Fire regime, climate and logging impacts on soil carbon in fire-prone forests of S. E. Australia

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Fire Regime, Climate and Logging Impacts on Soil Carbon in Fire-prone Forests of S.E. Australia.

A thesis submitted in fulfilment of the requirements for the degree

DOCTOR OF PHILOSOPHY

from

University of Wollongong

by

Robert B. Sawyer, BSc. Hon.

Submitted December, 2017
Certification

I, Robert Sawyer, declare that this thesis, submitted in partial fulfillment for the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other institution.

Robert B. Sawyer
15th December 2017
Statement of Candidate Contribution

Three of the four data chapters (i.e., chapters 3, 4 and 5) presented in this thesis have been prepared as manuscripts in collaboration with several co-authors. The contribution of each author is as follows:

- Chapters 3 & 4 - Robert Sawyer (70%), Ross Bradstock (15%), Michael Bedward (10%), R. John Morrison (5%);
- Chapter 5 - Robert Sawyer (55%), Ross Bradstock (15%), Michael Bedward (5%), Luke Collins (5%), Bronwyn Horsey (5%), Trent Penman (15%).
Abstract

Climatic change will potentially have wide ranging consequences for the impact of fire regimes on soil carbon pools in fire prone forests. Shifts in fire regimes are likely to result both from changing weather conditions and anthropogenic responses. Global change may require increased use of prescribed burning by land managers to mitigate the potential losses from an increased likelihood of wildfires. Such a change in management may entail trade-offs in other management goals such as maintenance of carbon stocks. Knowledge of the role of fire regimes in determining soil C concentrations is vital for predicting the consequences of changes to climate and land management options, as each of these processes will alter fire regimes. A series of studies in SE Australian eucalypt forests was carried out to provide a basis for evaluating how stocks of soil C may respond to coupled changes in climate, fire regimes and management. Specifically, the following studies were done to investigate how soil C concentration responds to fire regimes, climate and other disturbances:

1. Time since fire (TSF) following fires of different intensity i.e., prescribed fires (TSF up to 43 years) and wildfires (TSF up to 38 years);
2. Fire frequency (one, two and four fires in 30 years with TSF fixed at about 10 years) and variation in climate based on mean annual temperature (MAT) and mean annual precipitation (MAP);
3. The impacts of four fire severity combinations from two successive wildfires with TSF fixed. The severity classes were: low (fire confined to the understorey), and high (fire causing substantial crown damage) and the combinations were low low, low high, high low and high high.
4. Effects of experimental combinations of timber harvesting and varying frequencies of low intensity prescribed fires.

In all four studies the total C concentration in the mineral soil was estimated as a % by weight (%C_Tot). Recalcitrant pyrogenic C (%RPC) was estimated from C surviving a dilute Nitric acid and Hydrogen Peroxide digestion for experiments 1, 2 and 3. Total soil N expressed as % by weight (%N_Tot) was also estimated for experiment 4.

A positive effect of TSF on %C_Tot was apparent in sites last burnt by wildfire more than 20 years previously, contrasting to prescribed fire which showed no effect as TSF increased. Climate had a stronger effect on %C_Tot than either fire frequency or intensity
combinations of successive fires, with higher $\%C_{\text{Tot}}$ evident under cool and wet conditions. Relatively small fire frequency effects were climate region dependent. $\%C_{\text{Tot}}$ was higher after successive fires of low then high intensity compared with other intensity combinations. RPC was effectively a constant fraction of the $C_{\text{Tot}}$, irrespective of TSF, fire frequency and fire intensity. This questions how persistent RPC is in the landscape. Neither, timber harvesting or increased frequency of low intensity fire significantly impacted $\%C_{\text{Tot}}$. By contrast $\%N_{\text{Tot}}$ showed a significant decline with increasing fire frequency in sites without timber harvesting. The response of $\%N_{\text{Tot}}$ in harvested sites, as a function of increasing fire frequency, was non-linear. Sites experiencing one or two post harvest fires had the highest $\%N_{\text{Tot}}$ but this declined quickly with additional fires reaching a minimum for sites with 4 or more post harvest burns. C:N ratios responded strongly to very high frequencies of low intensity fire, reaching values around 60, which may be sufficient to retard ecosystem function through nutrient immobilisation.

Overall trends in C and N metrics were discussed in terms of theoretical interplay between net primary productivity, vegetation decomposition and soil respiration and the way they were affected by climate, fire and timber harvesting, although these parameters were not directly quantified.

It was concluded that soil C (either total or recalcitrant fractions) was unlikely to be strongly affected by wide variations in fire regimes in these forests. The divergent response to fire type (wild fire or prescribed fire) in $\%C_{\text{Tot}}$ was only apparent at TSF longer than the average fire return interval for this landscape ($i.e.$, 15 to 20 years). Thus, any attempt to substantially increase C sequestration in soils would require long-term exclusion of fire, the feasibility of which is questionable. Conversely, increased application of prescribed fire or occurrence of wildfires is likely to have negligible impact on soil C stocks in these forests. Soil C may therefore be robust to differing management strategies and future changes to fire regimes driven by climate change. By contrast, there is potential for climate change to strongly affect soil C, with a decline likely under future warmer and drier conditions, though the latter is uncertain.
Acknowledgements

This research has been conducted with the support of the Australian Government Research Training Program Scholarship. I received additional funding through the ARC Discovery project (DP130102576): ‘When fire and water mix: Do CO2-related water savings drive woody plant thickening and fire dynamics in a grassy woodland?’ I would like to thank the NSW Office of Environment and Heritage (OEH) for licencing this research and permitting access to lands under their control (licence number: SL100068). Thank you to the staff of regional offices and depots of OEH, including National Parks and Wildlife Service, for their support and permissions in accessing the lands under their control for the purpose of this research.

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1 General Introduction

1.1 Introduction

The mitigation of global climate change by offsetting greenhouse gas (GHG) emissions from human activity has become a key objective for environmental management in recent decades. Forest management occupies a substantive role in achieving these objectives, via the goals of increasing or maintaining terrestrial biomass. Carbon (C) and nitrogen (N) based GHGs are captured from the atmosphere and fixed into biomass by autotrophic organisms. Perturbation of terrestrial vegetation systems and heterotrophic respiration release GHGs back to the atmosphere at varying rates dependent on the intensity and type of disturbance. Managing this flux cycle to maximise biological fixation of GHG, and minimising the efflux of GHG back to the atmosphere, is the overarching strategy for land management mitigation of climate change (Santín et al., 2016, Bradshaw et al., 2013, Hurteau and Brooks, 2011). Improving the understanding of the impact of disturbance regimes and climate shifts on terrestrial C and N pools is critical for making informed decisions that maximise C and N sequestration.

The metric of ecosystem C sequestration potential (carbon carrying capacity) discounts the importance of anthropogenic influence in modern disturbance regimes. “Carbon carrying capacity (CCC) is defined as the mass of carbon able to be stored in a forest ecosystem under prevailing environmental conditions and natural disturbance regimes, but excluding anthropogenic disturbance” (Gupta & Rao 1994 cited in: Roxburgh et al., 2006 p.1150). Disturbance regimes are an integral part of life in forests and woodlands, with vegetation fires having duplicitous roles as a management tool (prescribed fires) and as an unplanned impact through wildfire (Bowman et al., 2011). Both fire types release GHGs into atmosphere including, carbon dioxide (CO$_2$), carbon monoxide (CO), methane (CH$_4$) and nitrogen oxides (NO$_x$). Biomass burning represents around 40% of global emissions of CO (Arneth et al., 2010) and 15% of NO$_x$ emissions (Arneth et al., 2010). Not only do fires release GHGs from aboveground biomass, they also shift the form and location of the C in organic matter within the plant soil complex. In the post-fire environment surviving organic matter includes living plants, newly killed plant...
tissue, charred litter and a range of more extensively carbonised pyrogenic products (Santín et al., 2012, de la Rosa and Knicker, 2011). Some forms of pyrogenic C (PyC: produced by fire) are resistant to further oxidation and these may form a buffer in the flux from ecosystem to the atmosphere.

As previously mentioned, wildfire is a common disturbance in many forest and woodland ecosystems worldwide; with those in Australia considered to be particularly fire-prone (Pausas and Keeley, 2009, Kemp, 1981). Vegetation fires have been part of the landscape since a time when plant cover was sufficient to sustain them (Bond, 2010, Pausas and Keeley, 2009, Glasspool et al., 2004) and atmospheric oxygen was sufficient to sustain ignition (>13% oxygen in atmosphere: Jones and Chaloner, 1991) in the Silurian ca. 400 My (Ball, 2014). Consequently, the woody vegetation of Australia has become highly adapted to regimes of relatively frequent fire (Mooney et al., 2012, Ashton, 1981).

Plant groups exhibit many complexities and subtleties of adaptations to, and responsive interactions with, fire regimes (Keith et al., 2007, Pausas and Bradstock, 2007, Christensen et al., 1981). The fecundity and survivorship of different plants experiencing the same fire regime is dependent on the nature of their specific fire adaptations (Moreira et al., 2014). From a biodiversity perspective in land management, it is generally accepted that the use of fire should be applied with intelligence and sophistication to maximise the expression of life forms in the area under management (Bradstock et al., 2012a). Aside from biodiversity, fire response traits have had a direct bearing on biomass recovery and resilience to shifts in fire regime and climate change (Backnäs et al., 2012). Much work is still needed to identify and better understand the mechanisms and pathways that drive biodiversity and biomass storage relating to fire regimes.

1.2 Carbon emissions and vegetation fires

Globally, it has been estimated that 148.8 M km² of vegetation was affected by fire between 1997 and 2016, resulting in the emission of 2.2 Pg C to the atmosphere (Van Der Werf et al., 2017). By comparison, this represented 23% of the 2014 global
emissions from burning fossil fuels and cement production (Boden et al., 2017). It is important to acknowledge that there is substantial temporal and spatial variation in estimates of the area burnt and mass of emissions (Van Der Werf et al., 2017). A portion of the C in vegetation affected by fire is converted to PyC, the production of which is estimated to be ca. 116–385 Tg C yr⁻¹ (Santín et al., 2016).

Fire in “natural systems” is currently only partly considered in the global C accounting systems (Hurteau and North, 2009). Emissions from fires are often treated as “natural” phenomena however in the light of the high number of ignitions from anthropogenic sources in many fire-prone ecosystems, it is difficult to sustain this argument (Doerr and Santín, 2016, Santín et al., 2012, Bowman et al., 2011, Bowman et al., 2009). While the C in natural systems comes from the atmosphere (Anderson-Teixeira and DeLucia, 2011), the rate at which it is cycled and the overall balance of C stocks in each component of the system can be affected by the fire regime. For this reason, it is important to find ways of examining the effect of variable fire regimes on total C storage.

1.3 The importance of belowground flux

Forests and woodlands, including their soil, are substantial stores of C both above and belowground. While the soil pool is potentially the largest C store in forests and woodlands (Bélanger and Pinno, 2008, Beringer et al., 2007, Raison, 1980), it is the least well investigated on both global and local scales (Santín and Doerr, 2016, Bradstock et al., 2012a, González-Pérez et al., 2004). The soil C pool includes inorganic C sources and organic matter sources. In most soil organic matter estimates, C from decaying organic matter, exudates from living organisms and fine root material (i.e., <2 mm) are included (González-Pérez et al., 2004). The below ground C pool may also include estimates of living biomass. Such estimates are usually limited to living root biomass of a particular diameter e.g., Vargas et al. (2008) used an upper limit of 3 mm.

An example from northern Australia found 75% of total ecosystem C resided in the soil pool (Beringer et al., 2007). Soil C flux has often been ignored or only loosely
approximated in the models for emissions/sequestration of C in terrestrial systems (Norris et al., 2010, Hurteau and North, 2009, Vargas et al., 2008). In a study on tropical seasonally dry forests, large differences in recovery time of the aboveground and belowground measures of ecosystem biomass were detected (Vargas et al., 2008). Aboveground recovery took 70 years for biomass and 50 years for C, whilst belowground recovery took < 19 years for both biomass and C (Vargas et al., 2008). This study was a chronosequence of time since fire (TSF) over a 29-year period. The chronosequence was used to estimate the aboveground C recovery rate at 2.5 Mg C ha⁻¹y⁻¹, compared to the belowground rate of 20 Mg C ha⁻¹y⁻¹. Given the size of the soil C pool in the landscape this is an important difference when assessing the GHG implications of fire events.

The form C takes in the soil has a strong influence on ecosystem C longevity and efflux rate to the atmosphere. There are some detailed studies on the relative importance and resistance to oxidation of the different forms of C in the soil (Bird et al., 2015, Saiz et al., 2015, Fang et al., 2014, González-Pérez et al., 2004, Krull et al., 2003). Pyrogenic C, charcoal and soot in particular, as a proportion of the total soil organic matter (SOM) is considered important to the residence time of C in the soil (Santin et al., 2016). The woodiness of vegetative tissues and the conditions under which PyC is formed from them yields C products whose degree of recalcitrance varies widely (see section 7). Nevertheless, the persistence of any organic matter is increased by its incorporation into stable soil (Bradshaw et al., 2013). Investigations that illuminate the size and complexity of the soil C pool and its drivers in natural systems would further enhance the knowledge base informing land management policy.

1.4 Manipulation of fire regime for organic matter sequestration

A current dilemma in forest management is how to assess the impact of fire regime on C cycling. There have been hopes of reducing GHG emissions from the overall ecosystem by using prescribed fires in a systematic way that could result in lower overall biomass consumed by fire (Volkova et al., 2014, Adams, 2013, Gibson et al., 2011). A study by Bradstock et al. (2012a) investigated the parameters under which prescribed burning
could enable C sequestration. This study identified tropical savannah in northern Australia as the only *Eucalyptus* spp. dominated ecosystem where this appeared possible. The “leverage” (Bradstock et al., 2012a, p.630) gained by prescribed fire (the reduction in area of unplanned fire divided by the area of prescribed fire) needed to be close to unity or higher for there to be positive sequestration of C in the ecosystem. Achieving a leverage outcome of this magnitude was shown to be improbable for the temperate forests of south-eastern Australia (Bradstock et al., 2012a, Bradstock et al., 2014). A study in Victorian managed public lands considered the GHG importance of prescribed burning to be negligible, as the extent of these fires was small (Norris et al., 2010). Given very low reductions in the extent of unplanned fires associated with prescribed burning in these forests, the impact on GHGs is likely to be small, but additive, to the unplanned fire flux.

1.4.1 **Fire regime components**

French naturalists first used the concept of fire regime in the 19th Century but it was not until the 1960s that the term was widely adopted by disturbance ecologists in the English speaking scientific community (Krebs et al., 2010). While Krebs et al. (2010) indicated divergent usage and definition of the concept; the common practice was the use of a selection of measurable fire parameters over a sequence of fires. Broadly, fire regimes are considered to consist of the following factors: frequency, intensity, time since fire, spatial extent and seasonality (Bowman et al., 2011, Bradstock, 2008, Gill and Allan, 2008).

Frequency is the number of times an area is affected by fire over a given period. Intensity is the amount of energy released by the fire and is generally expressed as kWm⁻¹ (Luke and McArthur, 1978). This is a very impractical measure for field studies in ecology, so estimates of fire severity are sometimes used as a proxy. Severity is a relatively coarse indicator of the effect of fire intensity on the vegetation (Bowman et al., 2009). Time since fire (TSF) is important in interpreting the post-fire recovery state of ecosystems. In some studies, a short TSF has been equated to high frequency fire and conversely a long TSF has been equated to low frequency fire (e.g., Adams et al., 1994). In such cases, the disparity in TSF can confound attempts to interpret effects of fire frequency. To disentangle effects of TSF from longer term effects of fire frequency an
appropriate study design is required: e.g. sampling across areas which have experienced differing frequencies of fire needs to be conducted at the same TSF. At a landscape scale, fires are often not homogenous in their severity and impact on soils and biota (Bradstock, 2008). The size of severe burn patches has implications for erosion (Bloom et al., 2016), general species survival and post-fire recruitment (Bradstock, 2008, Bradstock et al., 2005). Seasonality is the time of year in which fires occur. This timing has implications for fire intensity and biological activity of individual species and assemblages. In summary, studies that only look at a single fire *i.e.*, burnt or not burnt, are prolific but add little to our knowledge of fire regime effects (e.g., Fang et al., 2015, Maksimova et al., 2014, Smithwick et al., 2012, Blong et al., 1982).

1.5 Fire response traits in vegetation and community composition

Fire regimes have many ecological effects of probable significance in the development of fire-prone and fire-tolerant traits in plants (Pausas and Keeley, 2009). Xeromorphic and particularly sclerophyll vegetation morphology have developed widely in fire-prone ecosystems (Mooney et al., 2012). The search for evolutionary drivers of fire tolerance trait development, initially focused on nutrient status and climatic factors (Christensen, 1985, Beadle, 1966, Loveless, 1962, Loveless, 1961). More recent studies utilising molecular phylogenetic analyses, have attributed selective properties to fire regimes in the development of fire tolerance traits in the Proteaceae (Adams, 2013), *Pinus* (Pausas, 2015) and eucalypts (Attiwill et al., 2014b). It remains difficult to separate exaption (traits that increase a species fire tolerance but are selected by other drivers) from adaptation in relation to evolutionary drivers (Bowman et al., 2011, Bradshaw et al., 2011). Species are not adapted to fire *per se* but rather to a fire regime (Bowman et al., 2011).

The nature of the vegetative tissue influences its flammability at the individual plant scale (Keeley et al., 2011). Three plant scale flammability strategies were identified and represent a departure in thinking from considering flammability as a single entity (Keeley et al., 2011). The non-flammable, fast flammable and hot flammable strategies are alternate response traits to recurrent fire. At the community level all of these strategies maybe expressed (Keeley et al., 2011). The forest and woodlands that are the
subject of this thesis exhibit a mix of fast flammable and hot flammable characteristics, with hard leaves and leaves that contain volatile compounds considered more flammable than those with mesic traits (Grootemaat et al., 2015). More flammable leaves are common in the biota of fire-prone ecosystems (Midgley and Bond, 2013). The lignin:cellulose of litter has a negative relationship to its decomposition rate (Grootemaat et al., 2015, Krebs et al., 2010), as do volatile phenolic compounds (Grootemaat et al., 2015). One of the key differences in plant response traits and fire regime sensitivity is the regeneration mechanisms of individual species. Tree, shrub and herbaceous species are broadly divided into two groups. Non-resprouters which rely entirely on replacement by new individuals germinating from seeds, and resprouters that regenerate foliage from above or belowground buds, increasing survivorship of mature individuals and reducing dependency on seedling success for persistence (Moreira et al., 2014). In particular, variations in ecosystem and biomass resilience following fires may be a function of the resilience traits of dominant, canopy species: i.e. resprouters or non-resprouters (Nolan et al., 2015, Backnäs et al., 2012, Pausas and Keeley, 2009). Resprouters exhibit a rapid rebound in biomass following fires of any intensity (Backnäs et al., 2012). By contrast, non-reprouters suffer high mortality in high intensity fires with longer recovery of biomass which itself may be vulnerable to subsequent fires. Non-resprouter’s recovery trajectory is dependent on seedling recruitment and survivorship to sexual maturity (Backnäs et al., 2012).

