lead to a reduction in diversity, functional redundancy within the microbial biomass means a small decrease in diversity is not necessarily a significant issue.

Functional redundancy and changes in microbial function continue to be difficult to determine in soil systems due to their complex structures, materials, and organisms (Stanley et al. 2016, Ma et al. 2018). Although measuring simple microbial functions such as bulk microbial respiration or the presence of functional genes are easily achieved, the functions of specific members of the soil microbial community and their relationships with the environment are difficult to establish – particularly as many functions rely on multiple organism or substrate relationships (Jansson and Hofmockel 2018). It is also difficult to combine disparate datasets in a mathematically sound and scientifically meaningful manner. These difficulties are being addressed both methodologically and computationally through advances in microbial ecology techniques such as microfluidics, ‘chip’ technologies, and predictive ecology (Jansson and Hofmockel 2018, Oshiki et al. 2018, Otwell et al. 2018, Zhalnina et al. 2018). Even in the absence of these advanced techniques, however, it is clear that thermal alteration of foliage affects microbial community responses.

The similarity in responses to high temperature thermally altered needles for nitrogen, microbial activity, and microbial community indicate that these needles are effectively inert during the first 14–43 days. While the low temperature (40–150ºC) needles reacted in a broadly similar manner, subtle differences in nitrogen and microbial responses indicate the presence of thermally sensitive compounds in the needles. Therefore, relatively small differences in canopy heat intensity may have substantial effects on post-fire successional processes.

Forest fires are an increasing risk under warming conditions of climate change. Where there is incomplete combustion of the canopy and tree or branch death, leaves that fall during or soon after a fire may add to the overall emissions risk. Moreover, thermally altered needles can strongly affect microbial communities, including causing significant shifts in the fungal community, depending on canopy temperatures reached during the fire. This may have significant consequences for interpreting post-fire impacts as simply classifying sites by presence/absence of fire will be insufficient for soil ecological responses to the event.

8.4 Pseudo-replication

The preliminary research conducted at the start of this project (Chapters 3 and 4) is limited by pseudoreplication of the sites (Schank and Koehnle 2009); i.e. in ecological studies sites must be replicated in an independent manner. In this study, therefore, I would be required to find multiple Long Leaf Box woodlands and *Pinus radiata* plantations that had each experienced independent fires while holding all other variables relatively constant. This was not possible due to limited site availability, and so while the data in these chapters provide a valuable foundation for the thesis, this limitation needs to be kept in mind. Issues with pseudoreplication were anticipated during the experimental design stage and was initially addressed by locating four plots within each site with the argument that, as the soil properties I was investigating are highly spatially variable, microsite differences would be such that these plots could be considered statistically independent. After the change in project direction (from soil focussed to litter/foliage focussed; Chapter 4), pseudoreplication was addressed by using individual sites or soil treatments as replicates while limiting the strength of claims on site specific responses to treatments. Pseudoreplication reduces the power of statistical tests, leading to a higher proportion of errors (type I or II) (Colegrave and Ruxton 2018); therefore, further work in this area should be carefully planned to avoid such limitations.

8.5 Further research options

Several important questions are raised on abiotic nitrogen cycling and the role of the microbial biomass in this work. Obvious questions include whether other plant litter are capable of absorbing mineral nitrogen and whether particular soil properties allow this phenomenon to occur. On a more fundamental basis, research should be conducted into the chemical processes that allow pine needles to prevent the detection of mineral nitrogen to ensure this method is suitable for mineral N detection in fire affected forest soils. The temporal effects of abiotic reactions are also in important variable that requires investigation; the reactions that remove mineral nitrogen from solution are rapid, but it is not known how difficult (or over what timescale) the reverse reactions might be. Additionally, further research should be conducted into the effects of these nitrogen perturbations on microbial community and function. Finally, a difficulty that was repeatedly raised during this work was the large amounts of complementary, but not integrable, data that were produced. There is a need to develop analyses methods for relating disparate datasets in a mathematically sound and scientifically meaningful manner.

References

Abraham, J., and J. A. Chudek. 2008. Studies on litter characterization using ^{13}C NMR and assessment of microbial activity in natural forest and plantation crops' (teak and rubber) soil ecosystems of Kerala, India. Plant and Soil **303**:265-273.

Ager, A. A., N. M. Vaillant, M. A. Finney, and H. K. Preisler. 2012. Analyzing wildfire exposure and source-sink relationships on a fire prone forest landscape. Forest Ecology and Management **267**:271-283.

Águas, A., G. Incerti, A. Saracino, V. Lanzotti, J. S. Silva, F. C. Rego, S. Mazzoleni, and G. Bonanomi. 2018. Fire effects on litter chemistry and early development of *Eucalyptus globulus*. Plant and Soil **422**:495-514.

Alexis, M. A., D. P. Rasse, H. Knicker, C. Anquetil, and C. Rumpel. 2012. Evolution of soil organic matter after prescribed fire: a 20-year chronosequence. Geoderma **189-190**:98-107.

Alexis, M. A., D. P. Rasse, C. Rumpel, G. Bardoux, N. Péchot, P. Schmalzer, B. Drake, and A. Mariotti. 2007. Fire impact on C and N losses and charcoal production in a scrub oak ecosystem. Biogeochemistry **82**:201-216.

Alexis, M. A., C. Rumpel, H. Knicker, J. Leifeld, D. Rasse, N. Péchot, G. Bardoux, and A. Mariotti. 2010. Thermal alteration of organic matter during a shrubland fire: a field study. Organic Geochemistry **41**:690-697.

Anderson, J. M., and J. S. I. Ingram. 1989. Tropical soil biology and fertility: a handbook of methods. C.A.B. International.

Ansley, R. J., T. W. Boutton, and J. O. Skjemstad. 2006. Soil organic carbon and black carbon storage and dynamics under different fire regimes in temperate mixed-grass savanna. Global Biogeochemical Cycles **20**.

Arocena, J. M., and C. Opio. 2003. Prescribed fire-induced changes in properties of sub-boreal forest soils. Geoderma **113**:1-16.

Attiwill, P. M., H. B. Guthrie, and R. Leuning. 1978. Nutrient cycling in a *Eucalyptus obliqua* (L'Hérit.) forest. I. Litter production and nutrient return. Australian Journal of Botany **26**:79-91.

Attiwill, P. M., and B. M. May. 2001. Does nitrogen limit the growth of native eucalypt forests: some observations for mountain ash (*Eucalyptus regnans*). Marine and Freshwater Research **52**:111-117.

Bååth, E., Å. Frostegård, T. Pennanen, and H. Fritze. 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biology and Biochemistry **27**:229-240.

Badía, D., S. López-García, C. Martí, O. Ortíz-Perpiñá, A. Girona-García, and J. Casanova-Gascón. 2017. Burn effects on soil properties associated to heat transfer under contrasting moisture content. Science of the Total Environment **601-602**:1119-1128.