1.6 Drivers of total soil carbon

The source of all soil organic C is from the atmosphere via vegetation net primary productivity (NPP) and the subsequent flux to soil (Bradshaw et al., 2013). In the absence of disturbance, the mineral soil C stock is determined by the balance between net community productivity (NCP), litter decomposition rates, soil respiration and the balances of fluxes in organic matter transported in and out of the system via fluvial and aeolian processes (Bradshaw et al., 2013, Roxburgh et al., 2005). Ecosystem disturbance, climatic variables and nutrient availability are all known to have interrelated effects on the components that determine total soil C stocks (Bradshaw et al., 2013).
Disturbance regimes have a strong influence on the amount and nature of organic matter available for flux into the soil. Different disturbance types have contrasting effects on this flux. For example, wind damage will increase the amount of litter but its incorporation into soil will be determined by decomposer activity (Buma et al., 2014). Conversely, fire instantly oxidises organic matter releasing mineralised nutrients and PyC in ash which can be rapidly incorporated into the soil via infiltration and bioturbation (Santín and Doerr, 2016), while gaseous combustion products are immediately returned to the atmosphere. The nature of the disturbance regime, and climatic factors throughout the year, are likely to influence both the necromass pool and its decomposition rate (Penman et al., 2011). The complex and numerous interactions driving soil C flux necessitate careful consideration, as discussed below.

1.6.1 Soil carbon response to fire regimes

Prior studies have found that the level of total soil C including charcoal varies with fire regimes (Gibson et al., 2011, González-Pérez et al., 2004). Amongst the fire regime factors, fire frequency had the greatest bearing on total soil C. Fire intensity also had an influence on the production and consumption of soil charcoal (Gibson et al., 2011, Roxburgh et al., 2006, González-Pérez et al., 2004).

The direct emissions from soils during moving fires are assumed to be small as soil heating effects are known to be confined to the upper most horizons i.e., typically < 50 mm (Mondal and Sukumar, 2014, Bradstock and Auld, 1995, Raison et al., 1986a). Despite the limited size of the direct efflux from soil, there is an increasing demand for better knowledge and quantification of soil C processes as they relate to fire regimes (Ascough et al., 2016, Santín et al., 2016, Bird et al., 2015, Keeley et al., 2012, Gibson et al., 2011, Roxburgh et al., 2006, Steinmann et al., 2004). Therefore, knowledge about the way in which a varying series of fires in landscapes will affect SOM over longer temporal scales, is required to be able to determine long-term fire regime effects.
Standardising TSF within an experiment is important for comparing ecological impacts (Bradstock, 2008); however, the status of C pool sizes may differ at different TSF (Adams et al., 1994, Foote and Grogan, 2010, Kaye et al., 2010, Vargas et al., 2008). It is important to acknowledge this when comparing studies of fire regime effects that have different TSF. When designing studies, testing for the effect of TSF using chronosequences may be a useful tool for interpreting ecosystem resilience to fire and in providing a reference scale for interpreting results in comparison to other studies. Studies that focused on post-fire fuel accumulation estimate that litter fall and decomposition rates approach equivalence *i.e.* steady-state, in south-eastern Australian dry sclerophyll forest (DSF) at *ca.* 10-15 years (Watson and Hulley, 2011, Bridges, 2004, Fox et al., 1979).

When PyC is recalcitrant and has become incorporated into soil, it is unlikely to contribute substantially to GHG emissions (Fang et al., 2014, Santín et al., 2012), although it is uncertain that this PyC reservoir constitutes a global C sink (Ball, 2014). If the recalcitrant PyC flux can be accurately modelled in relation to fire regime (both frequency and severity) this may give scope to land managers for mitigating their GHG emissions. However, it has been argued that the incorporation of SOM into protective soil aggregates is potentially more important to its persistence than the chemical recalcitrance of the SOM (Bradshaw et al., 2013). Understanding the role of fire regimes in the fate of soil C requires models that estimate both the recalcitrant PyC and total C.

**1.6.2 Nutrient availability, litter quality as drivers of decomposition and soil respiration**

There is a complex relationship between nutrient availability and the establishment of vegetation communities that tend to be fire-prone *e.g.*, sclerophyll vegetation (Midgley and Bond, 2013). Within this broad paradigm there are subtle shifts in the balance of nutrient content in forest floor litter and the amount available in the soil matrix. Elevation of the lignin:cellulose of plant tissue can be induced by restricted nutrient availability during growth. The higher lignin content provides greater resistance of litter to decomposition (Krebs et al., 2010). The elevation of C:N in litter and soil has the
potential to immobilise nutrients, via reduced litter decomposition rates, thereby reducing soil nutrient availability (Soong and Cotrufo, 2015). This potentially creates a feedback loop resulting in more decomposition-resistant plant tissues being produced. Conversely, a lack of system perturbation is thought to lower C:N to levels that may be detrimental to individual trees (Turner et al., 2008) although this evidence was not supported in a subsequent study (Turner and Smith, 2016). Ecosystem CCC is positively correlated to nutrient availability e.g. forests and their soils on basalt store more C than those on granite under the same climatic conditions (Orgill et al., 2017).

1.6.3 Timber harvesting and forest thinning as compound disturbance regimes
While fire regimes have an important influence on forest C stocks there are other substantial impacts from natural hazards (e.g., extreme winds) and human management such as timber harvesting and fire fuel reduction (Buma et al., 2014). These disturbances may interact with fire regimes resulting in an alteration to the trajectory of C and N stock recovery in unexpected ways.

There has been some effort to assess the potential of timber harvesting and forest thinning in conjunction with prescribed burning to mitigate potential C losses from severe wildfires (Hurteau et al., 2016, Earles et al., 2014, Hurteau and North, 2009). These studies in mixed conifer forests (predominately obligate seeders) used selective timber harvesting to reset forests to a theoretical 1865 (pre-European settlement) C baseline. The subsequent simulated treatments included: understorey thinning, overstorey thinning and no thinning. A simulated prescribed burning regime was applied in conjunction with the thinning treatments, at the rate of one fire every 20 years over a century. The model included a wildfire event at the end of the century. Direct emissions from the wildfire were less than the total emissions calculated for the prescribed fire, timber harvesting, under scrubbing and thinning treatments in total. Significantly, in untreated plots the mortality of large trees was high, and the C emitted from their subsequent decomposition increased the total wildfire impact to an extent that exceeded the cumulative treatment emissions. The outcome of this study reflected...
the non-sprouting status of overstorey conifer species. Different responses may be anticipated in forests dominated by resprouting tree species, such as *Eucalyptus* spp.

### 1.6.4 Climate influence on carbon carrying capacity

Among the environmental conditions that limit CCC, climate has a substantial effect. In southern Australian cool temperate and warm temperate eucalypt forests, C stocks were consistently higher in living biomass, litter and the top 16 cm of soil compared to all other Australian biomes (Haverd et al., 2013). Net primary productivity was higher in moist tropical biomes, but turnover rates were greater, limiting C accumulation. By contrast in more arid biomes moisture availability severely restricted NPP. Within the two temperate forest classes, the cool climate forest demonstrated longer residence times for C in litter and soil. Total C stocks were highest in the cool forest type. A likely explanation for this is lower decomposition rates in litter and soil, induced by lower temperatures especially in winter (Bradshaw et al., 2013).

### 1.6.5 Erosion and leaching and their effect on soil carbon

The sources and sinks of C subject to fluvial and aeolian displacement are not well studied or quantified. This is particularly the case for PyC (Santín et al., 2016, Bradshaw et al., 2013). Ecosystems in an early TSF state are particularly prone to erosion, leaching and other dispersal forces (Shakesby et al., 2015, Morris et al., 2014). Pyrogenic C production rates were estimated to be between 4% and 28% of the vegetation scorched or consumed by fire (Santín et al., 2015). This contrasted with the relatively small amount observed on or in forest soil, which inferred that a substantial proportion of PyC was not finding its way into the soil profile. Alternatively, if PyC was largely incorporated into soil, then its mean residence time (Santín et al., 2016) must be short, which in turn inferred low recalcitrance. A study by Hobley et al. (2017) assessed soil erosion rates while estimating soil C pools and found low soil erosion in, sandy podsols within a dry sclerophyll forest in eastern Australia. If erosion rates were low and the PyC was highly recalcitrant, the accumulation of PyC in soil from successive fires should have been considerably higher than observed (Jenkins et al., 2016a) and strongly
correlated to fire frequency. The literature evidence is equivocal on this point, necessitating further study if reliable predictions are to be made for a given landscape.

1.6.6 Spatial distribution of soil carbon
There are numerous limitations and difficulties in measuring total C in forest soils due to large spatial heterogeneity, as remarked in numerous studies (García-Palacios et al., 2011, de la Rosa and Knicker, 2011, Merbold et al., 2011, Kaye et al., 2010, Bassett et al., 2017, González-Pérez et al., 2004, Conant et al., 2003, Perruchoud et al., 2000). The sources of spatial variation in the distribution of C are often related to biological activity, e.g., a study of wood ants' nest material showed a significant difference in both C and N storage compared to the surrounding forest floor (Lenoir et al., 2001). The nature of resource gathering and storage by both autotrophic and heterotrophic organisms will dictate the location of both C and N within the soil.

When considering SOM spatial heterogeneity, the use of a stratified and categorical approach to site selection was found to require a much lower sampling intensity than a fully random approach (de la Rosa and Knicker, 2011). This study informed the careful consideration of the biological influences on the distribution of resources within the habitat used to develop a stratified and targeted sampling approach for the work in this current thesis.

It is important to note that even when a stratified approach is used the number of samples required for work on a landscape scale is likely to be high. There are difficulties and considerable expense associated with analysing the total C constituents, particularly charcoal (Kurth et al., 2006, Kavdir et al., 2005). The available analytical techniques, their application and limitations were reviewed by Zimmerman and Mitra (2017), subsequent to the experimental work in this thesis. This highlights that all techniques have their limitations in their ability to quantify PyC. A review of the state of knowledge and available techniques at the time of this study are in section 1.7.
1.7 Charcoal as a component of soil carbon

It is a reasonable expectation that for a given ecosystem type, the proportion of the soil C pool that is in the form of charcoal should vary according to the frequency and severity of fires experienced over time (Roxburgh et al., 2006, González-Pérez et al., 2004). The most common assumption found in the literature is that higher intensity fires ignite more woody material and therefore produce more charcoal (Kavdir et al., 2005). By contrast, higher fire frequency leads to a decline in total soil C (Gibson et al., 2011, Ball et al., 2010, González-Pérez et al., 2004). While useful, these generalisations do not hold universally e.g., in northern Australian tropical savannas recalcitrant PyC formed the greater portion of the C pool exposed to fire in a C₄ dominated vegetation system compared to C₃ vegetation. This difference was attributed to short fire exposure in the finer fuel systems (Saiz et al., 2015).

The factors that may affect charcoal production in a fire are likely to include: the size of the area burnt (which could have a strong relationship to the time allowed for material on the ground to smoulder before being blacked out), the amount of combustible woody material available to be converted to charcoal and the intensity of the fire and its ability to ignite coarse woody material. Regardless of these factors, sites of similar vegetation types that are burnt more often are likely to have a greater charcoal concentration but lower total C (Gibson et al., 2011).

1.7.1 Influence of char formation conditions on recalcitrance

There are several factors that drive the subsequent recalcitrance of PyC. The extent to which the organic matter is charred or consumed is determined by fire residence time and oxygen availability (Saiz et al., 2015). Lightly charred material having similar residence times (decadal) to organic matter that is not charred (Bird et al., 2015, Norwood et al., 2013). The lignin:cellulose is positively correlated to recalcitrance, this property being analogous to the woodiness of the vegetative tissue that was charred (Bird et al., 2015, de la Rosa and Knicker, 2011, Hammes et al., 2007). The formation temperature of the chars and soots is also of importance, with the greatest recalcitrance from formation at > 750 °C relative to low formation temperatures < 450 °C (Norwood
et al., 2013, de la Rosa and Knicker, 2011, Hammes et al., 2007). The most recalcitrant forms of PyC have been shown to persist in soils for centennial or even millennial time scales (Bodi et al., 2014, Adams et al., 1994, Hammes et al., 2007).

1.8 Charcoal analytical techniques

It is only during the past few decades that alternatives to wet digestion chemistry for charcoal analysis have been available (Poirier et al., 2000, Skjemstad et al., 1998). An array of spectroscopic techniques has been developed to estimate and characterise the C content in soils. These include Nuclear Magnetic Resonance (NMR) spectroscopy, Infra-Red (IR) spectroscopic particle modelling estimates and X-ray crystallography. In Australia during this period, NMR and IR modelling have been largely used and mostly applied to agricultural soils (Forouzangohar et al., 2015, Baldock et al., 2013a, Clark Ehlers et al., 2010, Fang et al., 2014, Krull et al., 2003, Skjemstad et al., 2002). In a North American study of soil charcoal in native forests affected by fire regimes Kurth et al. (2006) reverted to using refined wet chemistry digestion, utilising hydrogen peroxide (H2O2) and dilute nitric acid (HNO3), hereafter referred to as the KMD method. This continued use of wet chemistry in ecological work is important because of the ready availability of equipment, expertise and ultimately cost. The uneven distribution of SOM in fire-prone forests necessitates sampling of high numbers of replicates, in comparison to more homogeneous agricultural systems (de la Rosa and Knicker, 2011). A study by Hammes et al. (2007) was conducted as a “ring-trial” between 17 independent laboratories, using a range of analytical techniques for black C determination. Calibration for the degree of overestimation of pyrogenic C associated with each technique was possible, provided an initial sample of material was thoroughly characterised to identify the transition point between interfering material and pyrogenic C. From these data, calibration of any selected method of black C analysis was possible. This calibration process was too costly to be utilised at the time this present thesis study was commenced. The analytical techniques of digestion chemistry and enhanced digestion chemistry were the only methods with the potential to provide quantitative data at the landscape scale without excessive cost.
1.8.1 Digestion chemistry without enhancement

H₂O₂ and dilute HNO₃ digestion achieved a consistent recovery of black C at 80% when soil concentrations of charcoal were in the range of 0.5%-5% (w/w) (Kurth et al., 2006). This was a considerable improvement in recovery over more traditional organic C digestion techniques, such as the Walkley-Black method. The fundamental limitation of the KMD method was the required digestion time of 16 hours. To overcome this limitation, I considered possible enhancement techniques including: sonication, chemical catalysts and microwave digestion. Microwave digestion appeared to offer the most control over reaction temperature and pressure and avoided the need for additional reagents that may have complicated subsequent analyses (Schoenfeld and Michel, 2006, Richter et al., 2001).

1.8.2 Microwave digestion

Traditionally microwave digestion has been used for the aggressive breakdown of organic matter prior to elemental analysis of components (Tatro, 1998, Milestone Microwave Laboratory Systems, 1995). There is no question that microwave digestion provides a far more rapid oxidation of organic matter than other techniques (Schoenfeld and Michel, 2006, Richter et al., 2001). However, there are key limitations such as consistently controlling the rate of the process. In addition, the apparatus available for use in this thesis had a low sample run capacity i.e., 6 samples. Controlling cross contamination required meticulous cleaning and drying of the pressurised sample tubes between each run negating much of the time saved.

1.8.3 Most appropriate recalcitrant carbon assay method

After carefully considering all options, the Kurth et al (2006) KMD method held the most promise for employment in analysis of the type of soil samples likely to be encountered in this project, particularly with the emergence of a technique using a programmable block digester with a high capacity to hold reflux tubes capped with teardrop condensers (Licata and Sanford, 2012). This addressed concerns about the digest running dry, meaning it could be left unattended. The technique required refining to ensure a more even distribution of charcoal in the sub-samples and better residue
recovery. The PyC origin of residue could be verified by electron microscope observation (Poirier et al., 2000).

1.9 Fire history data

This study required quantification of patterns of fire regimes across diverse landscapes, as a pre-requisite for determination of effects on soil C. A combination of good recordkeeping, Geographic Information Systems (GIS) and remote sensing techniques, enabled fire regime analysis with a degree of accuracy and precision for the study area.

1.9.1 Frequency

In the south-eastern Australian State of New South Wales (NSW), there is a comprehensive record of fire occurrence and perimeter mapping since 1967 (NSW fire database). Records, however tend to be more reliable and comprehensive from about 1975. This enables the use of these records to estimate the frequency of recent fires at various spatial scales: i.e. patches, landscapes, and regions.

1.9.2 Severity and Intensity

It is important to make the distinction between fire intensity and fire severity measures (Bowman et al., 2009). As indicated in Section 4, intensity is a measure of energy output from the fire. Severity on the other hand is a measure of the damage caused by the fire to the vegetation. Severity is linked to intensity and considered more practical for historic evaluation of fire events. There are remote sensing techniques in the literature that attempt to estimate either intensity or severity (Godwin and Kobziar, 2011, Bradstock et al., 2010, Murphy et al., 2010). The available methods for estimation of severity use analysis of change in indices of vegetation cover following fire. Such data may be sourced from aerial photography or satellite imagery. Some techniques rely on visual assessment while others rely on comparative reflectance value to estimate indices of cover change. The methods can be divided broadly into two groups (Brewer et al., 2005), those that compare pre-fire and post-fire imagery or values (Godwin and Kobziar, 2011, Coluzzi et al., 2010, Weber et al., 2008, Chafer et al., 2004) and those that
rely on comparison of post-fire imagery against a generic general state (Lanorte et al., 2011, Bradstock et al., 2010, Veraverbeke et al., 2010, Hammill and Bradstock, 2006).

A study by Chafer (2008) compared two methodologies based on reflectance indices generated from Landsat 5 Thematic Mapper data pre and post fire. This study looked at two wildfires near Sydney in 2001, and found a significant difference in performance for identifying the low severity categories, concluding that Normalized Difference Reflectance (NDRdif) was better than Normalized Difference Vegetation Index (NDVIdif) (Chafer, 2008). For the same fires, and in similar vegetation and topography, Hammill and Bradstock (2007) utilised NDVIdif for a comparison of severity estimates derived from SPOT 2 and Landsat 7 thematic mapper images. The SPOT 2 data were at 10 m resolution while the Landsat 7 resolution was 30 m. In addition, there was only two months separation between the images for the SPOT 2 while there was nine months between the images for Landsat 7. Severity was more reliably estimated using the finer resolution imagery. Differing levels of severity were also more clearly differentiated in forests and woodlands compared with sedgeland swamps and heathlands. In the latter vegetation types, it was more difficult to differentiate between low severity and unburned areas.

The other group of fire severity assessment methods relies on direct measurement of on-site scarring, material removal and other data such as rate of spread. A detailed study in the boreal forests of Alaska used the exposure of adventitious roots as a measure of organic ground matter consumed during fire events (Boby et al., 2010). In Australia, the thickness of stems that have been burnt through is often used to evaluate fire severity (Norris et al., 2010, Hammill and Bradstock, 2006). While these methods can produce some precise results, their application is impractical beyond the plot scale. They require physical site assessment and data collection soon after each fire.

The appropriateness of each technique can be limited by factors such as vegetation type and structure, topographic influences and atmospheric conditions. The ultimate limitation in any study is the availability of comprehensive estimates across a sufficiently broad range of landscapes and fires to allow development of appropriate
study designs. A method of estimating fire severity in the dry sclerophyll forest of the Greater Blue Mountains World Heritage Area (GBM-WHA), NSW, and its subsequent refinements was found to be the best available technique for use in the topographically diverse and forested ecosystems that are the subject of this current thesis (Bradstock et al., 2010, Hammill and Bradstock, 2009, Hammill and Bradstock, 2006).

1.10 Summary of key issues, study questions and thesis structure

This review of knowledge about C and N fluxes in soils of fire-prone forests has presented many general assumptions, however serious gaps in the knowledge have also been identified. Preeminent among these was the lack of experiments that manage the potentially complex interactions between disturbance regime elements and their impact on soil C and N stocks. It was apparent that many of the assumptions relating to manipulation of disturbance regimes (including fire) for positive C sequestration in soils, were developed in forest systems that have a low proportion of species adapted to survive fires of crown consuming intensity (Hurteau and Brooks, 2011, Hurteau and North, 2009). The trees in forests of south-eastern Australia are mostly well adapted to survive frequent but irregular high intensity fires. Thus, these assumptions may not be applicable to Australian forests (Bradstock et al., 2014). Climate change was also likely to influence the number of days with severe fire weather (Bradstock et al., 2014) and management responses to perceived fire risk, including more extensive or frequent use of prescribed fire and other disturbances to mitigate the risk (Attiwill et al., 2014b). Studies incorporating changing climate as a driver of likely soil C and N pool shifts and the potential feedbacks from altered fire regimes are lacking.

1.10.1 Summary of key issues leading into study questions

The thrust of this present thesis was to improve the understanding of the role of fire regimes, climate, timber harvesting and their interactions in determining the nature and amount of C in mineral soils of fire-prone forests in south-eastern Australia. Changing future weather conditions and the anthropogenic responses to them, are likely to result in fire regime shifts. Improving knowledge of the response of the soil C pool to likely
changes in climate, fire regimes and land management was the basis of the following specific studies:

1.10.2 Study 1
Given the potential importance of recalcitrant PyC to the persistence of C in soils, it was necessary to identify an effective and reliable method for assaying the most recalcitrant fraction of the C pool at a landscape scale. Section 1.7 indicated that the KMD method (Kurth et al., 2006) was the best available for assaying the most recalcitrant fraction of the C pool. There were still issues around control and residue recovery, so it was decided to modify the method and test its efficacy using a range of standard mixes of sand and various organic materials. These modifications and tests are described fully in Chapter 2 and the technique was applied to the fire regime studies in Chapters 3 and 4 of this thesis.

1.10.3 Study 2
It was important to understand how soil C concentrations change over TSF in order to interpret the storage potential of forest soils and to provide a base-line understanding of the soil C responses that are required to underpin broader analyses of effects of differing fire regimes. Any differences between low intensity prescribed fire and high intensity wildfire on the pattern of soil C change, with increasing TSF, were also considered critical for informing the use of fire in landscape management decisions. Determination of the response of the total C pool and the recalcitrant C fraction to TSF and fire type (wild or prescribed) was also required to assess long-term C sequestration implications. These themes were explored in Chapter 3 of this thesis.

1.10.4 Study 3
If the frequency of prescribed and unplanned fires is likely to increase in the future, then insights into the effects of varying fire regimes on soil C concentrations will be required to predict the outcome. In addition, climatic change in interaction with changes to fire regimes may affect soil C concentrations. Thus, a study was designed to examine
soil C responses to differing combinations of mean annual temperature (MAT), mean annual rainfall and variations in recent fire frequency. In addition, a further study of cumulative effects of differing fire severity combinations in successive fires on soil C pools was carried out to provide further insights relevant to future changes, not evident elsewhere in the literature. These combined studies were presented in Chapter 4 of this thesis.

1.10.5 Study 4
In forests utilised for timber harvesting, low intensity prescribed fires are lit frequently to reduce fuel with the aim of reducing the risk and impact of wildfires. This introduces the concept of compound disturbance (Buma et al., 2014), where the effect of high fire frequency and disturbances associated with timber harvesting may interact and change the effects on soil C and N stocks. These interactive effects need further study in the Australian context. The study presented in Chapter 5 of this thesis explored differences in soil C and N in response the frequency of low intensity fires and timber harvesting.

1.10.6 Synthesis
The final chapter of this thesis (Ch 6) provides a summary of the key findings from preceding chapters. The interactions effects of TSF, fire intensity and fire frequency, and climatic and timber harvesting on soil C and N pools are then further discussed, particularly in relation to future changes of management and climate. Landscape-scale scenarios of future changes to the soil C stocks within dry sclerophyll of the Sydney basin Bioregion under selected future climate scenarios were estimated, by scaling up the site based estimates presented in the preceding chapters. From this synthesis, management implications and future research directions are identified, discussed and presented.
2 Refinement of the KMD analytical technique for recalcitrant pyrogenic carbon in forest soils

2.1 Introduction

Soil organic matter (SOM) is a commonly used indicator of soil quality, however pyrogenic C (PyC) in the form of charcoal, can be a considerable portion of total SOM (Vyšná et al., 2014). It is generally considered that PyC is less biologically available than other forms of SOM and it persists in soils for long periods (Santín et al., 2015, Vyšná et al., 2014). The biological availability of PyC relates to its recalcitrance, which in turn is proportional to the coarseness of the material it is formed from (Mastrolonardo et al., 2017) and the formation conditions (Bird et al., 2015, Saiz et al., 2015, de la Rosa and Knicker, 2011). The persistence of PyC and its proportion in the total SOM pool may have implications for global C cycles (Bird et al., 2015, Santín et al., 2015). The total SOM pool and the PyC pool may respond independently to shifts in fire regime (Santín et al., 2016). The substantial but potentially dynamic contribution of PyC to total soil C requires its estimation by the most practical analytical technique available for the scale of the experiment (i.e., landscape).

It is only during the past few decades that alternatives to wet digestion chemistry for charcoal analysis have been available (Poirier et al., 2000, Skjemstad et al., 1998). An array of spectroscopic techniques has been developed to estimate and characterise the C content in soils. These include Nuclear Magnetic Resonance (NMR) spectroscopy, Infra-Red (IR) spectroscopic particle modelling estimates and X-ray crystallography. In Australia during this period, NMR and IR modelling have been largely used and mostly applied to agricultural soils (Forouzangohar et al., 2015, Baldock et al., 2013a, Clark Ehlers et al., 2010, Fang et al., 2014, Krull et al., 2003, Skjemstad et al., 2002). In a North American study of soil charcoal in native forests affected by fire regimes Kurth et al. (2006) reverted to using refined wet chemistry digestion, utilising hydrogen peroxide (H₂O₂) and dilute nitric acid (HNO₃), hereafter referred to as the KMD method. This continued use of wet chemistry in ecological work is important because of the ready availability of equipment, expertise and ultimately cost. The uneven distribution of SOM in fire-prone forests necessitates sampling of high numbers of replicates, in comparison to more homogeneous agricultural systems (de la Rosa and Knicker, 2011). A study by Hammes et al. (2007) was conducted as a "ring-trial" between 17 independent
laboratories, using a range of analytical techniques for black C determination. Calibration for the degree of overestimation of pyrogenic C associated with each technique was possible, provided an initial sample of material was thoroughly characterised to identify the transition point between interfering material and pyrogenic C. From these data, calibration of any selected method of black C analysis was possible. This calibration process was too costly to be utilised at the time this present thesis study was commenced. The analytical techniques of digestion chemistry and enhanced digestion chemistry were the only methods with the potential to provide quantitative data at the landscape scale without excessive cost.

The assessment of PyC assay techniques (Chapter 1), and the limited access to high technology equipment, resulted in the adoption of an approach involving direct digestion of oxidizable SOM and assaying C concentrations in the post-digest soil residue. We assumed that under appropriate conditions, this residue C would be a reasonable approximation of the most recalcitrant PyC fraction. Of the published digest techniques; the KMD method (Kurth et al., 2006), which used dilute nitric acid (1M HNO₃) and hydrogen peroxide (35% H₂O₂) was the most appropriate for our landscape scale studies. One concern with this digest method was controlling evaporative losses during the required 16 hours heating at 100°C. This issue was solved by the use of a block digester holding a reasonable number of close fitting reflux tubes capped with tear drop condensers (Licata and Sanford, 2012). In order to ensure that our assumption about C residue was valid it was necessary to assess the extent to which non-pyrogenic organic matter would be removed from samples by the digest. It was also essential to estimate the extent to which PyC survived the digest and to observe the properties of organic matter in the post digest residue.

2.2 Method

2.2.1 Development of digest process
Previously published versions of the KMD method have used filter paper to recover the post-digest soil residue (Licata and Sanford, 2012, Ball et al., 2010, Kurth et al., 2006). Two key problems were encountered when these methods were applied in our studies.
Firstly, fine organic matter travelled up and over the filter paper by capillary action and secondly, the paper pores tightly bound the fine soil material, hampering recovery of the residue incurring considerable risk of contamination from fragments of filter paper. In addition to these inconsistencies, the technique was very slow and demanded attention. Consequently, recovery by evaporation from glass beakers in ovens at 85°C was trialled. While the evaporating process was slow, it did not require attention other than loading and unloading the oven. A clean stainless-steel spatula was successful in dislodging the residue from the glass beaker without the risk of C contamination.

2.2.2 Establishing charcoal standards and recovery rate

In order to establish standards, a consistent source and quality of charcoal, lignified organic matter and biologically available organic matter were required. The charcoal and lignified organic matter were obtained by collecting fallen branches from *Eucalyptus grandis*. These were dried and sawn into 10 cm lengths with the resulting sawdust collected (the lignified organic matter). The branch segments were converted to charcoal at 900 °C in a muffle furnace. The resulting charcoal was crushed, passed through a 2 mm sieve and mixed with silicate sand free of organic matter to provide a base charcoal mix with a concentration of 20% by weight. The sawdust was similarly mixed with silicate sand to provide a base mix of 5% by weight. Laboratory grade humic acid was used as the biologically available organic matter in a sand mix at 20% by weight.

The ridge top soils of the study region are derived from Hawkesbury Sandstone which consists of quartz sand cemented by a clay matrix, of which kaolinite is the dominant mineral (Wray, 2011). The binding of organic matter in soil aggregates increases its resistance to decomposition (Bradshaw et al., 2013). To address this issue the range of standards tested was duplicated by a set that included kaolinite at 1.5% by weight, based on a stock mix of 10% by weight in sand. The mixtures used in the range of standards are listed in Table 2.1 expressed in grams of the base mixes. All base mixes had been pulverised in a ball mill (TEMA brand), resulting in particles < 120μm.
2.2.3 Carbon content of undigested standards

After thorough mixing of the standard constituents (Table 2.1), three replicate subsamples of ca. 1.5 g were taken from each standard and % total carbon ($%C_{\text{Tot}}$) analysis was conducted by 1250 °C dry combustion spectrometry (LECO Tru-Spec, USA, China).

**Table 2.1:** Components of the standards used. The percentages are %/w of the organic substrate in the silicate sand.

<table>
<thead>
<tr>
<th>Standard label</th>
<th>20% charcoal (g)</th>
<th>5% sawdust (g)</th>
<th>20% humic acid (g)</th>
<th>10% kaolin (g)</th>
<th>TEMA'd sand (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% char</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>1% char</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>0.5% char</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>5% char + kaolin</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>1% char + kaolin</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>32</td>
</tr>
<tr>
<td>0.5% char + kaolin</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>A + kaolin</td>
<td>5</td>
<td>16</td>
<td>0</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>B + kaolin</td>
<td>2</td>
<td>16</td>
<td>1</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>C + kaolin</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>D + kaolin</td>
<td>1</td>
<td>16</td>
<td>1</td>
<td>6</td>
<td>16</td>
</tr>
</tbody>
</table>

2.2.4 Post-digest C content of standards

The standards were used in two ways. First, to determine the amount and type of C that would survive the digest process and secondly, to monitor variation between digest batch runs. Three sub-samples from each of the mixes (ca. 1.5 g each) were weighed out and digested. After recovery, the residue was analysed for $%C_{\text{Tot}}$ (see Section 2.2.7).

2.2.5 Final digest method

Samples for digestion were carefully weighed in a fresh weigh boat to fall within the range of 1.5000 g to 1.5009 g. The sample was then transferred to a clean reflux tube
where it remained covered to prevent dust contamination until the oxidation reagents were added.

A solution of HNO₃ (1 mol/L = 1M) was prepared from concentrated HNO₃. A total of 10 mL of the HNO₃ was added to each sample using a multipipette to dispense two 5 mL aliquots. Tubes were swirled during the addition of 1M HNO₃. Each reflux tube was then capped with a tear drop condenser. The acid sample mix in the tubes was left to stand at room temperature and observed for volatility. After observable effervescence in the tubes had subsided at ca. 30 minutes, the tear drop condensers were removed and kept in order so they could be replaced without cross contamination of samples.

Hydrogen peroxide (H₂O₂ 35% solution) was then added to each sample/acid mix in a staged series of six 5 mL aliquots, to control volatility (30 mL of H₂O₂ in total). The reflux tubes were arranged in a rack which was then slotted into the block digester. The block controller had been programmed to ramp up in slow stages starting at 25°C for 45 minutes before ramping to 50°C over 5 hours and 25 minutes. Considerable effervescence produced foam at the surface of the digestion solution, rising high in the tubes, so replacing of the tear drop condensers had to be stalled until the foam had begun to subside, this usually occurred at ca. 70°C depending on the organic content of the sample. The ramping from 50°C to 100°C occurred over 1 hour and the digester remained at this temperature for 15 hours 40 minutes before it automatically switched off. The rack of tubes was then lifted clear of the block to cool.

Once cooled, the supernatant was carefully drawn off the settled samples. A fresh aliquot of 20 mL Reverse Osmosis water was added and the sample left to settle. Most of this secondary supernatant was then drawn off before the residual sample was washed into a glass beaker. The labelled beakers were placed into drying ovens set to 85°C.

Once evaporation was complete, after ca. 4 days, the beakers were removed from the oven and the samples were dislodged with a clean stainless-steel spatula. The recovered
samples were placed in labelled vials and sealed. The samples were subsequently analysed for total C by isotope ratio mass 296 spectrometer (IRMS; Delta V, Thermo Finnigan).

In order to identify the nature of residue recovered after the digest, a randomly selected group of treatment samples were selected. These were examined under a Scanning Electron Microscope (SEM; Desktop Phenom XL, The Netherlands) in order to identify residual organic matter of plant origin. A number of random points were generated within each slide and the chemical components of the nearest particles were recorded. The colour and angularity of all particles that had visual evidence of cellular structure and high C content were noted.

2.2.6 Data analysis
The measured $%C_{Tot}$ for the undigested and digested standards were used to estimate the mean and standard deviation of normal distributions in their respective populations fitted by Markov Chain Monte Carlo. Lower and upper bounds were for the 95% Bayesian credible interval. We were confident that the estimate for the mean ("mu") would be very close to the mean of the sample values, but some variation in the actual extent of deviation in the bounds from a standard confidence interval was possible due to differences between the actual distribution of the sample values and the model distribution.

2.2.7 Analysis of recovery rate
The amount of C to survive the digest was termed the ‘recovery rate’ and was modelled to provide a bounded estimate calculated from both the pre-digest and digest data sets for each standard. Markov Chain Monte Carlo, with uninformative priors, fitted a normal distribution to the pre-digest values. Digest values were then related to the pre-digest values by the assumption that they were drawn from a second normal distribution, with mean equal to the pre-digest mean multiplied by an unknown recovery rate (in the interval $[0, 1]$).
After a particular standard was identified and fitted to the model, the model was checked for convergence before posterior samples were returned as a data frame. Once the model was fitted for each standard and convergence established it then reported the ‘recovery rate’.

N.B. Full R scripts and data are available at the following link: https://github.com/Hot-Eco/carbon_standards/tree/sawyer_2018

2.3 Results

The results of analysis for total C in the standards produced clusters of data around the expected level based on theoretical C content. The extent of scatter indicated the degree to which the C sources had not been evenly distributed within the sand matrix in spite of thorough mixing. The results for the 5%, 1% and 0.5% charcoal standards had indicated C content very close to these percentages. The other forms of organic matter (sawdust and humic acid), showed C content to be approx. 50% of their mass in the standards (Fig. 2.1)

The C content of the standards after the digestion process exhibited a wider variation that may relate to the increased number of sample points (see Fig.2.2). The non-charcoal organic matter was consumed to trace or non-detectable concentrations in all standards.
Figure 2.1: Undigested standards %CTot. Mean indicated by vertical bar. Open circles indicate individual analyses.

Figure 2.2: Digested standards %CTot Mean indicated by vertical bar. Open circles indicate individual analyses.
The recovery rates estimated in standards without added kaolin were generally in the range ca. 0.526 to 0.005. The charcoal only standards ranged from ca. 0.526 (1% char) to 0.408 (5% char). By contrast the recovery rate was higher for the same charcoal concentrations in the standards with kaolin added (0.63- 1% and 0.59-5%). Table 2.2 provides distribution of the recovery rates for the full range of standards tested.

Table 2.2: Estimated recovery rates from standards displayed with mean and quantiles. Standard labels with “+k” indicate those that included the clay mineral kaolinite which is the main cement of the soil parent material in the study area (Wray, 2011).

<table>
<thead>
<tr>
<th>Standard label</th>
<th>lower_2.5%</th>
<th>median</th>
<th>mean</th>
<th>upper_97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.2790101</td>
<td>0.3345707</td>
<td>0.3359639</td>
<td>0.4013822</td>
</tr>
<tr>
<td>B</td>
<td>0.1997282</td>
<td>0.2420918</td>
<td>0.2431304</td>
<td>0.2914660</td>
</tr>
<tr>
<td>C</td>
<td>0.1803493</td>
<td>0.2433539</td>
<td>0.2492490</td>
<td>0.3465244</td>
</tr>
<tr>
<td>D</td>
<td>0.1447889</td>
<td>0.1719120</td>
<td>0.1727242</td>
<td>0.2061778</td>
</tr>
<tr>
<td>charcoal_1%</td>
<td>0.4283536</td>
<td>0.5246311</td>
<td>0.5255868</td>
<td>0.6268994</td>
</tr>
<tr>
<td>charcoal_5%</td>
<td>0.1647014</td>
<td>0.4045056</td>
<td>0.4080676</td>
<td>0.6784954</td>
</tr>
<tr>
<td>sawdust_5%</td>
<td>0.0011666</td>
<td>0.0050088</td>
<td>0.0052698</td>
<td>0.0107686</td>
</tr>
<tr>
<td>charcoal_1% + k</td>
<td>0.52</td>
<td>0.63</td>
<td>0.63</td>
<td>0.75</td>
</tr>
<tr>
<td>charcoal_5% + k</td>
<td>0.48</td>
<td>0.59</td>
<td>0.59</td>
<td>0.70</td>
</tr>
</tbody>
</table>

2.4 Discussion

A consistent removal of non-char organic matter from the standards was achieved through the application of the modified KMD digest (Figure 2.2). While charcoal was recovered at a mean rate, it underestimated the known concentration. Nevertheless, reliable means and bounds for the recovery rate have been modelled (Table 2.2). The magnitude of the recovery rate increased for the mixes that contained kaolinite and charcoal with no other sources of organic matter (Table 2.2). The recovery rate achieved from the standards that contained kaolinite were the best analogue of the natural soil matrix of the study area, due to it being the cement in Hawkesbury sandstone (Wray, 2011).
In comparison with published studies, our recovery rates underestimated total C from pyrogenic sources but were less variable. Charcoal recovery by the benzene polycarboxylic acid method, was described as reliable when it achieved 69-107% average (Karunaratne et al., 2014). The developers of the KMD method reported a charcoal recovery rate of 92% when charcoal concentration was in the range of 0.5% to 5% (w/w), with variability between runs ranging from 2.37% to 31.1% (Kurth et al., 2006). This inter-run variability exceeded 11% in 8 out of 14 soils tested. Our charcoal recovery rate varied between ca. 48% to 75% (95% confidence interval) with means of 63% (1% char + k) and 59% (5% char + k). The grinding of our soil matrix analogue to a fine homogenous texture prior to digestion, may have increased the potential for charcoal to be oxidized. In addition, the increased duration of heating from the slow ramp up to run temperature may have had some bearing on the amount of charcoal digested. Importantly, both of these factors were applied consistently across all sample preparations and digests. Hence, the application this technique across the landscape for the comparison of ecological impacts on soils was valid.