Badía, D., and C. Martí. 2003. Plant ash and heat intensity effects on chemical and physical properties of two contrasting soils. Arid Land Research and Management **17**:23-41.

Baker, A. C., and B. R. Murray. 2012. Seasonal intrusion of litterfall from non-native pine plantations into surrounding native woodland: implications for management of an invasive plantation species. Forest Ecology and Management **277**:25-37.

Baker, T. G., and P. M. Attiwill. 1985. Above-ground nutrient distribution and cycling in *Pinus radiata* D. Don and *Eucalyptus obliqua* L'Hérit. forests in southeastern Australia. Forest Ecology and Management **13**:41-52.

Baldock, J. A., B. Hawke, J. Sanderman, and L. M. MacDonald. 2013. Predicting contents of carbon and its component fractions in Australian soils from diffuse reflectance mid-infrared spectra. Soil Research **51**:577-595.

Baldock, J. A., and R. J. Smernik. 2002. Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. Organic Geochemistry **33**:1093-1109.

Bárcenas-Moreno, G., and E. Bååth. 2009. Bacterial and fungal growth in soil heated at different temperatures to simulate a range of fire intensities. Soil Biology and Biochemistry **41**:2517-2526.

Barreiro, A., E. Bååth, and M. Díaz-Raviña. 2016. Bacterial and fungal growth in burnt acid soils amended with different high C/N mulch materials. Soil Biology and Biochemistry **97**:102-111.

Bárta, J., T. Melichová, D. Vaněk, T. Picek, and H. Šantrůčková. 2010. Effect of pH and dissolved organic matter on the abundance of *nirK* and *nirS* denitrifiers in spruce forest soil. Biogeochemistry **101**:123-132.

Bastias, B. A., Z. Q. Huang, T. Blumfield, Z. Xu, and J. W. G. Cairney. 2006. Influence of repeated prescribed burning on the soil fungal community in an eastern Australian wet sclerophyll forest. Soil Biology and Biochemistry **38**:3492-3501.

Bauhus, J., P. K. Khanna, and R. J. Raison. 1993. The effect of fire on carbon and nitrogen mineralization and nitrification in an Australian forest soil. Australian Journal of Soil Research **31**:621-639.

Bennett, L. T., C. Aponte, T. G. Baker, and K. G. Tolhurst. 2014. Evaluating long-term effects of prescribed fire regimes on carbon stocks in a temperate eucalypt forest. Forest Ecology and Management **328**:219-228.

Berkinshaw, T. 2009. Mangroves to mallee: the complete guide to the vegetation of temperate South Australia. Finsbury Green, Adelaide.

Bird, M. I., A. V. McBeath, P. L. Ascough, V. A. Levchenko, C. M. Wurster, N. C. Munksgaard, R. J. Smernik, and A. Williams. 2017. Loss and gain of carbon during char degradation. Soil Biology and Biochemistry **106**:80-89.

Bird, M. I., E. M. Veenendaal, C. Moyo, J. Lloyd, and P. Frost. 2000. Effect of fire and soil texture on soil carbon in a sub-humid savanna (Matopos, Zimbabwe). Geoderma **94**:71-90.

Blagodatskaya, E., M. Dannenmann, R. Gasche, and K. Butterbach-Bahl. 2010. Microclimate and forest management alter fungal-to-bacterial ratio and N2O-emission during rewetting in the forest floor and mineral soil of mountainous beech forests. Biogeochemistry **97**:55-70.

Blank, R. R., J. C. Chambers, and D. Zamudio. 2003. Restoring riparian corridors with fire: effects on soil and vegetation. Journal of Range Management **56**:388-396.

Bodí, M. B., D. A. Martin, V. N. Balfour, C. Santín, S. H. Doerr, P. Pereira, A. Cerdà, and J. Mataix-Solera. 2014. Wildland fire ash: production, composition and eco-hydro-geomorphic effects. Earth-Science Reviews **130**:103-127.

Bonanomi, G., F. De Filippis, G. Cesarano, A. La Storia, M. Zotti, S. Mazzoleni, and G. Incerti. 2019. Linking bacterial and eukaryotic microbiota to litter chemistry: Combining next generation sequencing with 13 C CPMAS NMR spectroscopy. Soil Biology and Biochemistry **129**:110-121.

Bonanomi, G., G. Incerti, A. M. Abd El-Gawad, G. Cesarano, T. C. Sarker, L. Saulino, V. Lanzotti, A. Saracino, F. C. Rego, and S. Mazzoleni. 2018. Comparing chemistry and bioactivity of burned vs. decomposed plant litter: different pathways but same result? Ecology **99**:158-171.

Boomsma, C. D. 1949. Phosphate for top dressing as a normal plantation operation. Australian Forestry **13**:108-112.

Bradstock, R. A., K. A. Hammill, L. Collins, and O. Price. 2010. Effects of weather, fuel and terrain on fire severity in topographically diverse landscapes of south-eastern Australia. Landscape Ecology **25**:607-619.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 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.

Brown, J., N. J. Enright, and B. P. Miller. 2003. Seed production and germination in two rare and three common co-occurring *Acacia* species from south-east Australia. Austral Ecology **28**:271-280.

Bureau of Meteorology. 2015a. Climate data online.

Bureau of Meteorology. 2015b. Mount Crawford, South Australia April 2015 daily weather observations. Australian Government Bureau of Meteorology.

Bureau of Meteorology. 2015c. Mount Crawford, South Australia February 2015 daily weather observations. Australian Government Bureau of Meteorology.

Bureau of Meteorology. 2015d. Mount Crawford, South Australia January 2015 daily weather observations. Australian Government Bureau of Meteorology.

Bureau of Meteorology. 2015e. Mount Crawford, South Australia March 2015 daily weather observations. Australian Government Bureau of Meteorology.

Butterly, C. R., E. K. Bünemann, A. M. McNeill, J. A. Baldock, and P. Marschner. 2009. Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biology and Biochemistry **41**:1406-1416.

Butterly, C. R., P. Marschner, A. M. McNeill, and J. A. Baldock. 2010. Rewetting CO_2 pulses in Australian agricultural soils and the influence of soil properties. Biology and Fertility of Soils **46**:739-753.

Caon, L., V. R. Vallejo, R. J. Coen, and V. Geissen. 2014. Effects of wildfire on soil nutrients in Mediterranean ecosystems. Earth-Science Reviews **139**:47-58.

Carrasco, B., A. Cabaneiro, and I. Fernandez. 2017. Exploring potential pine litter biodegradability as a natural tool for low-carbon forestry. Forest Ecology and Management **401**:166-176.

Carreira, J. A., F. X. Niell, and K. Lajtha. 1994. Soil nitrogen availability and nitrification in Mediterranean shrublands of varying fire history and successional stage. Biogeochemistry **26**:189-209.

Castelli, L. M., and M. A. Lazzari. 2002. Impact of fire on soil nutrients in central semiarid Argentina. Arid Land Research and Management **16**:349-364.