The C that was detected in our post digest samples was derived from charcoal. This was determined by the complete digestion of other forms of organic matter in standards and SEM examination of soil residues revealing the only observable organic matter was in the form of charcoal. Other forms of recalcitrant C that may survive this type of digest may be present in natural soil however, the strong correlation between charcoal addition and recovery rate from natural soils achieved by Kurth et al. (2006), suggest that these are of little consequence for landscape scale investigations of fire regime effects.

2.5 Conclusion

Our refinements of the KMD method have enabled investigations to focus on the highly recalcitrant fraction of PyC (RPC) in soils without recourse to highly specialised and expensive spectrographic methods. This permitted higher field sampling intensities than would be affordable otherwise. The RPC quantified by this analysis was the fraction most likely to have long term C sequestration implications.
3 Fire intensity drives post-fire temporal pattern of soil carbon accumulation in Australian fire-prone forests

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Wildfire

Litter and canopy removed and mineralised, nutrients in ash available. New growth produces labile litter.

Prescribed fire

Considerable amounts of heat effected leaves and twigs fall and become ground cover resistant to decomposition.

Patterns of mineral soil carbon with increasing time since fire responds to higher net primary productivity and more readily decomposed litter following stronger system reset associated with wildfires.
3.1 Introduction

The temporal and spatial dynamics of organic matter in landscapes affected by fires are of fundamental interest in global ecological management (Santín et al., 2016, Arneth et al., 2014, Arneth et al., 2010). As biologically stored carbon (C) and nitrogen (N) pools shrink they release organically derived greenhouse gases that build up in the atmosphere contributing to global warming trends (Anderson-Teixeira and DeLucia, 2011). Ecosystem disturbances are strong drivers of the movement of C and N between terrestrial systems and the atmosphere. Vegetation fires are one such important disturbance and have been part of the landscape since there was sufficient vegetation cover and atmospheric oxygen to sustain combustion (Ball, 2014, Glasspool et al., 2004, Jones and Chaloner, 1991).

There are uncertainties about the fate of C pools affected by fire as not all materials are consumed and transferred to the atmosphere as gases. Fine particulates such as ash, soot (combustion condensation products) and charred vegetation fragments (sometimes of considerable mass) are dispersed by convection currents, wind and fluvial processes at varying spatial scales (Santín et al., 2015, Bradshaw et al., 2013). Also, there is organic matter left in-situ including surviving vegetation, standing charred material and variably charred plant remains in ash deposits (Santín et al., 2016, Knicker, 2011). Very little of the organic matter in the mineral soil is affected directly by fire, as high heat penetration is limited to the soil’s surface (i.e., 0 to 5 cm depth: Mondal and Sukumar, 2014, Bradstock and Auld, 1995, Raison et al., 1986a). The degree of consumption of the overlying humus is determined by its moisture content and the intensity of fire at the time, both factors being driven by weather (Santín et al., 2015, Boby et al., 2010, Bradstock et al., 2010). This results in a spectrum of biologically fixed C in the landscape ranging from living plant tissue to fully charred material and soots.

Within this spectrum the portion ranging from partially to fully charred plant tissue and soots constitutes pyrogenic carbon (PyC: Jenkins et al., 2016a) as described by Hammes et al. (2007). The recalcitrant properties attributed to the PyC fraction are determined by initial fuel source and formation conditions. Chars and soots formed at high
temperatures (>750°C) are the most unreactive and have been shown to persist in soils for centennial and millennial time periods (Bird et al., 2015, Bodí et al., 2014, Hammes et al., 2007). At the lower end of the recalcitrant range partially charred material and chars formed at lower temperatures (<450°C), or from fine materials (lower lignin: cellulose), have been found to decompose at similar rates (decadal) to organic matter of similar size unaffected by fire (Knicker, 2011, Norwood et al., 2013, Hammes et al., 2007). In terrestrial ecosystems, the recalcitrance of PyC is enhanced when incorporated into the mineral soil where it is protected from volatilization by fire and may be bound into soil aggregates that shield it from oxidation and other degradation processes (Bradshaw et al., 2013). This has attracted attention as a mechanism for potential sequestration of C to offset emissions from combustion of fossil fuels (Santín et al., 2016).

The focus on potential C sequestration in soils has led to considerable effort in quantifying and understanding the flux of soil C in agricultural systems (Ballock et al., 2013a, Krull et al., 2003, Skjemstad et al., 1998). In contrast, soil C dynamics of natural systems are less commonly studied. Where mineral soil C pools have been estimated, they can exceed the amount aboveground, emphasizing their importance in the global C cycle (Santín et al., 2015, Vargas et al., 2008, Beringer et al., 2007). For soil total C to increase, the input flux must be greater than the output flux, *i.e.*, Net Primary Productivity (NPP), aboveground decomposition and char translocation rates must exceed soil respiration and fluvial displacement (Santín et al., 2016, Roxburgh et al., 2005).

Little is known about the way these processes respond to variations in fire regimes (Keeley et al., 2012). This includes: time since fire (TSF; the period following the last fire occurrence on a site); fire frequency (the number of fires an area is subject to over a given time); fire intensity (the amount of energy released in a length of fire line); and seasonality (the time of burning in relation to annual climatic cycles) (Keeley et al., 2012). Fire intensity is commonly replaced by the proxy severity, as determined by fire type (*i.e.*, wildfire, WF, or prescribed fire, PF) or the extent of damage to the vegetation.
(e.g., crown scorch). At the broader scale fire severity and seasonality are interrelated; in temperate regions fires occurring in late spring or summer (when most WFIs occur) are usually more severe than fires in the cooler months (when PFIs are usually implemented) (Volkova et al., 2014).

Current knowledge indicates that fire intensity has variable effects on factors determining soil C accumulation. Fire can increase NPP by enhancing the availability of key resources for growth (nutrients, light, moisture), as well as stimulating germination and resprouting (Whelan, 1995). Increases in the availability of key plant nutrients such as phosphorus (P) (Lagerström et al., 2009), can in turn facilitate microbial processes that enhance the availability of other plant nutrients (Alexis et al., 2012). Fire opens the canopy, reduces soil cover and kills existing roots and shoots stimulating NPP through increased light penetration, higher soil temperatures and reduced resource competition for new growth (Bradstock et al., 2012a, Raison et al., 1986a). Given adequate temperature and moisture conditions, decomposition rates depend on litter quality, protective chemicals in the tissue and adequate labile nutrients. High lignin: cellulose and/or the presence of polyphenolic compounds, both associated with sclerophyll vegetation, retard decomposition rates (Krebs et al., 2010). Differences in fire intensity affect the nature of the subsequent litter and the extent to which decomposition resistant chemicals are driven out (Alexis et al., 2012, Alexis et al., 2007). Another source of C input to the soil, over TSF, is the breakdown of charred materials suspended above ground, e.g., on charred rough barked trees. The extent of this type of charring increases with fire intensity (Gould et al., 2011). PyC from this source is shed to the soil surface continuously over several decades TSF, until depleted (Bluff, 2014). Gould et al. (2011) recorded char on the boles of stringybark trees (i.e., a form of rough bark *Eucalyptus* spp.) had declined from 100% to 50% by ca. TSF 20 years.

An understanding of the TSF effect on soil C accumulation is therefore required to provide a foundation for investigation of long term fire regime effects on soil C. Studies of soil C accumulation as a function of TSF in fire prone forests are relatively rare. Whilst most studies are limited to simple pre-fire and single time postfire comparisons,
there are some studies that have investigated longer chronosequences (i.e., TSF > 13 years: Hobley et al., 2017, Alexis et al., 2012, Vargas et al., 2008) allowing for temporal trends to be examined. Studies of patterns of soil C that transcend a longer period after fire (i.e., > 20 years) while considering the influence of variations in fire intensity are lacking.

In this study, we examine responses of soil C to relatively long chronosequences (i.e., > 30 years) of PFs and WFs in dry sclerophyll forests dominated by *Eucalyptus* spp., in south eastern Australia. Such forests are highly fire prone and subject to occasional high intensity WFs, along with regular low intensity PFs targeted at fuel reduction and asset protection. The long-term consequences of variations in fire intensity in these extensive forest ecosystems, as a function of different sources of fire, are poorly known, but are crucial for understanding the consequences of management and future climatic change (Bradstock et al., 2012a). We predicted that total soil C and the concentration of recalcitrant PyC would be elevated in the early TSF phase (i.e., 10 years) and that subsequent soil C concentrations would be dependent on the intensity of the last fire. Considering all factors, we predicted higher fire intensity to enhance longer term total soil C accumulation. To test these predictions, we estimated patterns of total soil C and recalcitrant PyC across TSF chronosequences following prescribed and wild fires (ca. 43 and 38 years, respectively). The differing types of fire were chosen to represent characteristic, contrasting fire intensities.

3.2 Methods

3.2.1 Study sites

The study was carried out in the dissected landscapes of the Sydney Basin Bioregion of south eastern Australia (Fig. 3.1). These landscapes are dominated by sedimentary geology of mainly Permian/Triassic origins that result in predominantly shallow, sandy soils. Climate is cool-warm temperate with non-seasonal rainfall. Vegetation is predominantly open forest and woodland (i.e., dry sclerophyll forest (DSF) and woodland), dominated by *Eucalyptus, Corymbia* and *Angophora* spp., with an understorey often rich in sclerophyllous shrub species, graminoids and sedges
Figure 3.1: Map of time since fire (TSF) study sites with insert locating Sydney region within Australia. Sites last affected by prescribed fire are identified by a diamond; sites last affected by wildfire are identified by a star.
(Keith, 2004). The region is fire-prone with an average interval of ca. 15 to 20 years between fires (Bradstock et al., 2012a).

This study used a chronosequence approach to investigate temporal changes in soil C following fire (i.e., TSF effects). We contrasted responses of soil C across alternative chronosequences of sites burnt by WFs and PFs. WFs in the forested landscapes of the study region often burn at high intensities under severe weather conditions (e.g., > 10,000 kW/m) resulting in extensive areas of crown fire (i.e., high levels of scorch and consumption of tree crowns: Bradstock et al., 2010, Hammill and Bradstock, 2006). By contrast, PFs are lit under mild weather conditions to primarily reduce surface fuel loads (Murphy et al., 2013, Gill et al., 1987, McArthur, 1966). Inherently these fires are of lower average intensity, e.g., the typically aimed for flame height of around 1 m (NSW Rural Fire Service, 2006) is consistent with the lower end of intensity estimates ranging from <500 kW/m (Bradstock et al., 2005) to 2500kW/m (Attiwill et al., 2014b). Thus, we sampled sites that were previously burnt by both types of fires, based on the assumption that average intensity of the last fire would be strongly contrasting between types. By necessity, we used a chronosequence approach to estimate effects of TSF, because intervals between successive fires vary widely (e.g., 1 to > 20 years). Thus, it is not logistically feasible to examine TSF effects on soil C via longitudinal studies. The range of variation in incidence of fire across the region provided scope for exploration of TSF effects via sites with widely varying post-fire ages following both types of fires. In addition, the extensive expanse of DSF (i.e., > 70% of area of the region) and the dominant sedimentary geology offered scope for a standardised approach to site selection that minimised confounding effects of terrain and vegetation variations.

3.2.2  Time since fire site selection
Fire history across the region was characterised using fire records since 1972 (New South Wales Office of Environment and Heritage, unpublished data). These were mapped using ArcMap 10.2 (ESRI, 2013). All sites sampled were situated in DSF on sandstone derived soils, on ridge tops with slopes of < 10°.
A total of 33 sites were selected, 18 of these last experienced WFs, while 15 last experienced PFs. Within the limits of the available information, sites were selected to cover the widest range of TSF possible (< 1 year to 43 years). In this study, TSF was treated as a continuous variable (locations displayed in Fig. 1 & Appendix A, Table 1).

3.2.3 Field sampling
In natural forest and woodlands, soil organic matter distribution exhibits substantial spatial heterogeneity (de la Rosa and Knicker, 2011, García-Palacios et al., 2011, Fraterrigo et al., 2005). This spatial heterogeneity presents difficulties in examining natural systems in contrast to agricultural systems. However, there is a degree of predictability in the distribution of C and other biologically cycled elements associated with concentrated biological activity, such as upper soil horizon and proximity to trees (Gallardo, 2003, Stoyan et al., 2000). This study utilised this concept through a stratified approach to sampling that accounted for the principal sources of heterogeneity.

It was assumed that soil organic matter (SOM) distribution was a function of soil depth, proximity to trees and variations in tree bark type. Soils were sampled in two depth classes (0 to 5 cm and 6 to 15 cm) and in three micro-sites based on proximity to trees and their bark type. These were: Open, patches largely between tree crowns; Rough, adjacent to base of rough barked trees and; Smooth, adjacent to base of smooth barked trees.

Within each of these 6 sampling combinations, 4 replicate cores were collected and pooled. For each core, sites were randomly selected within the categories above and the litter was carefully scraped away to expose the surface of the mineral soil. Soil cores were then taken in stainless steel tubes of ≈5 cm inner diameter. Initially, the upper soil surface was sampled (i.e., 0 to 5 cm). The same hole was then used to remove the lower soil sample (6 to 15 cm).
3.2.4 Soil processing
One week after collection the soils were thoroughly air dried then passed through a 2 mm sieve. At this stage gravel and charcoal >2 mm and root and plant parts were separated. All these components were weighed and stored. After weighing, the >2 mm charcoal fraction was crushed to pass through a 2 mm sieve and mixed thoroughly into the <2 mm soil fraction. The soil and charcoal mix was sub-sampled (~50 g) and oven dried to constant weight (80°C). This was ground in a ball mill (TEMA brand) to <150 μm and stored in airtight jars.

3.2.5 Soil total C assay
Total soil C concentration was analysed for three replicate samples (1.5 g each), using a LECO dry combustion spectrometer at 1250°C (LECO TruSpec CHN, USA), with results expressed as % C by dry weight (% C\text{\textsubscript{tot}}).

3.2.6 Recalcitrant Pyrogenic C Assay
To remove the biologically and chemically degradable C, leaving the most resistant pyrogenic C (RPC), a modified 1M nitric acid (HNO\textsubscript{3}) and 35% hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) digest (KMD) method was used (Kurth et al., 2006).

The major modifications to this procedure were: 1) pre-grinding of the samples to a fine texture (\textit{i.e.}, <150 μm); 2) use of a block digester to control heat and reflux tubes capped by teardrop condensers for control of moisture loss (Licata and Sanford, 2012); 3) ramping up of temperature in slow stages of digestion to control volatility, and; 4) samples recovered by evaporation at 85°C in beakers rather than by filter paper.

In summary, the method involved weighing three replicates, each consisting of 1.5 g of the ground, oven dried sample into a digest tube. To each replicate 10 mL of 1 M HNO\textsubscript{3} was added and swirled. This was left to stand for 30 minutes prior to the addition of 30 mL of 35% H\textsubscript{2}O\textsubscript{2}. Each tube was again swirled and introduced to the block digester at room temperature. The array of 50 tubes was then brought to 100°C and maintained at
this temperature for 16 hours. At the end of the digestion the tubes were lifted clear of the block to cool, supernatant was decanted and residual soil washed into beakers for evaporative drying in ovens at 85°C.

The recovered dry residue was analysed for C content on an isotope ratio mass spectrometer (IRMS; Delta V, Thermo Finnigan). These results were expressed as the % C by dry weight in the residual digested soil material (% RPC). To confirm that the residual C was RPC, a selection of the digested material was examined by Scanning Electron Microscope (SEM; Desktop Phenom XL, The Netherlands) to verify that the residual organic matter was plant-derived charcoal (author’s unpublished data).

3.2.7 Statistical Analyses
Generalized additive models (GAMs: Wood, 2011, Wood, 2006) were used to relate the amount of soil C to TSF, considering the type of fire (wild or prescribed), soil depth class and micro-site. GAMs were chosen to allow for non-linear trends in C over time without needing to make prior assumptions about their form.

Data from analytical replicates for each site and sub-category were expressed as % $C_{Tot}$ by weight in mineral soil. Inspection of the empirical data led to the choice of log-transformed response values for % $C_{Tot}$ and a Gaussian identity link function for initial modelling. This resulted in better homogeneity of residuals compared to Gaussian or Gamma log-link functions. Inspection of residuals across sites showed that a random effect term for sites was necessary to account for spatially correlated errors. As sampling was based on a space for time substitution no issues of serial auto-correlation were expected, this was confirmed by inspecting model residuals.

Fire type, soil depth and micro-sites were included as fixed categorical terms. TSF was included as a smooth term allowing for an interaction with fire type and soil depth. The smooth terms for TSF and % $C_{Tot}$ were fitted using penalized thin-plate regression splines and REML estimation. All models were fitted using the ‘mgcv’ package (version 1.8-15) in the R statistical environment (R Core Team, 2016). In our preliminary
modelling, the smooth function for TSF sometimes had a markedly non-linear shape
within the interval where data were absent (missing / not available). To avoid this, we
set a conservative upper limit on the degrees of freedom for that term so that the
smooth function could still display some non-linearity but only to an extent supported
by the available data. Model validity was checked by inspecting residuals for normality
and an acceptably even distribution over fitted values and predictors.

For % RPC, GAMs were fitted using the same predictors as for the % C\textsubscript{Tot} analysis plus
an additional smooth term for % C\textsubscript{Tot}. This treated % C\textsubscript{Tot} as a conditioning variable,
thereby allowing the response of %RPC to fire type and TSF to be examined
independently. The smooth terms for TSF and % C\textsubscript{Tot} were fitted using penalized thin-
plate regression splines and REML estimation.

Predicted differences for the two C metrics (\% C\textsubscript{Tot} and \% RPC) between the fire types
were generated using posterior simulation of a set of 1000 credible mean trends from
the fitted GAM and, for each trend, the difference in C metric was calculated along the
TSF interval.

N.B. Full R scripts and data are available at the following link: https://github.com/Hot-
Eco/chrono_sequence/tree/sawyer_2018

3.3 Results

3.3.1 The effect of time since fire on total \% C\textsubscript{Tot}
The GAM analysis identified significant interactions between TSF, fire type and soil
depth (Appendix B, Table 1a). In particular, \% C\textsubscript{Tot} in the 0-5 cm depth class was
significantly higher than in the 6-15 cm depth class, with major differences in response
to TSF and fire type being apparent between these depth classes (Fig. 3.2 & Appendix B,
Table 1b.).
Figure 3.2: The relationship between soil total carbon as % by weight (% $C_{\text{Tot}}$) and time since fire (TSF) represented by generalised additive model trend lines for wildfire (WF, solid black) and prescribed fire (PF, dashed black). Two soil depths 0 - 5 cm and 6 - 15 cm for each of three microsites based on proximity to trees: open, largely between tree crowns; rough, adjacent to trees with rough bark; smooth, adjacent to trees with shedding bark. The 95% confidence bands for the trends are represented by grey shading. Mean measured data are represented by single points (solid for WF and hollow for PF).