Catalanotti, A. E., E. Giuditta, R. Marzaioli, D. Ascoli, A. Esposito, S. Strumia, S. Mazzoleni, and F. A. Rutigliano. 2018. Effects of single and repeated prescribed burns on soil organic C and microbial activity in a Pinus halepensis plantation of Southern Italy. Applied Soil Ecology **125**:108-116.

Certini, G. 2005. Effects of fire on properties of forest soils: A review. Oecologia **143**:1-10.

Certini, G. 2014. Fire as a soil-forming factor. Ambio **43**:191-195.

Certini, G., C. Nocentini, H. Knicker, P. Arfaioli, and C. Rumpel. 2011. Wildfire effects on soil organic matter quantity and quality in two fire-prone Mediterranean pine forests. Geoderma **167-168**:148-155.

Chao, S. C., D. G. Young, and C. J. Oberg. 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. Journal of Essential Oil Research **12**:639-649.

Chuvieco, E., C. Yue, A. Heil, F. Mouillot, I. Alonso-Canas, M. Padilla, J. M. Pereira, D. Oom, and K. Tansey. 2016. A new global burned area product for climate assessment of fire impacts. Global Ecology and Biogeography **25**:619-629.

Clarke, P. J., K. J. E. Knox, R. A. Bradstock, C. Munoz-Robles, and L. Kumar. 2014. Vegetation, terrain and fire history shape the impact of extreme weather on fire severity and ecosystem response. Journal of Vegetation Science **25**:1033-1044.

Clarke, P. J., M. J. Lawes, B. P. Murphy, J. Russell-Smith, C. E. M. Nano, R. Bradstock, N. J. Enright, J. B. Fontaine, C. R. Gosper, I. Radford, J. J. Midgley, and R. M. Gunton. 2015. A synthesis of postfire recovery traits of woody plants in Australian ecosystems. Science of the Total Environment **534**:31-42.

Close, D. C., N. J. Davidson, P. W. Swanborough, and R. Corkrey. 2011. Does low-intensity surface fire increase water- and nutrient-availability to overstorey *Eucalyptus gomphocephala*? Plant and Soil **349**:203-214.

Cohn, J. S., J. Di Stefano, F. Christie, G. Cheers, and A. York. 2015. How do heterogeneity in vegetation types and post-fire age-classes contribute to plant diversity at the landscape scale? Forest Ecology and Management **346**:22-30.

Colegrave, N., and G. D. Ruxton. 2018. Using biological insight and pragmatism when thinking about pseudoreplication. Trends in Ecology and Evolution **33**:28-35.

Compton, J. E., and R. D. Boone. 2002. Soil nitrogen transformations and the role of light fraction organic matter in forest soils. Soil Biology and Biochemistry **34**:933-943.

Cookson, W. R., D. A. Abaye, P. Marschner, D. V. Murphy, E. A. Stockdale, and K. W. T. Goulding. 2005. The contribution of soil organic matter fractions to carbon and nitrogen mineralization and microbial community size and structure. Soil Biology and Biochemistry **37**:1726-1737.

Cookson, W. R., M. Osman, P. Marschner, D. A. Abaye, I. Clark, D. V. Murphy, E. A. Stockdale, and C. A. Watson. 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. Soil Biology and Biochemistry **39**:744-756.

Crockford, R. H., and D. P. Richardson. 2002. Decomposition of litter in a dry sclerophyll eucalypt forest and a *Pinus radiata* plantation in southeastern Australia. Hydrological Processes **16**:3317-3327.

Dai, X., T. W. Boutton, M. Hailemichael, R. J. Ansley, and K. E. Jessup. 2006. Soil carbon and nitrogen storage in response to fire in a temperate mixed-grass savanna. Journal of Environmental Quality **35**:1620-1628.

de Gonzalo, G., D. I. Colpa, M. H. M. Habib, and M. W. Fraaije. 2016. Bacterial enzymes involved in lignin degradation. Journal of Biotechnology **236**:110-119.

De la Rosa, J. M., J. A. González-Pérez, R. González-Vázquez, H. Knicker, E. López-Capel, D. A. C. Manning, and F. J. González-Vila. 2008. Use of pyrolysis/GC-MS combined with thermal analysis to monitor C and N changes in soil organic matter from a Mediterranean fire affected forest. Catena **74**:296-303.

de Mendiburu, F. 2017. agricolae: statistical procedures for agricultural research.

Dean, S., E. C. Farrer, and E. S. Menges. 2015. Fire effects on soil biogeochemistry in Florida scrubby flatwoods. American Midland Naturalist **174**:49-64.

Demoling, F., D. Figueroa, and E. Bååth. 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biology and Biochemistry **39**:2485-2495.

Díaz-Delgado, R., F. Lloret, and X. Pons. 2003. Influence of fire severity on plant regeneration by means of remote sensing imagery. International Journal of Remote Sensing **24**:1751-1763.

Dixon, R. K., S. Brown, R. A. Houghton, A. M. Solomon, M. C. Trexler, and J. Wisniewski. 1994. Carbon pools and flux of global forest ecosystems. Science **263**:185-190.

Doerner, K. C., and B. P. Mason. 2006. Nutritional deprivation increases intracellular phosphate and polyphosphate in poultry litter microflora. Letters in Applied Microbiology **42**:527-531.

Doolette, A. L., and R. J. Smernik. 2018. Facile decomposition of phytate in the solid-state: Kinetics and decomposition pathways. Phosphorus, Sulfur and Silicon and the Related Elements **193**:192-199.

Doolette, A. L., R. J. Smernik, and T. I. McLaren. 2017. The composition of organic phosphorus in soils of the Snowy Mountains region of south-eastern Australia. Soil Research **55**:10-18.

Dooley, S. R., and K. K. Treseder. 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry **109**:49-61.

Duguy, B., P. Rovira, and R. Vallejo. 2007. Land-use history and fire effects on soil fertility in eastern Spain. European Journal of Soil Science **58**:83-91.

Dunn, R. M., J. Mikola, R. Bol, and R. D. Bardgett. 2006. Influence of microbial activity on plant-microbial competition for organic and inorganic nitrogen. Plant and Soil **289**:321-334.

Durán, J., A. Rodríguez, J. M. Fernández-Palacios, and A. Gallardo. 2009. Changes in net N mineralization rates and soil N and P pools in a pine forest wildfire chronosequence. Biology and Fertility of Soils **45**:781-788.

Elmajdoub, B., P. Marschner, and R. G. Burns. 2014. Addition of glucose increases the activity of microbes in saline soils. Soil Research **52**:568-574.

Farrell, M., T. K. Kuhn, L. M. Macdonald, T. M. Maddern, D. V. Murphy, P. A. Hall, B. P. Singh, K. Baumann, E. S. Krull, and J. A. Baldock. 2013. Microbial utilisation of biochar-derived carbon. Science of the Total Environment **465**:288-297.

Fernandes, P. M., J. A. Vega, E. Jiménez, and E. Rigolot. 2008. Fire resistance of European pines. Forest Ecology and Management **256**:246-255.

Fernandez, I., A. Cabaneiro, and T. Carballas. 2001. Thermal resistance to high temperatures of different organic fractions from soils under pine forests. Geoderma **104**:281-298.

Fernandez-Manso, A., C. Quintano, and D. A. Roberts. 2016. Burn severity influence on post-fire vegetation cover resilience from Landsat MESMA fraction images time series in Mediterranean forest ecosystems. Remote Sensing of Environment **184**:112-123.

Ferreira, A. J. D., C. O. A. Coelho, A. K. Boulet, G. Leighton-Boyce, J. J. Keizer, and C. J. Ritsema. 2005. Influence of burning intensity on water repellency and hydrological processes at forest and shrub sites in Portugal. Australian Journal of Soil Research **43**:327-336.

Ferreira, A. J. D., C. O. A. Coelho, C. J. Ritsema, A. K. Boulet, and J. J. Keizer. 2008. Soil and water degradation processes in burned areas: Lessons learned from a nested approach. Catena **74**:273-285.

Ferreira, R. V., D. Serpa, M. A. Cerqueira, and J. J. Keizer. 2016. Short-time phosphorus losses by overland flow in burnt pine and eucalypt plantations in north-central Portugal: A study at micro-plot scale. Science of the Total Environment **551-552**:631-639.

Flannigan, M. D., M. A. Krawchuk, W. J. De Groot, B. M. Wotton, and L. M. Gowman. 2009. Implications of changing climate for global wildland fire. International Journal of Wildland Fire **18**:483-507.

Florence, R. G., and D. Lamb. 1974. Influence of stand and site on radiata pine litter in South Australia. New Zealand Journal of Forestry Science **4**:205-210.

Fontúrbel, M. T., A. Barreiro, J. A. Vega, A. Martín, E. Jiménez, T. Carballas, C. Fernández, and M. Díaz-Raviña. 2012. Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. Geoderma **191**:51-60.

Forster, J. C. 1995. Soil nitrogen. Pages 79-87 *in* K. Alef and P. Nannipieri, editors. Methods in applied soil microbiology and biochemistry. Academic Press, London.

Frey, S. D., J. Six, and E. T. Elliott. 2003. Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil-litter interface. Soil Biology and Biochemistry **35**:1001-1004.

Fritze, H., T. Pennanen, and V. Kitunen. 1998. Characterization of dissolved organic carbon from burned humus and its effects on microbial activity and community structure. Soil Biology and Biochemistry **30**:687-693.

Giardina, C. P., R. L. Sanford Jr, and I. C. Døckersmith. 2000. Changes in soil phosphorus and nitrogen daring slash-and-burn clearing of a dry tropical forest. Soil Science Society of America Journal **64**:399-405.

Girisha, G. K., L. M. Condron, P. W. Clinton, and M. R. Davis. 2003. Decomposition and nutrient dynamics of green and freshly fallen radiata pine (*Pinus radiata*) needles. Forest Ecology and Management **179**:169-181.

Goberna, M., C. García, H. Insam, M. T. Hernández, and M. Verdú. 2012. Burning fire-prone Mediterranean shrublands: immediate changes in soil microbial community structure and ecosystem functions. Microbial Ecology **64**:242-255.

González-Pérez, J. A., F. J. González-Vila, G. Almendros, and H. Knicker. 2004. The effect of fire on soil organic matter - a review. Environment International **30**:855-870.

Granged, A. J. P., A. Jordán, L. M. Zavala, M. Muñoz-Rojas, and J. Mataix-Solera. 2011. Short-term effects of experimental fire for a soil under *Eucalyptus* forest (SE Australia). Geoderma **167-168**:125-134.

Greene, R. S. B., C. J. Chartres, and K. C. Hodgkinson. 1990. The effects of fire on the soil in a degraded semi-arid woodland. I. Cryptogam cover and physical and micromorphological properties. Australian Journal of Soil Research **28**:755-777.

Guénon, R., M. Vennetier, N. Dupuy, S. Roussos, A. Pailler, and R. Gros. 2013. Trends in recovery of mediterranean soil chemical properties and microbial activities after infrequent and frequent wildfires. Land Degradation and Development **24**:115-128.

Guinto, D. F., P. G. Saffigna, Z. H. Xu, A. P. N. House, and M. C. S. Perera. 1999. Soil nitrogen mineralisation and organic matter composition revealed by ^{13}C NMR spectroscopy under repeated prescribed burning in eucalypt forests of south-east Queensland. Australian Journal of Soil Research **37**:123-135.

Guri, A., P. Kefalas, and V. Roussis. 2006. Antioxidant potential of six pine species. Phytotherapy Research **20**:263-266.

Hamer, U., B. Marschner, S. Brodowski, and W. Amelung. 2004. Interactive priming of black carbon and glucose mineralisation. Organic Geochemistry **35**:823-830.

Hamman, S. T., I. C. Burke, and M. E. Stromberger. 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biology and Biochemistry **39**:1703-1711.

Han, L., K. Sun, J. Jin, and B. Xing. 2016. Some concepts of soil organic carbon characteristics and mineral interaction from a review of literature. Soil Biology and Biochemistry **94**:107-121.

Hättenschwiler, S., and H. B. Jørgensen. 2010. Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. Journal of Ecology **98**:754-763.

Heal, O. W., J. M. Anderson, and M. J. Swift. 1997. 1. Plant litter quality and decomposition: an historical overview. Pages 3-30 *in* G. Cadisch and K. E. Giller, editors. Driven by nature: plant litter quality and decomposition. CABI Publishing, Eastbourne, UK.

Heath, J. T., C. J. Chafer, T. F. A. Bishop, and F. F. Van Ogtrop. 2015. Wildfire effects on soil carbon and water repellency under *Eucalyptus* forest in eastern Australia. Soil Research **53**:13-23.

Hestrin, R., D. Torres-Rojas, J. J. Dynes, J. M. Hook, T. Z. Regier, A. W. Gillespie, R. J. Smernik, and J. Lehmann. 2019. Fire-derived organic matter retains ammonia through covalent bond formation. Nature Communications **10**.

Hilscher, A., K. Heister, C. Siewert, and H. Knicker. 2009. Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. Organic Geochemistry **40**:332-342.

Hinojosa, M. B., A. Parra, D. A. Ramírez, J. A. Carreira, R. García-Ruiz, and J. M. Moreno. 2012. Effects of drought on soil phosphorus availability and fluxes in a burned Mediterranean shrubland. Geoderma **191**:61-69.

Hobley, E. U., A. J. Le Gay Brereton, and B. Wilson. 2017. Forest burning affects quality and quantity of soil organic matter. Science of the Total Environment **575**:41-49.