3.3.1.1 Soil depth 0-5 cm

For the open microsites, early in the TSF continuum (i.e., < 15 years), both WF and PF had similar, relatively constant % $C_{\text{Tot}}$ of ca. 3.4% (Fig. 3.2). Later in the TSF (i.e., > 15 years) % $C_{\text{Tot}}$ estimates for WF increased to a maximum at TSF 38 years of ca. 6.0% (Fig 3.2). By contrast, PF % $C_{\text{Tot}}$ remained relatively constant at ca. 3.0%. The estimated difference in % $C_{\text{Tot}}$ between WF and PF ranged from ca. 0.1% (earliest TSF i.e., < 1 year) to ca. 3.3% at TSF 38 years (Fig. 3.3).
Figure 3.3: The relationship between soil total carbon as % by weight (% $C_{Tot}$) between wildfire and prescribed fire along time since fire (TSF), limited to the extent of common TSF values. These are based on posterior simulation generating a 1000 credible mean trends from the fitted generalised additive model and the fire type difference calculated for each trend. The solid curve represents the mean difference and the shading is the 95% confidence band. Individual plots for two soil depths 0 - 5 cm and 6 - 15 cm for each of three microsites based on proximity to trees: open, largely between tree crowns; rough, adjacent to trees with rough bark; smooth, adjacent to trees with shedding bark.

For the rough barked microsites, the relationships between % $C_{Tot}$, fire type and TSF were similar to those described for the open microsites but the magnitude of % $C_{Tot}$ was significantly (P< 0.001; Appendix B, Table 1b) higher (e.g., ca. 4.5% and 4.4% respectively for WF and PF at TSF < 15 years, rising to ca. 8.0% for WF at TSF 38 years: Fig. 3.2). The estimated difference in % $C_{Tot}$ between WF and PF ranged from ca. 0.1% at TSF < 1 year, to ca. 4.5% at TSF 38 years (Fig. 3.3).

For smooth bark microsites, the relationships between fire type and TSF were again similar to the open microsites but the PF trend was truncated due to lack of data at
higher TSF values. Estimated % $C_{Tot}$ was similar to rough barked microsites for both fire types across the range of TSF (Fig. 3.2). The estimated difference in % $C_{Tot}$ between WF and PF ranged from ca. 0.1% at TSF < 1 year, to ca. 1.9% at TSF 24 years (Fig. 3.3).

3.3.1.2 Soil depth 6-15 cm
Across all three microsites there was a similar pattern of response for % $C_{Tot}$ to TSF for each fire type (Fig. 3.2). While the patterns remained similar in all microsites, the magnitude of %$C_{Tot}$ differed.

For open microsites WF % $C_{Tot}$ estimates rose from ca. 1.9% early in the chronosequence to ca. 2.9% by TSF 38 years. The PF %$C_{Tot}$ remained near constant at ca. 1.8% throughout the TSF range. The estimated difference in % $C_{Tot}$ between WF and PF ranged from ca. 0.1% at TSF <1 year, to ca. 1.4% at TSF 38 years, though 95% confidence bands were only consistently positive after ca. TSF 10 years (Fig. 3.3).

For rough microsites, similar peaks occurred at the same TSF values as described for open microsites but % $C_{Tot}$ was ca. 4.0% in both instances. The PF % $C_{Tot}$ remained constant at ca. 2.5% throughout the TSF range. The estimated difference in % $C_{Tot}$ between WF and PF ranged from ca. 0.1% (earliest TSF) to ca. 2.0% at TSF 38 years (Fig. 3.3).

In the smooth microsites, the overall patterns and magnitude of % $C_{Tot}$ estimates for WF in relation to TSF were the same as in the rough microsites. The average for PF % $C_{Tot}$ was ca. 2.4%, though the estimate was truncated at TSF 24 years due to lack of data. The estimated difference in % $C_{Tot}$ between WF and PF, peaked between TSF 15 and 20 years at 1.6% (Fig. 3.3).
3.3.2 The effect of time since fire on %RPC

The GAM analysis identified significant interaction between TSF, type and soil depth (Table 2a in Appendix B, Fig. 3.4). In particular, % RPC in the 0-5 cm depth class was significantly higher than in the 6-15 cm depth class, with major differences in response to TSF and fire type being apparent between these depth classes (Table 2b in Appendix B, Fig. 3.4).

Figure 3.4: The relationship between relative abundance of recalcitrant pyrogenic carbon (%RPC adjusted for soil total carbon (% C\text{tot}) and time since fire (TSF) represented by generalised additive model trend lines for wildfire (WF, solid black) and prescribed fire (PF, dashed black). Two soil depths 0 - 5 cm and 6 - 15 cm for each of three microsites based on proximity to trees: open, largely between tree crowns; rough, adjacent to trees with rough bark; smooth, adjacent to trees with shedding bark. The 95% confidence bands for the trends are represented by grey shading. Mean measured data are represented by single points (WF- solid and PF hollow).
3.3.2.1 Soil depth 0-5 cm

Across all three microsites there was a similar pattern of response for % RPC to TSF for each fire type (Fig. 3.4). In particular, % RPC for WF was estimated to be non-linear as a function of TSF, in all microsites, whereas the response to PF was relatively constant. This is reflected in the % RPC estimated difference between the fire types (Fig. 3.5).

![Figure 3.5](image)

**Figure 3.5**: The predicted difference in relative abundance of recalcitrant (RPC) between wildfire and prescribed fire as a function of time since fire (TSF), limited to the extent of common TSF values. These are based on posterior simulation generating a 1000 credible mean trends from the fitted generalised additive model and the fire type difference calculated for each trend. The solid curve represents the mean difference and the shading is the 95% confidence band. Individual plots for two soil depths 0 - 5 cm and 6 - 15 cm for each of three microsites based on proximity to trees: open, largely between tree crowns; rough, adjacent to trees with rough bark; smooth, adjacent to trees with shedding bark.

For open microsites, at TSF < 10 years % RPC was similar for both fire types (*ca. 0.5%, Fig. 3.4). For WF, there was a decline in % RPC to a minimum of about 0.3% at TSF 25
years followed by an increase to original levels (i.e., 0.5%) at TSF 38 years. For PF, % RPC remained relatively constant (ca. 0.5%, Fig. 3.4) across the range of TSF (Fig.3. 4).

In the rough microsites, the % RPC was higher than in open microsites but the patterns for both fire types across the TSF range were similar (Fig. 3.4). % RPC for WF was initially ca. 0.9% at TSF < 10 years, dropping to 0.5% at TSF 25 years, then rising to ca. 0.7% at TSF 38 years. For PF, % RPC was relatively constant at ca. 0.6% throughout the TSF range.

For the smooth microsites, the magnitude and pattern of % RPC response to both WF and PF was similar to that of the rough microsites (Fig. 3.4), though PF estimates were truncated to 24 years.

3.3.2.2 Soil depth 6-15 cm
Estimates of % RPC for both fire types were relatively constant (i.e., ca. 0.25% to 0.5%) across the TSF range and among the microsites (Fig. 3.4). Estimated % RPC for WF tended to be higher than for PF up to ca. TSF 20 years (Fig. 3.5), with the strongest elevation being apparent in the rough microsites.

3.4 Discussion
Fire type (intensity) had divergent effects on soil C in the dry sclerophyll forests of the study region. Over the initial 15 years TSF, little effect of fire type (intensity) on % $C_{Tot}$ was evident. Beyond this range, however, there was a steady increase in % $C_{Tot}$ after WF whereas estimates for PF remained relatively constant. Thus, there is a fundamental difference in soil C storage after high intensity wildfires, with potential for soil C to increase substantially if fires are absent for long periods (i.e., > 30 years). Importantly, there was little evidence that these trends were driven by major variations in the recalcitrant fraction (i.e., % RPC). Small differences in % RPC attributable to fire type were mainly apparent in the early phase of the response and had diminished to be negligible by TSF 15 years (Fig. 3.5). These findings are important because average fire
return intervals in the study landscape are about 15 to 20 years. Thus, a major shift in fire activity would be required to alter soil C storages to a substantial degree. Importantly, any attempt to increase soil C storage would implicitly entail a significant reduction in the frequency of fires.

Differences in the stimulation of net primary productivity (NPP), decomposer efficiency in the litter and soil respiration to fire type (intensity) may partly explain these divergent responses in C metrics as a function of TSF. The degree to which surface litter is consumed and hence mineralized is comparatively moderate in low intensity fires (Morris et al., 2014, Goforth et al., 2005). The ash loads typically contain lightly charred and uncharred plant parts. Canopies are rarely consumed but scorch damage is common, with subsequent deposition of the dead leaves increasing soil surface cover. This has consequences for NPP through smaller post fire soil heating effects (Raison et al., 1986a) and a surface litter load skewed toward high lignin: cellulose which is slow to decay, locking up nutrients that would otherwise be available for NPP (Krebs et al., 2010, Alexis et al., 2007).

By contrast, WFs consume a large proportion of surface and aboveground fuels (Hammill and Bradstock, 2006, Chafer et al., 2004). Ash deposits are typically white/grey with visible char mainly fully carbonized (Brewer et al., 2013, Goforth et al., 2005). Substantial consumption of tree crowns is also common and any residual scorched crown provides a much lighter soil cover when it subsequently falls (Alexis et al., 2010, Hammill and Bradstock, 2006). The nutrients derived from ash on the soil surface that percolate into the soil structure are more likely to be available for plant uptake as soil surface decomposition activity is temporally suspended (i.e., surface litter is absent or sparse) (Alexis et al., 2012). A higher proportion of the subsequent above ground litter is derived from new growth which has lower lignin: cellulose, increasing amenability to decomposition (Krebs et al., 2010). Extra sunlight reaches the soil surface due to the reduced canopy foliage and soil cover, raising soil temperatures and boosting ground level photosynthetic potential (Hammill and Bradstock, 2006, Raison et al., 1986a). The greater mortality and consumption of leaves in WFs reduces
transpiration and potentially increases soil moisture availability. Soil texture
determines the vulnerability to evaporative deep drying from increased insolation after
fire; open sandy loams (the most abundant soil type in this study) are less susceptible
than fine clay based soils (Bradstock et al., 2012a). Any such effects are short lived as
foliage cover is restored within a few years and the recruitment and regrowth of trees
and shrubs is enhanced (Pickup et al., 2013, Antunes et al., 2009).

These early successional differences in response to fire intensity do not have an
immediate impact on soil C but do provide a boost to above ground biomass
accumulation rates. As the vegetation grows and other small scale disturbances
increase, fine and coarse litterfall rates accelerate, reaching 4 t ha\(^{-1}\) yr\(^{-1}\) at TSF 3 years
(Bridges, 2004). These accumulations change the soil surface conditions, inducing
greater decomposition rates via enhanced microbial activity and begin to add C from
this source to the soil. Estimated fine fuel (mostly surface litter) accumulation for
similar DSF, at TSF 15 years was ca. 30 t ha\(^{-1}\) but declined to ca. 20 t ha\(^{-1}\) at TSF 30 years
(Morrison et al., 1996). Other studies measured a maximum load at TSF 10 years and
declined to a slightly lower steady state between TSF 10 and 20 years (Bridges, 2004,
Fox et al., 1979). After the TSF 10 year peak in litter accumulation, the maximum rate
for addition of C to soil would be expected, which is consistent with our findings.

Based on the mechanisms described, we contend that the early successional conditions
following low intensity fires, immobilise nutrients and retard decomposition and
therefore the flux of new labile litter is low. Under such conditions (\textit{i.e.} a high C, low
mineral nutrient environment) microbial activity becomes self-limiting when the
available N and other mineral nutrients are exhausted (Lagerström et al., 2009). In
contrast the high intensity fire resets the board by releasing a pulse of mineral nutrients
and leaving little high C content litter that could lead to nutrient immobilisation. Thus,
the new growth litter sets a faster decomposition cycle. This is self-perpetuating, with
the rate of C addition to soil peaking when litter loads peak.
The difference in the extent of charring on above ground material (e.g., coarse woody debris and rough barked trees) is another factor explaining the fire intensity-based divergent C accumulation patterns. Charring associated with WF typically covers a higher proportion of the tree bole and penetrates deeper into the bark than PF charring (Gould et al., 2007, Gill et al., 1986). The input of PyC from bark char is likely to continue until the char is largely shed, i.e., up to 40 yrs TSF (Gould et al., 2011). Tree trunk diameter growth is the likely mechanism causing the char on bark to be shed (Bluff, 2014). Increased % $C_{\text{Tot}}$ is only apparent in the upper soil layer of longer unburnt sites (i.e., TSF > 25 years) affected by higher intensity fire. The importance of % RPC within the % $C_{\text{Tot}}$ pool began increasing from a low at TSF 25 years (Fig. 4). This is additional to the decomposition products of coarse woody debris and standing dead trees, the amount of which is also known to increase with fire intensity (Bennett et al., 2016). Consumption and charring of coarse woody debris during fire is proportional to fire intensity (Bi et al., 2015).

Patterns of soil C accumulation and the contribution of PyC are described in studies conducted across a range of forest types using a post disturbance chronosequence (Alexis et al., 2012, Foote and Grogan, 2010, Vargas et al., 2008). These identify a C pulse from PyC addition and newly killed SOM with a subsequent decline in both PyC and total C until the contribution of litter from new growth again dominates (i.e., TSF > 11 years) (Rao et al., 2014, Alexis et al., 2012). Hobley et al. (2017) reported increased soil PyC: total C (PyC not necessarily recalcitrant) in *Eucalyptus* dominated forests. While both PyC and total C declined over the 14 year chronosequence studied by Hobley et al. (2017), the rate of decline for total C was higher. This effect underpinned the change in the PyC: total C. By contrast, our RPC estimates showed a relative elevation in the early TSF phase following WF, consistent with inputs of PyC described in these other studies. It is understandable that this highly recalcitrant PyC fraction is not elevated relative to total C in sites affected by the lower intensity PF, based on the expected differences in char formation material and conditions (less woody material consumed and lower overall heating) (Santín et al., 2012).
It has been demonstrated that substantial proportions of the above ground biomass are converted to PyC during the passage of fires (Santín et al., 2015). Our results indicate that this PyC is not being abundantly incorporated into the mineral soil. Both char production and its rate of removal from the soil surface (due to increased erodibility in the early post fire environment) are proportional to the intensity of the fire experienced (Morris et al., 2014, Santín et al., 2012). The reduced cover (vegetation and litter) associated with higher intensity fire increases the risk of material being transported off site through fluvial processes (Moody and Martin, 2001). However, the extent of cover removal is not uniform; burn patchiness may be inversely related to fire intensity but is also very dependent on topographic and geological variation (Penman et al., 2008, Hammill and Bradstock, 2006). In less severely burnt patches litter dams may form and act as filters and deposition zones, reducing sediment movement into stream flows (Denham et al., 2009). Moderate and relatively gentle rainfall will stimulate resprouting and germination quickly, boosting foliage cover which provides protection from subsequent rain (Inbar et al., 1998). If high intensity rainfall occurs before foliage cover is established, severe erosion can be expected following either fire type (Morris et al., 2014). The fate of PyC (which may or may not be recalcitrant) displaced from the forest floor remains indeterminate, requiring further investigation.

3.4.1 Limitations
There is considerable spatial heterogeneity in the distribution of soil C in forest ecosystems (Bradshaw et al., 2013, Foote and Grogan, 2010). This imposes numerous constraints on the logistics of sampling and the ability of feasible sampling programs to detect effects of influences such as TSF and fire type. We attempted to overcome these constraints, by stratifying sampling in areas where differences in total C were expected (e.g., microsites) and by pooling multiple samples from each microsite within any given site. While effects of variations in TSF across 43 years of recent fire history were examined, effects of prior fire regimes may be stored in the pool of soil C. It was assumed that longer term effects prior to the limits of the recorded fire history were homogenised across all the sites.
While we estimated an increase in $\%C_{Tot}$ up to about 40 years after WF, it is possible that $\% C_{Tot}$ will reach a steady state value at some time after WF beyond the range measured here. Eventually inputs and losses of soil C may come into balance but the likelihood of this occurring and its timing in these forests is unknown. Further detailed knowledge of the controls on these processes and the way they change as a function of time since disturbance is required.

Characterising SOM in soil, particularly PyC, remains complex, expensive and difficult (Ascough et al., 2016). Techniques of predicting average nuclear magnetic resonance spectral results from the mid infrared spectra have been published (Forouzangohar et al., 2015). While this technique shows considerable promise for applications in ecological management, at the time of our study they were neither fully developed or cost effective. The digestion process used in our study effectively left only the most recalcitrant C as the residual (post digestion). This is not a measure of total PyC as defined by Jenkins et al. (2016a), hence we used the term RPC. Reducing the soil and charcoal to a very fine fraction prior to digest possibly made the SOM more available to oxidation, though this was done consistently across the entire treatment range. In contrast, other studies have digested coarser material (i.e. not finely ground) containing a wider spectrum of particle sizes with differing oxidation potential (Licata and Sanford, 2012, Ball et al., 2010, Ares et al., 2007, Kurth et al., 2006). In addition, these studies have used filtration to recover the soil residue which introduces the potential for contamination. We observed strong capillary action on the finer organic matter during filtration attempts, causing variable material recovery (authors’ unpublished observations). It was more prudent to minimise the spectrum of particle sizes, by pre-grinding, and employ evaporative recovery to avoid contamination and other complications associated with filtration. This produced a consistent and conservative estimate of the recalcitrant PyC, providing a relatively robust basis for investigation of the effects of TSF, fire type and site characteristics.
3.4.2 Future directions, influences and conclusions

Our estimates of % RPC, adjusted for % $C_{Tot}$, were only slightly elevated in WF affected topsoil for ca. TSF < 10 years. Thus, further work may be best focussed in estimation of the total soil C pool rather than estimation of RPC, which is time consuming and expensive. This would potentially provide greater scope for further scrutiny of TSF and other fire regime effects on soil C.

There is also a need for catchment scale studies that relate fire history to total C stocks in multiple topographic positions (including sediment yields in water courses) and incorporate the compound effects of other land management practices and disturbances. Ultimately this will provide better understanding of the PyC fate and successional soil C accumulation patterns in the whole landscape.

Our results established a basis for an examination of effects of fire regime factors in the same landscapes. Based on the results that suggest little difference in soil C between fire types (intensities) for up to TSF 20 years, variations in fire frequency and intensity in the future may have to be large to strongly affect soil C in these forests. Current policies which mandate increased prescribed burning, will lead to average increases in fire frequency and decreases in fire intensity in such forests (Bradstock et al., 2012a). However, we predict, based on our results, that likely variations from current fire regimes produced by such policies (e.g., doubling in treatment rates from the current level of about 1.5% of landscapes per annum) will have few major effects on soil C. The results suggest that only long-term fire exclusion is likely to have a major effect on soil C. The likelihood of this occurring in these landscapes, which exhibit rapid fuel accumulation, regular severe fire weather and frequent ignitions from lightning and people (Bradshaw et al., 2013) is negligible. Further work on understanding effects of variations in fire frequency and intensity on soil C is required to test these predictions.

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4 Soil Carbon in Australian fire-prone forests determined by climate more than fire regimes.