Hoegh-Guldberg, O., D. Jacob, M. Taylor, M. Bindi, S. Brown, I. Camilloni, A. Diedhiou, R. Djalante, K. Ebi, F. Engelbrecht, J. Guiot, Y. Hijioka, S. Mehrotra, A. Payne, S. I. Seneviratne, A. Thomas, R. Warren, and G. Zhou. 2018. Impacts of 1.5°C global warming on natural and human systems.*in* V. Masson-Delmotte, P. Zhai, H. O. Pörtner,

D. Roberts, J. Skea, P. R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T. Waterfield, editors. Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty.

Holden, S. R., and K. Treseder. 2013. A meta-analysis of soil microbial biomass responses to forest disturbances. Frontiers in Microbiology **4**.

Holloway, J. M., and R. A. Dahlgren. 2002. Nitrogen in rock: occurrences and biogeochemical implications. Global Biogeochemical Cycles **16**.

Hosking, J. S. 1938. The ignition at low temperatures of the organic matter in soils. The Journal of Agricultural Science **28**:393-400.

Hosseini, M., J. J. Keizer, O. G. Pelayo, S. A. Prats, C. Ritsema, and V. Geissen. 2016. Effect of fire frequency on runoff, soil erosion, and loss of organic matter at the micro-plot scale in north-central Portugal. Geoderma **269**:126-137.

Huang, W., Z. Xu, C. Chen, G. Zhou, J. Liu, K. M. Abdullah, F. Reverchon, and X. Liu. 2013. Short-term effects of prescribed burning on phosphorus availability in a suburban native forest of subtropical Australia. Journal of Soils and Sediments **13**:869-876.

Huffman, M. S., and M. D. Madritch. 2018. Soil microbial response following wildfires in thermic oak-pine forests. Biology and Fertility of Soils **54**:985-997.

Hume, A., H. Y. H. Chen, A. R. Taylor, G. J. Kayahara, and R. Man. 2016. Soil C:N:P dynamics during secondary succession following fire in the boreal forest of central Canada. Forest Ecology and Management **369**:1-9.

IPCC. 2014. Climate change 2014: synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland.

Isbell, R. 2002. The Australian soil classification. 2nd edition. CSIRO Publishing, Melbourne.

IUSS Working Group WRB. 2015. World reference base for soil resources. FAO, Rome.

Jansson, J. K., and K. S. Hofmockel. 2018. The soil microbiome — from metagenomics to metaphenomics. Current Opinion in Microbiology **43**:162-168.

Jenkins, M. E., T. L. Bell, J. Norris, and M. A. Adams. 2014. Pyrogenic carbon: the influence of particle size and chemical composition on soil carbon release. International Journal of Wildland Fire **23**:1027-1033.

Jenkins, M. E., T. L. Bell, L. F. Poon, C. Aponte, and M. A. Adams. 2016. Production of pyrogenic carbon during planned fires in forests of East Gippsland, Victoria. Forest Ecology and Management **373**:9-16.

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

Jones, A. G., and N. J. Davidson. 2014. Altered N, P and C dynamics with absence of fire in *Eucalyptus* forests affected by premature decline. Austral Ecology **39**:587-599.

Keeley, J. E. 2009. Fire intensity, fire severity and burn severity: A brief review and suggested usage. International Journal of Wildland Fire **18**:116-126.

Keiluweit, M., P. S. Nico, M. Johnson, and M. Kleber. 2010. Dynamic molecular structure of plant biomass-derived black carbon (biochar). Environmental Science and Technology **44**:1247-1253.

Khanna, P. K., R. J. Raison, and R. A. Falkiner. 1994. Chemical properties of ash derived from *Eucalyptus* litter and its effects on forest soils. Forest Ecology and Management **66**:107-125.

Knicker, H. 2010. "Black nitrogen" - an important fraction in determining the recalcitrance of charcoal. Organic Geochemistry **41**:947-950.
Knicker, H., F. J. González-Vila, O. Polvillo, J. A. González, and G. Almendros. 2005. Fire-induced transformation of C- and N- forms in different organic soil fractions from a Dystric Cambisol under a Mediterranean pine forest (*Pinus pinaster*). Soil Biology and Biochemistry **37**:701-718.
Knicker, H., A. Hilscher, F. J. González-Vila, and G. Almendros. 2008. A new conceptual model for the structural properties of char produced during vegetation fires. Organic Geochemistry **39**:935-939.
Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry **34**:139-162.
Kouno, K., Y. Tuchiya, and T. Ando. 1995. Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biology and Biochemistry **27**:1353-1357.
Koyama, A., K. L. Kavanagh, and K. Stephan. 2010. Wildfire effects on soil gross nitrogen transformation rates in coniferous forests of central Idaho, USA. Ecosystems **13**:1112-1126.
Krishnaraj, S. J., T. G. Baker, P. J. Polglase, L. Volkova, and C. J. Weston. 2016. Prescribed fire increases pyrogenic carbon in litter and surface soil in lowland *Eucalyptus* forests of south-eastern Australia. Forest Ecology and Management **366**:98-105.
Kuzyakov, Y., I. Subbotina, H. Chen, I. Bogomolova, and X. Xu. 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biology and Biochemistry **41**:210-219.
Lal, R. 2005. Forest soils and carbon sequestration. Forest Ecology and Management **220**:242-258.
Lee, K. E., and R. L. Correll. 1978. Litter fall and its relationship to nutrient cycling in a South Australian dry sclerophyll forest. Australian Journal of Ecology **3**:243-252.
Li, A., and T. J. Fahey. 2013. Nitrogen translocation to fresh litter in northern hardwood forest. Ecosystems **16**:521-528.
Li, W., J. M. H. Knops, X. Zuo, and R. Laungani. 2014a. Carbon and nitrogen cycling are resistant to fire in nutrient-poor grassland. Soil Science Society of America Journal **78**:825-831.
Li, W., K. W. Pan, N. Wu, J. C. Wang, Y. J. Wang, and L. Zhang. 2014b. Effect of litter type on soil microbial parameters and dissolved organic carbon in a laboratory microcosm experiment. Plant, Soil and Environment **60**:170-176.
Li, Y., N. Chen, M. E. Harmon, Y. Li, X. Cao, M. A. Chappell, and J. Mao. 2015. Plant species rather than climate greatly alters the temporal pattern of litter chemical composition during long-term decomposition. Scientific Reports **5**.
Lin, G., and D. H. Zeng. 2017. Heterogeneity in decomposition rates and annual litter inputs within fine-root architecture of tree species: Implications for forest soil carbon accumulation. Forest Ecology and Management **389**:386-394.
Liu, G., L. Wang, L. Jiang, X. Pan, Z. Huang, M. Dong, and J. H. C. Cornelissen. 2018. Specific leaf area predicts dryland litter decomposition via two mechanisms. Journal of Ecology **106**:218-229.
Liu, W., W. Xu, J. Hong, and S. Wan. 2010. Interannual variability of soil microbial biomass and respiration in responses to topography, annual burning and N addition in a semiarid temperate steppe. Geoderma **158**:259-267.
Liu, X., C. Chen, W. Wang, J. M. Hughes, T. Lewis, E. Hou, and J. Shen. 2015. Vertical distribution of soil denitrifying communities in a wet sclerophyll forest under long-term repeated burning. Microbial Ecology **70**:993-1003.

Lobert, J. M., D. H. Scharffe, W. M. Hao, and P. J. Grutzen. 1990. Importance of biomass burning in the atmospheric budgets of nitrogen-containing gases. Nature **346**:552-554.

Lu, W., W. Ding, J. Zhang, Y. Li, J. Luo, N. Bolan, and Z. Xie. 2014. Biochar suppressed the decomposition of organic carbon in a cultivated sandy loam soil: a negative priming effect. Soil Biology and Biochemistry **76**:12-21.

Ma, B., K. Zhao, X. Lv, W. Su, Z. Dai, J. A. Gilbert, P. C. Brookes, K. Faust, and J. Xu. 2018. Genetic correlation network prediction of forest soil microbial functional organization. ISME Journal **12**:2492-2505.

Ma, L., X. Rao, P. Lu, S. H. Bai, Z. Xu, X. Chen, T. Blumfield, and J. Xie. 2015. Ecophysiological and foliar nitrogen concentration responses of understorey *Acacia* spp. and *Eucalyptus* sp. to prescribed burning. Environmental Science and Pollution Research **22**:10254-10262.

Ma, Q., Z. Wu, F. Pan, J. Wang, H. Zhou, C. Jiang, Y. Xu, and W. Yu. 2016. Effect of glucose addition on the fate of urea-^{15}N in fixed ammonium and soil microbial biomass N pools. European Journal of Soil Biology **75**:168-173.

Mabuhay, J. A., Y. Isagi, and N. Nakagoshi. 2006. Wildfire effects on microbial biomass and diversity in pine forests at three topographic positions. Ecological Research **21**:54-63.

Martínez, A., A. Larrañaga, J. Pérez, A. Basaguren, and J. Pozo. 2013. Leaf-litter quality effects on stream ecosystem functioning: a comparison among five species. Fundamental and Applied Limnology **183**:239-248.

Mataix-Solera, J., A. Cerdà, V. Arcenegui, A. Jordán, and L. M. Zavala. 2011. Fire effects on soil aggregation: A review. Earth-Science Reviews **109**:44-60.

McBeath, A. V., R. J. Smernik, and E. S. Krull. 2013. A demonstration of the high variability of chars produced from wood in bushfires. Organic Geochemistry **55**:38-44.

McGroddy, M. E., T. Daufresne, and L. O. Hedin. 2004. Scaling of C:N:P stoichiometry in forests worldwide: implications of terrestrial redfield-type ratios. Ecology **85**:2390-2401.

McLaughlin, M. J., A. M. Alston, and J. K. Martin. 1986. Measurement of phosphorus in the soil microbial biomass: a modified procedure for field soils. Soil Biology and Biochemistry **18**:437-443.

McMullan-Fisher, S. J. M., T. W. May, R. M. Robinson, T. L. Bell, T. Lebel, P. Catcheside, and A. York. 2011. Fungi and fire in Australian ecosystems: a review of current knowledge, management implications and future directions. Australian Journal of Botany **59**:70-90.

Meaney, K. J. 2017. Proterozoic crustal growth in the southeastern Gawler Craton; the development of the Barossa Complex, and an assessment of the detrital zircon method. The University of Adelaide, Adelaide.

Merino, A., B. Chávez-Vergara, J. Salgado, M. T. Fonturbel, F. García-Oliva, and J. A. Vega. 2015. Variability in the composition of charred litter generated by wildfire in different ecosystems. Catena **133**:52-63.

Miranda, K. M., M. G. Espey, and D. A. Wink. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide - Biology and Chemistry **5**:62-71.

Moore, J. M., S. Klose, and M. A. Tabatabai. 2000. Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biology and Fertility of Soils **31**:200-210.

Muqaddas, B., X. Zhou, T. Lewis, C. Wild, and C. Chen. 2015. Long-term frequent prescribed fire decreases surface soil carbon and nitrogen pools in a wet sclerophyll forest of Southeast Queensland, Australia. Science of the Total Environment **536**:39-47.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta **27**:31-36.

Ng, E. L., A. F. Patti, M. T. Rose, C. R. Schefe, K. Wilkinson, R. J. Smernik, and T. R. Cavagnaro. 2014. Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? Soil Biology and Biochemistry **70**:54-61.

Nguyen, T. T. N., C. Y. Xu, I. Tahmasbian, R. Che, Z. Xu, X. Zhou, H. M. Wallace, and S. H. Bai. 2017. Effects of biochar on soil available inorganic nitrogen: a review and meta-analysis. Geoderma **288**:79-96.

Noble, J. C., D. J. Tongway, M. M. Roper, and W. G. Whitford. 1996. Fire studies in Mallee (*Eucalyptus* spp.) communities of western New South Wales: spatial and temporal fluxes in soil chemistry and soil biology following prescribed fire. Pacific Conservation Biology **2**:398-413.

Orians, G. H., and A. V. Milewski. 2007. Ecology of Australia: the effects of nutrient-poor soils and intense fires. Biological Reviews **82**:393-423.

Oshiki, M., T. Segawa, and S. Ishii. 2018. Nitrogen cycle evaluation (NiCE) chip for simultaneous analysis of multiple N cycle associated genes. Applied and Environmental Microbiology **84**.

Otwell, A. E., A. López García de Lomana, S. M. Gibbons, M. V. Orellana, and N. S. Baliga. 2018. Systems biology approaches towards predictive microbial ecology. Environmental Microbiology **20**:4197-4209.

Paine, C. E. T., T. R. Marthews, D. R. Vogt, D. Purves, M. Rees, A. Hector, and L. A. Turnbull. 2012. How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. Methods in Ecology and Evolution **3**:245-256.

Palese, A. M., G. Giovannini, S. Lucchesi, S. Dumontet, and P. Perucci. 2004. Effect of fire on soil C, N and microbial biomass. Agronomie **24**:47-53.

Penman, T. D., R. P. Kavanagh, D. L. Binns, and D. R. Melick. 2007. Patchiness of prescribed burns in dry sclerophyll eucalypt forests in south-eastern Australia. Forest Ecology and Management **252**:24-32.

Penman, T. D., and A. York. 2010. Climate and recent fire history affect fuel loads in *Eucalyptus* forests: implications for fire management in a changing climate. Forest Ecology and Management **260**:1791-1797.

Pérez-Valera, E., M. Goberna, K. Faust, J. Raes, C. García, and M. Verdú. 2017. Fire modifies the phylogenetic structure of soil bacterial co-occurrence networks. Environmental Microbiology **19**:317-327.

Pérez-Valera, E., M. Goberna, and M. Verdú. 2019. Fire modulates ecosystem functioning through the phylogenetic structure of soil bacterial communities. Soil Biology and Biochemistry **129**:80-89.

Pérez-Valera, E., M. Verdú, J. A. Navarro-Cano, and M. Goberna. 2018. Resilience to fire of phylogenetic diversity across biological domains. Molecular Ecology **27**:2896-2908.