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4.1 Introduction

Fires have major, immediate and longer-term effects on the turnover and make-up of organic carbon (C) via heating and consumption of biomass and the redistribution of residual, oxidised solids and gasses into the atmosphere and soil. There is fossil evidence of vegetation fires dating back to the Silurian (ca. 420 Mya); a time when vegetation cover and atmospheric oxygen concentrations became sufficient to sustain combustion (Glasspool et al., 2004, Jones and Chaloner, 1991). Rapid oxidation of organic matter (living and dead) facilitated by fire can release gasses directly to the atmosphere while the fate of non-gaseous pyrogenic products is more variable (Lal, 2013, Mouillot et al., 2006). This is determined by the composition and size of non-gaseous products, coupled with the timing and strength of dispersal forces applied to them (Santín et al., 2016, Lal, 2013). In some forests, very little soil organic matter (SOM) is directly altered by fire, as heating in soils under moving fires is limited to the near surface (i.e. 0 to 5 cm depth) (Mondal and Sukumar, 2014, Bradstock and Auld, 1995, Raison et al., 1986b). The degree of consumption of the overlying humus is determined by its moisture content and the intensity of fire at the time, both of these factors being partially driven by weather (Santín et al., 2016, Boby et al., 2010, Bradstock et al., 2010). Fires leave a spectrum of biologically fixed C in the landscape ranging from living plant tissue to fully charred material and soots.
The portion of biologically fixed C in the post-fire landscape consisting of partially to fully charred plant tissue and soots, is termed pyrogenic carbon, PyC (Jenkins et al., 2016b). Substantial research effort has been expended on the perceived C sequestration potential of PyC associated with its assumed recalcitrance (Balduck et al., 2013b, Krull et al., 2003, Skjemstad et al., 1998). PyC recalcitrance can be strong, with observed persistence for centenial and millenial timescales, but is dependent on formation at high temperatures (>750°C) from coarse, lignified fuels (Bird et al., 2015, Bodí et al., 2014, Hammes et al., 2007). Decomposition rates in chars formed at lower temperatures (<450°C) or from fine material (low lignin content) may have weaker recalcitrance, which in some cases may be similar to other SOM particles of the same dimensions (Norwood et al., 2013, Knicker, 2011, Hammes et al., 2007). The recalcitrance of all SOM, including PyC, is enhanced when incorporated into the mineral soil where it is shielded from oxidation and other chemically degrading processes associated with fire (Santín et al., 2016). It has been argued that this physical protection by location in soil is potentially more important to C mean residence time (MRT) than the chemical recalcitrance of the specific SOM under consideration (Lal, 2013).

In natural forests, woodlands and shrublands, estimates of mineral soil C pools can exceed the aboveground amount, emphasising its importance in the global C cycle (Santín and Doerr, 2016, Vargas et al., 2008, Beringer et al., 2007). Despite the potential importance of soil pools of C, little is known about the way these pools may respond to variations in fire regimes (i.e. the frequency, intensity and season of fires) (Keeley et al., 2012). Such knowledge is important given that many forests in differing parts of the world are fire-prone (Archibald et al., 2013, Keeley et al., 2012), and that future changes in fire regimes are predicted (Clarke et al., 2011, Flannigan et al., 2009). A fundamental understanding of how soil C pools are affected by fire regimes is needed to predict their long-term fate given anthropogenic global change.

This study is an examination of how variations in recent fire regimes in fire-prone 
Eucalyptus dominated forests, in south-eastern Australia, have affected soil pools of C
and recalcitrant pyrogenic C (RPC; the most recalcitrant fraction of PyC). This study also examines how such fire regime effects may be ameliorated by regional-scale variations in temperature and rainfall within a homogenous forest type. Climatic models suggest that temperatures are likely to increase across eucalypt dominated forests, with possible increases or decreases in precipitation at a local level (CSIRO and Bureau of Meteorology, 2015, Clarke et al., 2013, Clarke et al., 2011). Such shifts have the potential to change net primary productivity (NPP), fire-proneness of the vegetation through floristic shifts and the incidence of severe fire weather in the landscape (Attiwill et al., 2014a, Flannigan et al., 2009, Roxburgh et al., 2005). This study was designed to provide some integrated insight into the way that changes to fire regimes and climate may affect: i) soil C pools generally; and ii) the ability to enhance long-term C sequestration in soils through manipulation of the fire regime using planned burning. For example, Adams (2013) and Volkova and Weston (2015) have proposed that planned burning could be used to enhance soil pools of PyC, though the evidence for appreciable inputs from such fires is equivocal (Jenkins et al., 2016b).

This study focussed on the fire frequency and sequential fire intensity aspects of fire regime. Given evidence that C pools in the soil may vary as a function of time since fire (Sawyer et al., 2018) the study design held this effect constant by sampling only sites burnt 10 to 12 years previously. Over this time since fire interval, soil C pools were found to be stable after both unplanned and planned fires (Sawyer et al., 2018). The study was carried out across the Sydney Basin Bioregion, which is dominated by fire-prone eucalypt forests. Variations in fire frequency created by both unplanned and planned fires were used to explore effects on soil C pools at sites distributed across rainfall and temperature gradients. Remotely sensed estimates of fire severity (estimated damage to vegetation from fire) were used as a proxy of fire intensity in the sampling design.

The study therefore aimed to estimate how fire frequency and intensity combinations affected the distribution of total C and RPC in mineral soils across regional-scale variations in climate (i.e. mean annual precipitation and temperature).
4.2 Methods

4.2.1 Study Sites

The study was carried out in the Sydney Basin Bioregion in south-eastern Australia (Fig. 1). The dissected landscapes of this Bioregion are dominated by sedimentary geology of mainly Permian/Triassic origins that result in predominantly acidic (e.g. pH 4 to 4.5), shallow (ca. 20 to 30 cm), sandy soils. Climate is cool-warm temperate with aseasonal rainfall. Vegetation is predominantly open forest and woodland (i.e. dry sclerophyll forest (DSF), (Keith, 2004)), dominated by *Eucalyptus, Corymbia* and *Angophora* spp., with an understorey often rich in sclerophyllous shrub species, graminoids and sedges (Keith, 2004). The region is fire-prone with an average interval of 15 to 20 years between fires (Bradstock et al., 2012b).

The sites sampled for this study were a subset of those originally established to examine vegetation responses to climatic and fire regime gradients across the region (Hammill et al., 2016). Areas in several parts of the Bioregion characterised by contrasting combinations of mean annual precipitation (MAP) and mean annual temperature (MAT), were initially identified, in order to test the effects of variations in rainfall and temperature on soil C. Two areas with MAP of 1200 mm and contrasting MATs (12°C, 16°C) were classified as wet and cold (WC) and wet and warm (WW) respectively. A third area, with MAP of 1000 mm and MAT of 16°C, was classified as intermediate and warm (IW) (Fig. 1 and Appendix C). Study sites were then selected within these climatically differentiated areas (hereafter referred to as climatic regions) based on fire regime attributes (i.e. fire frequency and fire intensity) in order to test effects of fire regimes on soil C.

Three levels of fire frequency (FF; one, two or four fires between 1972 and 2003) were selected for each combination of MAT and MAP. These selections were based on mapped fire records since 1972 (New South Wales Office of Environment and Heritage unpublished data). Three replicate sites for each combination of MAT, MAP and FF class were sampled. All sites were situated in areas last burnt in the 2001/2002 or
2002/2003 peak fire seasons (i.e. likelihood of similar intensity). Sampling was conducted over a 2.5-year period (2011 – 2013), therefore equating to a time since fire range of 10 – 12 years.

![Map of fire frequency and fire intensity study sites with insert locating Sydney region within Australia. Sites are grouped within the climatic regions indicated: wet and cold (WC); intermediate and warm (IW); and wet and warm (WW).](image)

**Figure 4.1:** Map of fire frequency and fire intensity study sites with insert locating Sydney region within Australia. Sites are grouped within the climatic regions indicated: wet and cold (WC); intermediate and warm (IW); and wet and warm (WW).

A separate investigation of the effects of fire intensity on soil C was carried out in one climatic region (IW, Fig.1 and Appendix C) at sites burnt by 2 successive fires (1993/1994, 2001/2002 fire seasons). Indices of fire intensity were derived by estimating fire severity from remote sensing, using methods described by Hammill and Bradstock (2006). For both fire seasons, areas that were burnt at either high severity which infers high intensity (H, canopies completely scorched or consumed) or low severity which infers low intensity (L, fire confined to the understorey) (Hammill and Bradstock, 2006) were identified. Sites with combinations of successive high/high (HH), high/low (HL), low/high (LH) and low/low (LL) were then identified and sampled. Between three and five replicate sites for each combination were sampled. It was only
possible to obtain sufficient quality fire severity data at potentially accessible sites for this one climatic region.

All sites sampled in both studies (FF and intensity combinations) were situated in DSF on sandstone derived soils, on ridge tops with slopes of < 10°. To minimise the effects of spatial correlation and pseudo replication, sites within each experimental category (climate region, fire frequency and fire intensity combination) were separated by the maximum extent achievable (within practical constraints) with the minimum separation being approximately 500 m.

4.2.2 Field Sampling

In natural forests and woodlands SOM distribution exhibits substantial spatial heterogeneity (García-Palacios et al., 2011, Liu et al., 2011, Fraterrigo et al., 2005). This spatial heterogeneity creates difficulties and expense in experimental design and analysis, in contrast to agricultural systems. There is, however, a degree of predictability in the distribution of C and other biologically cycled elements associated with the degree of concentration in biological activity, e.g. soil horizon, and the proximity to trees (Gallardo, 2003, Stoyan et al., 2000). This study utilised this concept in a stratified approach to sampling that was aimed at accounting for some of this heterogeneity. It was assumed that SOM distribution was a function of soil depth, proximity to trees and variations in tree bark type.

The dominant *Eucalyptus, Corymbia* and *Angophora* species in the forests of the study area vary widely in their bark characteristics (Brooker et al., 2012). Some species commonly found across the region (e.g. *E. rossi, A. costata*) have smooth bark which is shed annually, whereas other common species have rough bark that is either tightly bound (e.g. *E. sieberii*) or loose and stringy (*E. globoidea*) but not regularly shed. Thus, the area surrounding the base of trees of species with contrasting bark types is subject to characteristically different patterns of bark accumulation, thereby creating fine-scale heterogeneity in potential C inputs to the soil during and after combustion. In each site all litter and any duff were carefully removed to expose the surface of the mineral soil.
prior to soil collection. Mineral soils were then sampled in two depth classes (0 to 5cm and 6 to 15cm) from the same core hole and in three micro-sites based on proximity to trees and their bark type. These were: Rough, adjacent to base of rough barked trees; Smooth, adjacent to base of smooth barked trees; and Open, patches largely between tree crowns.

Within each of these 6 sampling combinations, 4 replicate cores were collected, pooled and processed in accordance with the method described in Sawyer et al. (2018)

4.2.3 Soil Total C Assay
Prior to this assay a randomly selected subgroup of soil samples were subjected to the hydrochloric acid fizz test to detect the presence of inorganic C. Significant contributions from inorganic C were very unlikely in this soil type and this was confirmed by the lack of reactivity to the acid test. Analysis of the soil for its total C concentration was conducted using a LECO dry combustion spectrometer (1250°C) with results expressed as % mass of soil (% CTot).

4.2.4 Recalcitrant Pyrogenic C Assay
A modified 1N nitric acid (HNO3) and 35% hydrogen peroxide (H2O2) digest (KMD) method was used (Kurth et al., 2006) to remove the biologically and chemically degradable C, leaving the most recalcitrant pyrogenic C (RPC). Details of the modifications and digest method used have been previously published (Sawyer et al., 2018). In brief the method involved:

i) oven drying of samples to constant weight at 45°C;
ii) pre-grinding of samples in a ball mill (TEMA brand) to pass through 120 μm sieve;
iii) use of reflux tubes capped with teardrop condensers to control moisture loss (Licata and Sanford, 2012);
iv) use of a block digester with a pre-programmed temperature control unit to slowly ramp up (8 hours) to the operating temperature of 100°C for a duration of 16 hours, helping to control volatility;
v) samples recovered by evaporation in drying ovens (85°C) after decanting and washing the oxidising reagents away;
vi) recovered digested soil samples assayed for total C as % mass of soil, using dry combustion spectrometer at 1250°C;
vii) scanning electron microscopy to examine a random selection of post-digest examples to ensure all remaining identifiable organic matter had the appearance of charcoal;
viii) a range of standard mixes of chemically clean silicious sand with varying known amounts of charcoal, sawdust and humic acid were digested and underwent subsequent C assay. Effectively, all non-charcoal organic matter was consumed by the digest while approximately 60% of the charcoal C survived.

4.2.5 Statistical analyses

We employed Bayesian modelling to directly estimate differences in soil C components (% CTot and % RPC) between fire regime and climatic combinations, while explicitly accounting for all sources of uncertainty. Conventional hypothesis testing, such as analysis of variance, would be unable to detect subtle effects against a background of substantial natural variability. By using this Bayesian approach we were able to address variation in replicate measures of pyrogenic carbon, and to apply variable selection (akin to ensemble modelling) within a single, consistent analytical framework. This builds on the Bayesian modelling approach used by Leon and Gonzalez (2009) for prediction of C content of Scottish soils.

The analysis consisted of a Bayesian multiple linear regression model incorporating variable selection and fitted using Markov Chain Monte Carlo (MCMC) methods. The approach is similar to that referred to as Bayesian Variable Selection Regression by Guan and Stephens (2011) and to Indicator Model Selection by O’Hara and Sillanpää (2009). The choice of this approach was motivated by: i) recognition of the scarcity in
4.2.5.1 Model description
A two-step modelling process was used in the investigation of fire frequency and fire intensity effects. The first step was to estimate % \( C_{Tot} \), then using the output as an input to the second step, % RPC estimation. This enabled examination of the relationship between % RPC and % \( C_{Tot} \) and allowed discrimination of effects of fire regime, region, micro-site and soil depth on % RPC that were additional to any such influences on % \( C_{Tot} \).

4.2.5.1.1 Relative importance of fire frequency, intensity, region and site characteristics on % \( C_{Tot} \).
Ensemble modelling was used initially to derive predicted means of % \( C_{Tot} \) from log-transformed % C empirical data, as a function of fire frequency across all factors (soil depth, region, micro-site). Regression analysis was performed, ranking the importance of variables by inclusion rate, assigning upper and 95% credible intervals, determining whether the 95% credible interval included zero and indicating the direction of any non-zero effect. A similar approach was then used to derive predicted means of % \( C_{Tot} \) for each fire intensity combination, soil depth and micro-site.
4.2.5.1.2 Relative importance of fire frequency, intensity, region, site characteristics and % $C_{Tot}$ on RPC.

For the second step, ensemble modelling estimates for mean % RPC as a function of either fire frequency or fire intensity, across all relevant factors (soil depth, region, micro-site, % $C_{Tot}$), were generated using the same modelling process described above. Within each combination, mean %RPC was calculated for each field sample by fitting a heavy-tailed distribution (a t-distribution with unknown mean, standard deviation and shape parameter) to the replicate RPC assay values. This approach ensured that any outlying assay values would be down-weighted in a data-driven manner. The mean of the fitted distribution for each experimental category became the response value for that MCMC iteration.

4.3 Results

4.3.1 Relative importance of fire frequency, climatic region and site characteristics on % $C_{Tot}$.

The predicted mean concentration of C (% $C_{Tot}$) was less than 6.5% in all categories (Fig. 2). % $C_{Tot}$ was consistently higher in the upper soil layer (0 to 5 cm) compared with the lower soil layer (6 to 15 cm, Fig. 2). The patterns of differences among the other predictors were also strongest in the upper soil layer where % $C_{Tot}$ tended to be higher for two fires, compared with one or four fires in the IW and WC regions (Fig. 2), though the 95% credible intervals were large. This pattern was particularly evident in micro-sites beneath trees (Rough and Smooth barked micro-sites, Fig. 2), but less apparent in the micro-site in gaps between trees (Open, Fig. 2). In the WW region, there was a trend for higher % $C_{Tot}$ with four fires (FF4) (Fig. 2) but the inclusion rate for this effect was low (Appendix D, Table D.1) and 95% credible intervals were large. This pattern was generally repeated in the lower soil layer (6 to 15 cm) in the respective regions, though differences between micro-sites were more subdued. In general, the Open micro-site had lower % $C_{Tot}$ than the micro-sites positioned beneath trees (Fig. 2).

The strong inclusion rates in the ensemble modelling for interactions between fire frequency and region (ca. 39% to 91%, Appendix D, Table D.1) and the three-way interactions between fire frequency, region and micro-site (ca. 15% to 47%, Appendix
D, Table D.1) further support the patterns described. Three-way interactions between fire frequency, region and soil depth had inclusion rates ranging from ca. 13% to 31% (Appendix D, Table D.1).

![Figure 4.2](image)

**Figure 4.2**: Predicted mean total % C as % mass of soil (with upper and lower 95% credible intervals) from ensemble modelling categorized by fire frequency, region and site characteristics. Fire frequency was represented as the number of fires (1,2,4) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200mm and annual average temperature 12°C; wet, warm (WW) annual precipitation 1200mm, annual average temperature 16°C; intermediate precipitation, warm (IW) annual precipitation 1000mm, annual average temperature 16°C. Soil depth was characterized by two layers (0-5cm; 6-15cm). Micro-sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).

4.3.2 **Relative importance of fire intensity and site characteristics on % C\textsubscript{Tot}**

The % C\textsubscript{Tot} in all fire intensity categories, across all site characteristics, was less than ca. 4.1% by weight, based on the ensemble modelling (Fig. 3). The % C\textsubscript{Tot} for the upper soil layer (0 to 5 cm) was consistently greater than the lower soil layer (6 to 15 cm) with a mean difference of 1.57% when all other factors were combined. The pattern of
difference for the other predictors, *i.e.* intensity combination and micro-sites, was strongest in the upper soil layer as reflected by their two-way interaction inclusion rates (ca. 24% to 50%, Appendix D, Table D.2).

The combination of low fire intensity followed by high (LH) had greater C concentrations than those predicted for other combinations across all micro-sites, though the 95% credible intervals were large. The differences for % $C_{Tot}$ between the other intensity combinations were relatively small. However, the order of the remaining intensity combinations for % $C_{Tot}$ showed variation between the micro-sites (Fig. 3).

Within any one fire intensity combination the % $C_{Tot}$ was highest under trees with shedding bark (Smooth) followed by under trees with rough bark (Rough) and lowest in micro-sites between trees (Open). These patterns applied to both soil layers, though in the lower soil layer (6 to 15cm) differences were more subdued.

**Figure 4.3:** Predicted mean total % C as % mass of soil (with upper and lower 95% credible intervals) from ensemble modelling categorised by fire intensity and site characteristics. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years (soil collected 10 years post the last fire) yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (0-5cm; 6-15cm). Micro-sites sampled
were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).

4.3.3 **Relative importance of fire frequency, region and site characteristics on recalcitrant pyrogenic C.**

Predicted mean recalcitrant pyrogenic C concentration in the soil (% by weight; % RPC) was generally low (<1%) irrespective of fire history, region and site effects (Fig. 4). The strongest predictor of % RPC was % C<sub>Tot</sub> with 100% inclusion rate in the ensemble modelling (Appendix D, Table D.3). Soil depth 6 to 15cm and four fires (FF4) were the only predictors of mean % RPC to have inclusion rates above 5% (ca. 13% to 16.5%, respectively, Appendix D, Table D.3) in the ensemble modelling.

Effects of region and micro-site were considerably less influential with low inclusion rates (ca. 4% and 1% respectively, Appendix D, Table D.3). Interactions between these predictors had little influence based on very low inclusion rates in the ensemble modelling (i.e. < 1%, Appendix D, Table D.3). Therefore, the distribution of % RPC effectively mirrored that for % C<sub>Tot</sub> across all categories (Figs 2 and 4): i.e. % RPC was effectively a constant fraction of % C<sub>Tot</sub> across all categories (Appendix E, Fig. E.1).
Figure 4.4: Predicted mean % RPC as % mass of soil (with upper and lower 95% credible intervals) from ensemble modelling categorized by fire frequency, region and site characteristics. Fire frequency was represented as the number of fires (1,2,4) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200mm and annual average temperature 12°C; wet, warm (WW) annual precipitation 1200mm, annual average temperature 16°C; intermediate precipitation, warm (IW) annual precipitation 1000mm, annual average temperature 16°C. Soil depth was characterized by two layers (0-5cm; 6-15cm). Micro-sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).