Pingree, M. R. A., and L. N. Kobziar. 2019. The myth of the biological threshold: a review of biological responses to soil heating associated with wildland fire. Forest Ecology and Management **432**:1022-1029.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2018. nlme: linear and nonlinear mixed effects models. R Foundation for Statistical Computing, Vienna, Austria.

Pluchon, N., A. G. Vincent, M. J. Gundale, M. C. Nilsson, P. Kardol, and D. A. Wardle. 2016. The impact of charcoal and soil mixtures on decomposition and soil microbial communities in boreal forest. Applied Soil Ecology **99**:40-50.

Portillo-Estrada, M., M. Pihlatie, J. F. J. Korhonen, J. Levula, A. K. F. Frumau, A. Ibrom, J. J. Lembrechts, L. Morillas, L. Horváth, S. K. Jones, and Ü. Niinemets. 2016. Climatic controls on leaf litter decomposition across European forests and grasslands revealed by reciprocal litter transplantation experiments. Biogeosciences **13**:1621-1633.

Prendergast-Miller, M. T., A. B. de Menezes, L. M. Macdonald, P. Toscas, A. Bissett, G. Baker, M. Farrell, A. E. Richardson, T. Wark, and P. H. Thrall. 2017. Wildfire impact: natural

experiment reveals differential short-term changes in soil microbial communities. Soil Biology and Biochemistry **109**:1-13.
Prieto-Fernández, A., M. J. Acea, and T. Carballas. 1998. Soil microbial and extractable C and N after wildfire. Biology and Fertility of Soils **27**:132-142.
Prieto-Fernández, A., M. C. Villar, M. Carballas, and T. Carballas. 1993. Short-term effects of a wildfire on the nitrogen status and its mineralization kinetics in an atlantic forest soil. Soil Biology and Biochemistry **25**:1657-1664.
Prober, S. M., I. D. Lunt, and K. R. Thiele. 2008. Effects of fire frequency and mowing on a temperate, derived grassland soil in south-eastern Australia. International Journal of Wildland Fire **17**:586-594.
Quilliam, R. S., H. C. Glanville, S. C. Wade, and D. L. Jones. 2013. Life in the 'charosphere' - does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biology and Biochemistry **65**:287-293.
R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Raison, R. J., P. K. Khanna, and P. V. Woods. 1985. Mechanisms of element transfer to the atmosphere during vegetation fires. Canadian Journal of Forest Research **15**:132-140.
Reyes, O., J. García-Duro, and J. Salgado. 2015. Fire affects soil organic matter and the emergence of *Pinus radiata* seedlings. Annals of Forest Science **72**:267-275.
Richardson, D. M., R. M. Cowling, and D. C. Le Maitre. 1990. Assessing the risk of invasive success in *Pinus* and *Banksia* in South African mountain fynbos. Journal of Vegetation Science **1**:629-642.
Rodríguez, J., J. A. González-Pérez, A. Turmero, M. Hernández, A. S. Ball, F. J. González-Vila, and M. E. Arias. 2018. Physico-chemical and microbial perturbations of Andalusian pine forest soils following a wildfire. Science of the Total Environment **634**:650-660.
Rodríguez, J., J. A. González-Pérez, A. Turmero, M. Hernández, A. S. Ball, F. J. González-Vila, and M. Enriqueta Arias. 2017. Wildfire effects on the microbial activity and diversity in a Mediterranean forest soil. Catena **158**:82-88.
Romanyà, J., P. K. Khanna, and R. J. Raison. 1994. Effects of slash burning on soil phosphorus fractions and sorption and desorption of phosphorus. Forest Ecology and Management **65**:89-103.
Ruiz-González, A. D., and J. G. Álvarez-González. 2011. Canopy bulk density and canopy base height equations for assessing crown fire hazard in *Pinus radiata* plantations. Canadian Journal of Forest Research **41**:839-850.
Sacchetti, G., S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice, and R. Bruni. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry **91**:621-632.
Saha, B. K., M. T. Rose, V. Wong, T. R. Cavagnaro, and A. F. Patti. 2017. Hybrid brown coal-urea fertiliser reduces nitrogen loss compared to urea alone. Science of the Total Environment **601-602**:1496-1504.
Sawyer, R., R. Bradstock, M. Bedward, and R. J. Morrison. 2018. Fire intensity drives post-fire temporal pattern of soil carbon accumulation in Australian fire-prone forests. Science of the Total Environment **610-611**:1113-1124.
Schank, J. C., and T. J. Koehnle. 2009. Pseudoreplication is a pseudoproblem. Journal of Comparative Psychology **123**:421-433.
Schmidt, M. W. I., and A. G. Noack. 2000. Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. Global Biogeochemical Cycles **14**:777-793.

Schulten, H. R., and M. Schnitzer. 1998. The chemistry of soil organic nitrogen: a review. Biology and Fertility of Soils **26**:1-15.

Seifert, T., M. Meincken, and B. O. Odhiambo. 2017. The effect of surface fire on tree ring growth of *Pinus radiata* trees. Annals of Forest Science **74**.

Selosse, M. A., M. P. Dubois, and N. Alvarez. 2009. Do *Sebacinales* commonly associate with plant roots as endophytes? Mycological Research **113**:1062-1069.

Serra-Diaz, J. M., C. Maxwell, M. S. Lucash, R. M. Scheller, D. M. Laflower, A. D. Miller, A. J. Tepley, H. E. Epstein, K. J. Anderson-Teixeira, and J. R. Thompson. 2018. Disequilibrium of fire-prone forests sets the stage for a rapid decline in conifer dominance during the 21st century. Scientific Reports **8**.

Serrasolsas, I., and P. K. Khanna. 1995. Changes in heated and autoclaved forest soils of S.E. Australia. I. Carbon and nitrogen. Biogeochemistry **29**:3-24.

Shen, J. P., C. R. Chen, and T. Lewis. 2016. Long term repeated fire disturbance alters soil bacterial diversity but not the abundance in an Australian wet sclerophyll forest. Scientific Reports **6**.

Shi, A., and P. Marschner. 2014. Changes in microbial biomass C, extractable C and available N during the early stages of decomposition of residue mixtures. Soil Research **52**:366-372.

Sinsabaugh, R. L. 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry **42**:391-404.

Smith, M., P. Conte, A. E. Berns, J. R. Thomson, and T. R. Cavagnaro. 2012. Spatial patterns of, and environmental controls on, soil properties at a riparian-paddock interface. Soil Biology and Biochemistry **49**:38-45.

Smith, M. E., J. M. Facelli, and T. R. Cavagnaro. 2018. Interactions between soil properties, soil microbes and plants in remnant-grassland and old-field areas: a reciprocal transplant approach. Plant and Soil **433**:127-145.

Smits, M. M., S. Bonneville, L. G. Benning, S. A. Banwart, and J. R. Leake. 2012. Plant-driven weathering of apatite - the role of an ectomycorrhizal fungus. Geobiology **10**:445-456.