4.3.4 Relative importance of fire intensity and site characteristics on % RPC.
% RPC was less than 0.65% in all categories (Fig. 5). The strongest predictor of % RPC was % $C_{Tot}$ with 100% inclusion rate in the ensemble modelling (Appendix D, Table D.4). The fire intensity and micro-site effects on % RPC were negligible, with the highest inclusion rate for any factor being <5%. The inclusion rates for interactions quickly declined to zero (Appendix D, Table D.4). Effectively the pattern of distribution for % RPC mirrored that established for % $C_{Tot}$ (Figs 3 and 5): i.e. % RPC was effectively a constant fraction of % $C_{Tot}$ across all categories (Appendix E, Fig. E.2).
Figure 4.5: Predicted mean % RPC as % mass of soil (with upper and lower 95% credible intervals) from ensemble modelling categorized by fire intensity and site characteristics. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years (soil collected 10 years post fire) yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (0-5cm; 6-15cm). Micro-sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open)

4.4 Discussion

The results indicated that % RPC generally remained constant as a proportion of % C_{Tot} across fire regime, climate and site characteristic categories (Appendix E). Similarly, Wang et al. (2018) found that total soil organic carbon (SOC) was the strongest predictor of PyC across a range of environments in south eastern Australia. The consistency of this conversion rate of C to this recalcitrant form is important when considering both the measurement and the manipulation of soil C for sequestration.
Effects of fire regime and climatic variation on mineral soil C were therefore registered principally through responses of % $C_{Tot}$ rather than % RPC.

Climatic region was a much stronger determinant of % $C_{Tot}$ pools than any of the fire regime components (i.e. frequency and intensity) tested in this study. The highest % $C_{Tot}$ was estimated for the WC region. In both of the other regions the % $C_{Tot}$ levels were substantially lower than WC, with IW being the lowest. The detectability of the fire regime effect was small, and its direction driven by climatic factors. The role of climate is illustrated by the peak in % $C_{Tot}$ associated with the intermediate fire frequency treatment (two fires over 40 years) in the WC and IW regions, contrasted by little variation in % $C_{Tot}$ across the three fire frequency treatments in the WW region. This has important implications and needs careful interpretation when evaluating fire regime management impacts on C under various climate change scenarios. In the fire intensity study, the peak of % $C_{Tot}$ was observed in LH. Notably this work was confined to the one climatic region, IW, which had the lowest % $C_{Tot}$ of the three regions.

The higher % $C_{Tot}$ in the WC region was associated with lower MAT (driven by cold winter minimums), which may be sufficient to retard heterotrophic soil respiration rates, while higher MAP and adequate growing season temperatures may support strong NPP. The difference in soil C between this cool temperate forest region and the warm temperate counterparts on similar geology (i.e., the WW, IW) was within the range expected from the modelling of Haverd et al. (2013). This work also supports the NPP/heterotrophic soil respiration mechanism we proposed, with longer soil C residence time in the cool temperate versus warm temperate region (inferred lower soil microbial respiration). Similarly, Thomas et al. (2014) describe climatic trends for litterfall (positively correlated with MAP) and decomposition rate (positively correlated with MAT, subject to moisture availability) in DSF that further supports the theory of retarded heterotrophic soil respiration associated with lower MAT in the WC region.

The low detectability of fire regime effects (frequency and intensity combination) on SOM contrasts with many other studies (Bennett et al., 2014, Wanthongchai et al., 2008,
DeLuca and Sala, 2006, Adams and Attiwill, 1986). These studies claim to have examined fire frequency; however in these cases, fire frequency was confounded with substantial time since fire variation. The ability to infer fire regime effects beyond that of a single fire in these studies was therefore limited. Our finding of minimal effect from fire regime with time since fire held relatively constant was consistent with studies that have accounted for time since fire in evaluating fire frequency and fire intensity (e.g., Richards et al., 2012). The standardising of time since fire in this study therefore provides a more robust basis for understanding of the potential of other fire regime effects on soil C.

Higher estimated levels of $\% C_{\text{Tot}}$ under intermediate fire frequency in the WC and IW regions were possibly explained by the interplay between fire frequency, NPP and heterotrophic soil respiration, the latter two being driven by climate (Haverd et al., 2013, Penman and York, 2010, Lieth, 1975). Overall NPP is governed by precipitation and adequate growing season temperature (Lieth, 1975). Accumulation of C in the soil is determined by rates of aboveground material breakdown (inputs) and the rate of heterotrophic soil respiration, translocation and erosion (outputs) (Santín et al., 2016, Haverd et al., 2013), with the rate of heterotrophic soil respiration being most dependent on climate. In the WC and IW regions the limits to NPP are MAT and MAP respectively, with the precipitation effect being stronger (Haverd et al., 2013). Less frequent fire may lead to a drop in the availability of nutrients (e.g. phosphorus), elevating the C:N in surface debris, retarding the rate of decomposition and mineralisation (Lagerström et al., 2009). Combined with the absence of fire this would slow the movement of aboveground material to the soil. In the highest fire frequency, aboveground material may be consumed at a rate faster than it could be incorporated into the soil. The intermediate frequency represents a situation where aboveground material has sufficient time to provide soil inputs (breakdown accelerated by second fire and higher nutrient availability) at a rate higher than heterotrophic soil respiration. Heterotrophic soil respiration is more strongly inhibited by lower MAT, which may lead to higher total C values in the WC region. In the WW region both NPP and heterotrophic soil respiration are potentially at the maximum for these soil types (Haverd et al., 2013).
This has enabled soil C levels to fully recover between fires, even at the highest frequency treatment.

The peak in $\% C_{Tot}$ under the LH fire intensity combination may relate to low intensity fire killing but not consuming significant standing vegetation. This would have resulted in an elevated lignified tissue load on and above the soil surface. A subsequent high intensity fire is then likely to accelerate the charring and decomposition of this material enabling its incorporation into the mineral soil over the ten years prior to sampling. We estimated that a low intensity fire followed by a high intensity fire (LH) may enhance soil C storage by 2.9 Mg.ha$^{-1}$ with 95% credible intervals from -11.4 to 18.5 Mg.ha$^{-1}$, and a 0.66 probability of the difference being positive. The estimate was based on an approximate landscape average soil bulk density of 0.9 g.cm$^{-3}$ (from author’s unpublished data) limited to the top 5 cm of soil. This was applied to the difference in soil C concentration in comparison to two successive low intensity fires (LL). The similarity in size of soil C pools for high intensity fire followed by either high or low intensity fire (i.e., HH & HL respectively) to LL implies a similar difference to LH. However, these results infer that any gains in the $\% C_{Tot}$ pool from the LH combination could be subsequently lost following a third fire, irrespective of intensity.

This study was concerned with the C incorporated into the mineral soil where it is most protected from chemical, physical and biological degradation (Lal, 2013). The work has been done on ridgetops where C deposited on the soil surface is highly vulnerable to relocation and consumption (Santín et al., 2016, Santín et al., 2013). The fire regime may produce PyC, some of which is RPC, but unless it is retained on the site in a protected location, such as mineral soil, its fate is largely indeterminate and therefore not amenable to manipulation via the fire regime.

4.4.1 Limitations

The well documented variability in the distribution of soil C in natural systems demands a high level of targeted replication (Lal, 2013). Acting against this is the cost and time required to collect, process and analyse large numbers of samples. Potential variation in the results presented here was minimised by using a stratified sampling strategy that targeted micro-sites where differences in total C were expected. This potentially
maximises insights that could be gained from relatively limited replication. Greater replication of sites at the treatment level (i.e., climate, fire frequency and fire intensity) may have provided more precision in the estimation of the fire regime effects. Nonetheless, the magnitude of these effects is likely to remain relatively small even under higher levels of replication. Additionally, effects of variations on 40 years of recent fire history were examined but effects of prior fire regimes may be stored in the pool of soil C. It was assumed that longer-term effects prior to the limits of the recorded fire history were homogenised across the sites.

As Jenkins et al. (2016b) have argued there is a need for consistent definition of PyC. Accordingly, the term RPC was used here as only the most recalcitrant C was likely to have survived the digest process. Reducing the soil and charcoal to a very fine fraction prior to digest may have made the SOM readily available for oxidation, though this was done consistently across the entire treatment range. In contrast, other studies have digested coarser material (e.g. fraction < 2mm) containing a wider spectrum of particle sizes with differing oxidation potential (Licata and Sanford, 2012, Ball et al., 2010, Kurth et al., 2006). In addition, these studies have used filtration to recover the soil residue, which introduces the potential for organic contamination. Strong capillary action on the finer organic matter was observed in this study during filtration attempts, which resulted in its loss. It was considered more prudent to minimise the spectrum of particle sizes and avoid contamination and other complications associated with filtration. As a result, a highly consistent and conservative estimation of PyC was produced that provided a relatively robust basis for investigation of the effects of climate, fire regime and site characteristics on the most recalcitrant fraction of soil C as well as total soil C.

4.4.2 Implications and future directions

Based on our findings, future work on the manipulation of soil C pools via alteration of the fire regime in natural ecosystems needs experiments designed to explore more than just time since fire or single fire effects. A comprehensive and cumulative assessment of fire regime effects is required. The costs of future studies in natural systems, such as these eucalypt forests, may be constrained through a much greater emphasis on
measuring % \( C_{\text{Tot}} \) (relatively inexpensive analysis), based on the assumption that the recalcitrant C proportion and the inferred conversion rate is constant. This conclusion is further supported by the work of Lal (2013) who asserts that mean residence time for soil C is more driven by physical protection from erosion, consumption and abiotic factors unrelated to the chemical recalcitrance of the C in question.

This study suggests that potential for manipulating fire regime to enhance soil C sequestration, or even minimising soil C losses, is limited in this forest type. Climate variations were more influential determinants of estimated total soil C pools than fire frequency or intensity factors. By considering the difference in mean % \( C_{\text{Tot}} \) between climate regions and converting to Mg.ha\(^{-1}\) (using the same soil depth and bulk density applied to the differences in intensity combination, Section 4 above), a simple estimate of C stock shifts was generated. Climate shifts towards warmer MAT by up to 4°C (which is within the scope of fifty year climate models: CSIRO and Bureau of Meteorology, 2015) have the potential to substantially lower soil C pools in the WC region (ca 4.5 Mg.ha\(^{-1}\)). If temperature increases by this amount and MAP decreases by 200 mm, the predicted losses of C to the atmosphere are in the order of 7.3 Mg.ha\(^{-1}\). In the WC region, there was considerably more C in sites that had experienced 2 fires in 40 years compared to 4 fires in 40 years. Increasing fire frequency may therefore further exacerbate C losses in this region.

A whole ecosystem fire regime climate C model incorporating existing data on landscape factors such as soil cover, tree cover and estimations of above and belowground biomass can be developed on this basis, enabling an integrated understanding of fire regime and climate effects on the total C stores. Such an understanding is required to predict the consequences of landscape management decisions, in conjunction with future climate changes, on terrestrial C stores in these flammable ecosystems.

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5 Impacts on mineral soil C and N from fire frequency treatments between harvested and unharvested eucalypt forests in SE Australia

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5.1 Introduction

Increasing terrestrial carbon (C) sequestration through forest management is a global objective of growing importance (IPCC, 2014, McKinley et al., 2011). Given the widespread use of prescribed fire as a management tool in forests, an understanding of the impact of fire regimes on C stock dynamics is required to assess the potential to manipulate C to enhance sequestration. Changing climate is likely to increase the frequency of fires in many forest ecosystems (Bowman et al., 2017, Moritz et al., 2012). An increase in the number of days with severe fire weather is projected, leading to a greater likelihood of ignitions and larger fires (Attiwill et al., 2014a, Bradstock et al., 2014). In response, fire and land managers may consider increasing the use of prescribed burning for fuel reduction, in order to protect assets and timber resources (Adams, 2013).
The flux of C between terrestrial ecosystems and the atmosphere is a function of changes in biomass resulting from growth, decomposition and disturbance regimes, such as fire. Nitrogen (N) availability in terrestrial ecosystems influences net primary productivity (NPP) and the limit of ecosystem carbon carrying capacity (CCC: Roxburgh et al., 2006) via its function as a key nutrient for autotrophic organisms. C and N are fixed into terrestrial ecosystems primarily via biological activity (Adams, 2007). When organic matter oxidizes by decomposition or direct combustion, C and N based gases are returned to the atmosphere. These potentially contribute to the greenhouse gas pool, driving climate change (Anderson-Teixeira and DeLucia, 2011). Thus, consideration of the fate of both C and N in the soil under different fire regimes is required so that appropriate management strategies can be developed. Many fire prone forests are also subjected to timber harvesting, necessitating an understanding of the additive effects of fire and harvesting regimes on soil C and N dynamics, to aid the prediction of consequences of future management options in the context of global change.

Understanding the landscape-specific responses to disturbance scenarios is important given the role of soil C in global carbon cycles (Hobley et al., 2014) Considerable effort has been made in characterizing the types of C store in soil, i.e. measuring pyrogenic C (PyC) and assumed, or measured, degrees of recalcitrance (Hobley et al., 2017, Baldock et al., 2013a, Krull et al., 2003). However, the location of organic matter in the mineral soil may afford more protection from oxidization and breakdown (i.e. increased mean residence time), and thus may be a more influential determinant of the longevity of the pool of C stored than the specific chemical recalcitrance of PyC (Bradshaw et al., 2013,
Lal, 2013). In recent studies on forest soils, the recalcitrant PyC fraction was found to be relatively constant in the total C pool in mineral soils within eucalypt forest located on ridge tops, irrespective of substantial variations in fire regime (Time since fire (TSF), fire frequency, fire intensity), in the absence of other disturbances (Sawyer et al., 2018). Studies that have demonstrated relationships between soil C pools, including PyC, and fire regimes in combination with logging are rare.

A global metadata analysis found that timber harvesting effects on soil C and N pools in forests were dependent on whether saw-log or whole tree removal techniques were applied (i.e. positive and slightly negative on soil C and N respectively), with further variation determined by forest type (Johnson and Curtis, 2001). In a North American subalpine mixed conifer forest, examinations of compound disturbance from wind, logging and fire were shown to exacerbate C and N losses, which increased further at higher disturbance frequency (Buma et al., 2014). By contrast, burning had a slight positive effect on ecosystem net C and N stocks, while tree removal had a significant negative effect on these stocks in tropical savannas (Xiaoyong et al., 2004). Studies which have estimated soil organic accumulation in forests found that soil C pools were reduced (Johnson and Curtis, 2001), or remained unchanged (Sawyer et al., 2018) after low intensity fire. By contrast, soil C increased following high intensity fire (Johnson and Curtis, 2001) at TSF greater than 25 years (Sawyer et al., 2018).

The eucalypt (Eucalyptus, Corymbia and Angophora spp.) dominated forests of south-eastern Australia are fire prone and are subject to a relatively high frequency of burning
(Turner et al., 2008). Therefore, effects of any potential increase in fire activity on ecosystem functions via changes to climate and management requires evaluation (Driscoll et al., 2015). Our study used a long-term experiment, established in 1985 in a southeastern Australian dry sclerophyll forest, to investigate the effect that prescribed burning and timber harvesting had on soil C and N pools. We predicted that high numbers of low intensity fires would continually remove labile litter, thereby diminishing the potential for decomposition of this litter to recharge soil C and N. Similarly, we predicted that burning in combination with timber harvesting (i.e., whole tree removal) would diminish soil C and N pools, compared with soils exposed to infrequent burning in the absence of harvesting.

5.2 Methods
5.2.1 Site description
The study area location was in the Yambulla State Forest, in the southeastern corner of New South Wales, Australia, ca. 550 km south of Sydney and 30 km southwest of Eden (Figure 1). The Eden Burning Study Area (EBSA), established in 1985, is ca. 830 ha in size and contains 18 coupes (areas identified in forestry practice for block treatment) with an average area of 32 ha, ranging from 8 – 56 ha (Binns and Bridges, 2003).

The underlying geology was Devonian Wallagaraugh adamellite parent material with an altitudinal range of 180 m to 440 m. Climate was considered temperate with the maximum mean monthly temperature of 26.9°C in February, falling to 6.5°C in July with a mean annual precipitation of ca. 900 mm (Penman et al., 2008). These produced a coarse sandy loam soil with pH in the range 4.1 to 4.5 as measured by 1:4 soil water
extract by Turner et al. (2008). The geology and climate were relatively homogeneous, but substantial microhabitat and vegetation variability occurred. The vegetation conformed to the South-Eastern Dry Sclerophyll Forest classification of Keith (2004). The major overstorey species were *Eucalyptus consideniana, E. sieberi* and *E. agglomerate*. The understorey commonly contained the small tree *Allocasuarina littoralis*, shrubs *Daviesia buxifolia, Epacris impressa, Acacia terminalis, A. longifolia* and *Platysace lanceolate* and herbs *Gonocarpus teucrioides, Lomandra multiflora* and *Pteridium esculentum* (Binns and Bridges, 2003).
5.2.2 Experimental design

The entire EBSA experienced wildfire in 1972/73. Subsequently nine of the coupes were harvested in 1987/88 while nine were left unharvested (Binns and Bridges, 2003). Three post-harvest fire treatments were attempted in both harvested and unharvested coupes: no burning; routine burning (4 yearly); and frequent burning (2 yearly). Six permanent monitoring sites (25 m radius) in each of the 18 experimental coupes were established (108 sites: Binns and Bridges, 2003). Logistical constraints based around resources and weather led to inconsistencies in the application of the EBSA fire treatments. Nonetheless, a variable range of fire frequency, including no fires since
1973, has resulted to date. The prescribed fires varied considerably in their extent and patchiness. Ten burn evaluation plots were established within each site to quantify site burn coverage (Binns and Bridges, 2003). The number of fires affecting a site was estimated on the basis of the proportion of evaluation plots burnt following each attempted ignition: i.e. a site was considered to have burned if an ignition resulted in 20% or more of the evaluation plots being burnt (Penman et al., 2007). Of the 108 EBSA sites, 12 were excluded to avoid confounding factors (e.g. water logging and vegetation type), while the remaining 96 sites were utilised in our study. Of these, 47 were in harvested coupes, with 15 experiencing no fire and 32 experiencing from 1 to 7 fires between 1986 and 2009. A further 49 were in unharvested coupes with 13 of these unburnt and the remaining 36 experiencing between 1 to 7 fires in the period 1986 to 2009 (Details of site locations and treatments are given in Appendix F, Table 1).

5.2.3 Field collection
The primary determinant of spatial heterogeneity in soil organic matter was proximity to trees; with no significant effect on total soil % C (% C\text{Tot}) between microsites established beneath trees with contrasting bark types (rough or smooth bark) Based on previous work (Sawyer et al., 2018). Thus, in this study, stratified sampling was directed at only two microsites: Open, largely between tree crowns; and Trees, adjacent to the trunks of significant trees (i.e., > 20cm diameter at breast height). Three replicate cores from each of these categories were taken in random locations within two metres of a 50 m transect line perpendicular to the slope at the midpoint of the site. Litter, humus and duff were scrapped aside to expose the mineral soil surface. PVC pipe with an internal diameter of 2.3 cm was gently driven into the soil to a depth of 20 cm (where possible), using a hammer. If the 20cm penetration could not be achieved due to the presence of rock or woody roots, the actual depth of penetration was marked on the
outside of the tube (ca. 18% of cores). Both ends of the labelled tube were sealed to keep the core intact, placed in a sealed bag and stored until returned to the laboratory approximately one week later.