Stanley, C. E., G. Grossmann, X. CasadevallI Solvas, and A. J. DeMello. 2016. Soil-on-a-Chip: Microfluidic platforms for environmental organismal studies. Lab on a Chip **16**:228-241.

Stevens-Rumann, C. S., K. B. Kemp, P. E. Higuera, B. J. Harvey, M. T. Rother, D. C. Donato, P. Morgan, and T. T. Veblen. 2018. Evidence for declining forest resilience to wildfires under climate change. Ecology Letters **21**:243-252.

Stevenson, F. J., and M. A. Cole. 1999. The phosphorus cycle. Pages 279-329 Cycles of Soil. John Wiley & Sons, Inc., New York, Chichester, Weinheim, Brisbane, Toronto, Singapore.

Stirling, E. 2019. Nutrient Cycling Between Litters and Soil after Fire in Native Woodland and *Pinus radiata* Plantations. The University of Adelaide, Adelaide, South Australia.

Stirling, E., L. M. Macdonald, R. J. Smernik, and T. R. Cavagnaro. 2019a. Post fire litters are richer in water soluble carbon and lead to increased microbial activity. Applied Soil Ecology **136**:101-105.

Stirling, E., R. J. Smernik, L. M. Macdonald, and T. R. Cavagnaro. 2019b. The effect of fire affected Pinus radiata litter and char addition on soil nitrogen cycling. Science of the Total Environment **664**:276-282.

Stoof, C. R., A. I. Gevaert, C. Baver, B. Hassanpour, V. L. Morales, W. Zhang, D. Martin, S. K. Giri, and T. S. Steenhuis. 2016. Can pore-clogging by ash explain post-fire runoff? International Journal of Wildland Fire **25**:294-305.

Sun, H., M. Santalahti, J. Pumpanen, K. Köster, F. Berninger, T. Raffaello, F. O. Asiegbu, and J. Heinonsalo. 2016. Bacterial community structure and function shift across a northern boreal forest fire chronosequence. Scientific Reports **6**.

Sun, H., M. Santalahti, J. Pumpanen, K. Köster, F. Berninger, T. Raffaello, A. Jumpponen, F. O. Asiegbu, and J. Heinonsalo. 2015a. Fungal community shifts in structure and function across a boreal forest fire chronosequence. Applied and Environmental Microbiology **81**:7869-7880.

Sun, Q., W. S. Meyer, G. R. Koerber, and P. Marschner. 2015b. Response of respiration and nutrient availability to drying and rewetting in soil from a semi-arid woodland depends on vegetation patch and a recent wildfire. Biogeosciences **12**:5093-5101.

Toberman, H., C. Chen, T. Lewis, and J. J. Elser. 2014. High-frequency fire alters C : N : P stoichiometry in forest litter. Global Change Biology **20**:2321-2331.

Tongway, D. J., and K. C. Hodgkinson. 1992. The effects of fire on the soil in a degraded semi-arid woodland. III. Nutrient pool sizes, biological activity and herbage response. Australian Journal of Soil Research **30**:17-26.

Trehan, R., G. L. Kad, R. Jindal, K. Lal, and S. Arora. 2004. Thermal, spectral and morphological studies on cellulose diisoamylaminophosphate in air. Indian Journal of Chemical Technology **11**:213-219.

Turner, J., M. Lambert, V. Jurskis, and H. Bi. 2008. Long term accumulation of nitrogen in soils of dry mixed eucalypt forest in the absence of fire. Forest Ecology and Management **256**:1133-1142.

Ulery, A. L., R. C. Graham, and C. Amrhein. 1993. Wood-ash composition and soil ph following intense burning. Soil Science **156**:358-364.

Updyke, K. M., T. B. Nguyen, and S. A. Nizkorodov. 2012. Formation of brown carbon via reactions of ammonia with secondary organic aerosols from biogenic and anthropogenic precursors. Atmospheric Environment **63**:22-31.

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

Vázquez, F. J., M. J. Acea, and T. Carballas. 1993. Soil microbial populations after wildfire. FEMS Microbiology Ecology **13**:93-103.

Volkova, L., and C. J. Weston. 2015. Carbon loss from planned fires in southeastern Australian dry *Eucalyptus* forests. Forest Ecology and Management **336**:91-98.

Wagg, C., S. F. Bender, F. Widmer, and M. G. A. Van Der Heijden. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America **111**:5266-5270.

Walkley, A., and I. A. Black. 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.

Wan, S., D. Hui, and Y. Luo. 2001. Fire effects on nitrogen pools and dynamics in terrestrial ecosystems: a meta-analysis. Ecological Applications **11**:1349-1365.

Wang, J., Z. Xiong, and Y. Kuzyakov. 2016. Biochar stability in soil: meta-analysis of decomposition and priming effects. GCB Bioenergy **8**:512-523.

Wang, Q. K., M. C. Zhong, and S. L. Wang. 2012. A meta-analysis on the response of microbial biomass, dissolved organic matter, respiration, and N mineralization in mineral soil to fire in forest ecosystems. Forest Ecology and Management **271**:91-97.

Wang, Y., Z. Xu, and Q. Zhou. 2014. Impact of fire on soil gross nitrogen transformations in forest ecosystems. Journal of Soils and Sediments **14**:1030-1040.

Wanthongchai, K., J. Bauhus, and J. G. Goldammer. 2008. Nutrient losses through prescribed burning of aboveground litter and understorey in dry dipterocarp forests of different fire history. Catena **74**:321-332.

Weston, C. J., and P. M. Attiwill. 1990. Effects of fire and harvesting on nitrogen transformations and ionic mobility in soils of *Eucalyptus regnans* forests of south-eastern Australia. Oecologia **83**:20-26.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

Williams, M. C., and G. M. Wardle. 2007. Pine and eucalypt litterfall in a pine-invaded eucalypt woodland: the role of fire and canopy cover. Forest Ecology and Management **253**:1-10.

Xiong, Y., H. Zeng, H. Xia, and D. Guo. 2014. Interactions between leaf litter and soil organic matter on carbon and nitrogen mineralization in six forest litter-soil systems. Plant and Soil **379**:217-229.

Zhalnina, K., K. Zengler, D. Newman, and T. R. Northen. 2018. Need for laboratory ecosystems to unravel the structures and functions of soil microbial communities mediated by chemistry. mBio **9**.

Zheng, J., J. Chen, G. Pan, X. Liu, X. Zhang, L. Li, R. Bian, K. Cheng, and Z. Jinwei. 2016. Biochar decreased microbial metabolic quotient and shifted community composition four years after a single incorporation in a slightly acid rice paddy from southwest China. Science of the Total Environment **571**:206-217.

Zhou, Y., S. Wang, H. Lu, L. Xie, and D. Xiao. 2010. Forest soil heterogeneity and soil sampling protocols on limestone outctops: example from SW China. Acta Carsologica **39**:115-122.