5.2.3.1 Soil processing and carbon and nitrogen analysis
In the laboratory, the soil samples were carefully pushed out of the tubes into clean trays. The depth of core penetration (as indicated by field markings), total depth of soil in core (a measure of soil compaction), depth of top soil component (as determined by dark gray to chocolate colour) and overall soil colour (grey, grey-yellow, yellow) were recorded. The extracted soil samples, including any root material and gravel, were then oven dried at 85°C until they reached constant weight. The oven dried samples (including roots and gravel) were subsequently ground to a fine homogenous texture, i.e., < 150µm, in a ball mill (TEMA brand). From the ground samples, three replicate sub-samples of ca. 1 g were taken. These sub-samples were then analysed for % C and % N by dry combustion (1250 °C) spectrometer (LECO Tru Mac (CHN, USA)).

5.2.4 Statistical analysis
Generalized Additive Models (GAM’s: Wood, 2011, Wood, 2006) were used to relate the amount of C, N and the C:N of soils to fire history (number of fires recorded for the site), site treatment (harvested or unharvested) and microsite (Open or Tree). GAMs were chosen to allow for possible non-linear trends without needing to make prior assumptions about their form. Number of fires was fitted as a smooth term using a penalised thin-plate regression spline and including an interaction with site treatment. All models were fitted by REML estimation. Models included a random effect term to
account for spatially correlated values between field samples within each site. To check for any residual spatial patterns, spline correlograms based on Moran’s I statistic were plotted. Models were fitted using the ‘mgcv’ package (version 1.8-15) in the R statistical environment (R Core Team, 2016). Correlograms were plotted using the ‘ncf’ package (version 1.1-7, https://CRAN.R-project.org/package=ncf : Bjornstad, 2016).

The number of fires for sites varied between 0 and 7 but the sample coverage across the range was uneven; relatively few sites experienced 5 or more fires. A smooth term for number of fires was fitted with weights assigned to samples relative to their proportion of data for each number of fires. Weights were scaled to preserve the overall magnitude of model log likelihoods so that samples for poorly represented fire frequencies had less influence on the fitted model and the corresponding bounds on the smooth term were wider.

The site-level variance of C and N estimates tended to increase with mean site value, but using log-transformed response values with a Gaussian identity link function gave homogeneous residuals. Site treatment (harvested or unharvested) and microsite (Open or Tree) were included as fixed categorical terms. The smooth term fitted for number of fires, allowed for an interaction with site treatment. Model validity was checked by inspecting residuals for normality and an acceptably even distribution of fitted values and predictors. For C:N, the response variable was log-transformed and the model fitted using a scaled t-distribution (implemented as the scat family function in the ‘mgcv’ package) producing an even distribution of residuals.
5.3 Results

5.3.1 Total soil C
Total C ranged from 18.4 to 311.8 Mg ha\(^{-1}\) with a distinctly right-tailed distribution (median 59.7; lower quartile 46.8; upper quartile 79.9 Mg ha\(^{-1}\)). Total C differed significantly between microsites with samples taken near trees significantly higher than those in open sites (p<0.0001) (Appendix G, Table 1b). There was no significant effect of number of fires and harvesting on total C (p>0.17), though there was a slight tendency for total C to be lower when exposed to high numbers of fires (Figure 2, Appendix G, Table 1b). Total estimates of C ranged from ca. 40 to 70 Mg ha\(^{-1}\) in Open microsites, whereas in Tree microsites the range was ca. 50 to 85 Mg ha\(^{-1}\) (Figure 2).
Figure 5.2: The C (Mg ha\(^{-1}\)) trend lines (solid lines) from GAM models based on measurements (open circles) in 96 sites, divided between timber harvesting treatments (49 unharvested; 47 harvested) affected by a number of fires ranging from no fire to 7 fires, between 1986 and 2009. The data are presented for two microsites, Open (areas largely between tree crowns) and Tree (adjacent to the trunks of significant trees). The grey shading represents 95% confidence intervals for the GAM trend line.

5.3.2 Total soil N
Total N ranged from 0.3 to 18.8 Mg ha\(^{-1}\) with a distinctly right-tailed distribution (median 1.2; lower quartile 0.8; upper quartile 1.5 Mg ha\(^{-1}\)). Responses of total N were influenced by a significant interaction between number of fires and harvesting (Figure 3, Appendix G Table 2a). Although, the parametric regression indicated a significant effect of microsite on estimated N (Appendix G Table 2b), the net differences between the microsite means were small (mean 0.11 and Std error 0.04) (Figure 3). The relationship between estimates of total N and the number of fires for the unharvested treatments was linear and negative (Figure 3), with estimated N declining from ca. 1.5 Mg ha\(^{-1}\) in unburnt sites to ca. 0.9 Mg ha\(^{-1}\) in sites burnt 7 times. By contrast, for
harvested treatments, there was a non-linear relationship, with estimated total N peaking ca. 1.6 Mg ha\(^{-1}\) at sites burnt by 2 fires, then declining to ca. 1 Mg ha\(^{-1}\) in sites burnt by 7 fires (Figure 3).

![Figure 5.3: The N (Mg ha\(^{-1}\)) trend lines (solid lines) from GAM models based on measurements (open circles) in 96 sites, divided between timber harvesting treatments (49 unharvested; 47 harvested) affected by a number of fires, ranging from no fire to 7 fires, between 1986 and 2009. The data are presented for two microsites, Open (areas largely between tree crowns) and Tree (adjacent to the trunks of significant trees). The grey shading represents 95% confidence intervals for the GAM trend lines.](image)

5.3.3 Soil C:N
Estimated C:N was influenced by a significant interaction between number of fires and harvesting (Figure 4, Appendix G Table 3a,). In both harvested and unharvested sites, estimated C:N increased with increasing numbers of fires, though the rate of increase was low and there were differences in the trajectory of response between harvested and unharvested sites (Figure 4). For example, in unharvested sites, estimated C:N
increased from ca. 46 to 50 in unburnt sites to ca. 58 to 63 in sites burnt by 4 fires, with little change beyond this level evident in sites burnt by higher numbers of fires (Figure 4). By contrast, in harvested sites, estimated C:N was ca. 48 to 52 in unburnt sites and ca. 43 to 47 in sites burnt by 1 or 2 fires, rising to ca. 58 to 63 in sites burnt by 7 fires (Figure 4). Although, the parametric regression indicated a significant effect of microsite on estimated C:N (Appendix G, Table 3b), the net differences between the microsite means were small (mean 0.085 and SE 0.017) (Figure 4).

**Figure 5.4:** The C:N trend lines (solid lines) from GAM models based on measurements (open circles) in 96 sites, divided between timber harvesting treatments (49 unharvested; 47 harvested) affected by a number of fires, fires ranging from no fire to 7 fires, between 1986 and 2009. The data are presented for two microsites, Open (areas largely between tree crowns) and Tree (adjacent to the trunks of significant trees). The grey shading represents 95% confidence intervals for the GAM trend lines.
5.4 Discussion

Measuring soil C and N in this long term study site, where plot based disturbance histories for over 30 years have been recorded, provided a unique insight into the role disturbance has in altering these values. The prediction that an increase in the number of fires would reduce mineral soil total C was not supported, although there was a trend for a small decline in response to increasing number of fires (Figure 2). Total N declined as the number of fires increased in unharvested coupes as anticipated, but in harvested coupes the relationship with number of fires was more complex (i.e., non-linear) (Figure 3). The C:N pattern mirrored the N pattern. The negligible decline in C relative to N enhanced the C:N response to number of fires. Contrary to expectation, timber harvesting had no direct effect on mineral soil C.

The elevation in soil N observed following 1 or 2 fires in association with timber harvesting may be due in part to the large organic matter load on the forest floor following logging. The mass of material may have been high enough to induce nutrient immobilization (Lagerström et al., 2009) and likely had a high content of lignin and phenolic compounds (Cornelissen and Cornwell, 2014, Krebs et al., 2010) which may have combined to retard litter decomposition rates. The physical disturbance of timber harvesting was shown to boost shrub recruitment, including N fixing species (Penman et al., 2009, Penman et al., 2008). Our results have an interaction between fire and timber harvesting contrasting to the results of previous shrub species studies (Penman et al., 2009, Penman et al., 2008), which have a significant positive response to timber harvesting but no related response to fire treatment.
The resultant high fuel load, may have generated soil heating sufficient to break the dormancy (i.e., >60°C: Penman and Towerton, 2008, Bradstock and Auld, 1995) of seeds of leguminous species (e.g. Acacia longifolia, A. myrtifolia, A. terminalis, Aotus ericoïdes, Davesia buxifolia, Hovea linearis, Playlobium formosum (Penman et al., 2009)), during the first post-harvest prescribed fire, even under mild weather conditions. It is possible that the fire in post-harvest debris could have generated temperatures high enough to kill some of the seedbank. Following consumption of this initial input of post-harvest fuels, subsequent prescribed fires did not produce heating sufficient to break the dormancy of leguminous species, due to the mild weather at the time of burning and low surface fuel loads (Penman et al., 2008). The consumption of post-harvest litter load by the first fire, may have released key nutrients (P, calcium-Ca, potassium-K) likely to stimulate decomposition and N mineralisation processes (Lagerström et al., 2009, Bauhus et al., 1993), thereby boosting soil N. This effect would have been enhanced by the initial fire-stimulated recruitment of N fixing species from seed. It is possible that the subsequent decline in soil N with increasing fire frequency reflected the lower volatilisation temperature of N (DeBano, 1991). This effect may have been compounded by the reported progressive decline of shrub species (including several leguminous N fixing species) in the frequently burnt plots (Penman et al., 2009, Penman et al., 2008), due to short burn intervals (Matthews et al., 2012) and low fire intensities (i.e., lack of post-fire seed germination) resulting in lower N fixation.

The planned frequent burning treatments (i.e. 2 year burn frequency) proved difficult to implement in this experimental study area. Cumulative burn scores and the number of ignition attempts indicated that burn cover of individual fires was generally low in these
treatments (Penman et al., 2007, Binns and Bridges, 2003), probably due to a combination of low fuel levels and mild ambient weather conditions at the time of burning. However, each successful burn may have volatilised most of the surface and near-surface N, whether combustion of organic matter was complete or not. By contrast, losses of C (less volatile: DeBano, 1991) were more likely directly related to the degree of organic matter consumed. The resultant elevated C:N increased the potential for nutrient immobilisation retarding decomposition rates (Cornelissen and Cornwell, 2014, Lagerström et al., 2009). In the lower frequency treatment (routine burning), fuel loads were possibly higher at the time of each fire, which may have resulted in higher fire intensities and more even and complete consumption of litter.

In contrast, the unharvested areas did not initially have elevated fuel loads resulting from post-harvest debris. Thus from the commencement of the experiment, the temperatures experienced in the soil during the prescribed fires (very low intensity) may have been proved insufficient for breaking seed dormancy of N-fixing legumes and the transfer of litter nutrients to the soil (Penman et al., 2008). Recruitment of N fixing species was low, leading to a decline in shrub and herb species of this kind (Penman et al., 2009, Penman et al., 2008) resulting in likely reduced net N fixation from the outset. Coupled with the losses of N through volatilisation and litter consumption, soil N resupply was likely to be less than uptake rates and overall loss of N from the ecosystem, leading to a consistent declining trend in N as a function of fire frequency in the unharvested treatments.
In general, C sequestration potential in soil is known to be limited by nutrient availability: e.g., Orgill et al. (2017) found higher nutrient basalt soil had greater C sequestration potential than the lower nutrient granite soil and higher upper limits on C input rates. Given the nutrient poor, sandy soils of the EBSA study area, it is likely that effects of frequent fire would further drive down the inherent limited C sequestration potential of these soils. The high mineral soil C:N associated with high fire frequencies in our study, was at levels found by other studies to result in nutrient immobilisation (Soong and Cotrufo, 2015). In tallgrass prairie (USA), PyC with a C:N of around 50 was associated with N limitation effects on microbial activity, which was reversed by the addition of fresh litter with lower C:N (Soong and Cotrufo, 2015). This indicated that nutrient availability was limited in the presence of fire-affected SOM but that microbes were capable of responding to more inputs of unburnt material with more labile nutrients. In sub-alpine heath (Australia), antecedent fire induced increased soil C and C:N which may indicate that increased fire activity could result in N immobilisation (White-Monsant et al., 2017). The elevated C:N that was estimated in our study could be sufficient to retard NPP and if the frequency of burning is sustained, could lead to diminution of ecosystem C stocks including AGC.

The C:N recorded in our study (range 43 to 63) is above the C:N range of ca. 20 to 46 identified by Turner et al. (2008) in the EBSA. It was suggested that the absence of fire in these forests led to sufficiently low C:N (20 to 25) to result in declining tree health (Turner et al., 2008). This fire exclusion N eutrophication hypothesis was subsequently rejected by Turner and Smith (2016) who found no relationship between mistletoe infestation and fire frequency in the EBSA treatments. We reported C:N on the top 20
cm of soil as a whole (including fine roots) and this may be partially responsible for the
difference in our observed C:N values compared with those of Turner et al. (2008).
Notably the values reported by Turner et al. (2008) do not take into account long term
effects of burning frequency. There is a scarcity of published studies that provide
mineral soil C:N, estimated from the total organic C and N for comparable Australian
forests (Snowdon et al., 2005). The review by Snowdon et al. (2005), for dry sclerophyll
forests in Australia reported a mean C:N of 32.6 with a minimum of 3.0 and maximum of
59.6 compiled from 91 different studies and adjusted for variations in method of
calculation. The fire frequency dependent range of C:N ratios in our study had means
(43 – 63) which are at the upper end of, and in some cases, exceed this range. By
contrast, mineral soil C:N reported in a North American mixed oak pine forest (range 40
to 50) did not find a clear association with burn frequency (Neill et al., 2007).

5.4.1 Future directions, implications and conclusions
There is a need for further long-term monitoring of the interactive effects of timber
harvesting and fire management practices on ecosystem attributes, such as soil C. This
is particularly important where high fire frequency has the potential to deplete soil N
and diminish productivity and C sequestration potential. The 23-year history of
treatment explored in this study may only be an indicator of longer term trends if
harvesting and fire cycles are maintained at the same rates.

The vulnerability of soil C and N stocks to high rates of burning and other disturbances
may be linked to ecosystem recovery traits i.e., mode of regeneration (prevalence of re-
sprouting or those primarily dependent on seeding for recruitment i.e., non-
resprouting) for taxa associated with key ecosystem functions such as nitrogen fixation (Hammill et al., 2016, Bassett et al., 2015, Moreira et al., 2014). The vulnerability of such taxa will also vary with ecosystem productivity, making it important to examine combined disturbance effects across a range of factors known to impact on productivity e.g., climate, soil parent materials and topographic position (Pausas and Austin, 2001). There is also a need to consider the soil C and N response in conjunction with effects of timber harvesting or fire frequency in the aboveground C and N pools in order to derive a more complete understanding of the vulnerability and dynamics of these nutrient stocks.
6 Carbon cycles for SE Australian DSF mineral soils driven by climate and fire regime variables: The state of knowledge and future research directions.

6.1 Introduction

In the preceding chapters, the responses of total mineral soil C (Chapters 3, 4 and 5) and total mineral soil N (Chapter 5) to a range of influences, including contrasting climate factors, varying fire regimes and a compounding anthropogenic disturbance (timber harvesting), were estimated in fire-prone, dry sclerophyll forests (DSF) of south-eastern Australia. In chapters 3 and 4, the most recalcitrant fraction of pyrogenic C (% RPC) was estimated in addition to soil total C (% C$_{\text{Tot}}$). The insights gained from the individual studies were useful in promoting the understanding of land management implications and identifying future ecological investigation needs, however these insights were limited by the scope of the individual studies. This chapter provides an enhanced, holistic assessment of the wider role that climate, fire regimes and harvesting have on soil C across dry sclerophyll forested landscapes, including the potential effects of climate change on macro-scale carbon storage in the soil.

6.1.1 Summary of preceding investigations and findings

Chapter 3 investigated the influences of time since fire (TSF) and its interaction with fire intensity on the patterns of post-fire soil C accumulation in dry sclerophyll forest. The key finding was that there was no change in estimated mineral soil % C$_{\text{Tot}}$ for up to 20 years TSF irrespective of the inferred intensity of the last fire (i.e., low; prescribed fires & high; wildfires) but at longer TSF after wildfires, estimated soil % C$_{\text{Tot}}$ increased. For the first few years after wildfires, estimated % RPC was slightly higher than after prescribed fires but was effectively constant, irrespective of fire intensity, at longer TSF.

The different % C$_{\text{Tot}}$ responses to increasing TSF were largely explained by considering early post-fire successional differences. Wildfires were considered to have removed most of the decomposition resistant organic matter. This was subsequently replaced by litter from new growth (low lignin: cellulose) which is generally more labile than scorched or high lignin litter which is abundant following low to moderate intensity
prescribed fires (Krebs et al., 2010, Alexis et al., 2012, Alexis et al., 2010). These less labile litters can induce nutrient immobilisation (Soong and Cotrufo, 2015) causing potential feedback through low nutrient availability, generating lower quality (less decomposable) subsequent litter, perpetuating the problem.

In Chapter 4, I investigated fire frequency effects across three distinctive climate regions in one relatively homogeneous dry sclerophyll forest type. In addition, the effects of different fire severity (i.e. as an index of fire intensity) combinations from successive wildfires were investigated. The key finding was that climate had a stronger effect than fire frequency on the size of the estimated mineral soil C pool. The largest soil C pool was estimated to occur under a wet and cold (WC) climate, via presumed effects of high precipitation, an adequate growing season temperature (i.e. resulting in relatively high NPP) and winter conditions sufficiently cold to retard seasonal soil respiration rates. The smallest soil C pool was estimated in forests with lower precipitation but warmer mean annual temperature (MAT). The lower precipitation and higher temperature was likely to have retarded NPP (Haverd et al., 2013) and litter decomposition rates but may have had little effect on relative soil respiration (Thomas et al., 2014). Small effects associated with fire frequency were found but both their magnitude and direction were climate dependent. There was a significant increase in soil C associated with a low intensity fire being followed by a high intensity fire; irrespective of the intensity of any subsequent fire, the effect could not be repeated. In both the fire frequency and intensity treatments the response of % RPC mirrored that of % $C_{Tot}$: i.e. it was effectively a constant across all combinations of climate and fire regimes sampled.

In Chapter 5, a long-term experimental site was utilised to evaluate fire frequency treatments in combination with timber harvesting treatments on estimated total soil C (Mg.ha$^{-1}$) and total soil N (Mg.ha$^{-1}$). I found no direct effect on estimated total soil C from either fire frequency or timber harvesting. Increasing fire frequency had a linear, negative impact on estimated soil N stocks in unharvested sites, which contrasted with harvested sites where the relationship was non-linear but ultimately negative in
response to high frequencies of burning. The estimated C:N at very high frequencies of burning exceeded 60. These levels could potentially induce nutrient immobilisation that may lead to a decline in net ecosystem productivity, ultimately reducing ecosystem C capital (Soong and Cotrufo, 2015).

6.2 Macro-scale implications of fire regime variation for soil C

6.2.1 Soil C and N stocks and their response to shifts in fire regimes

In terms of soil C, shifts associated with fire frequency or fire intensity were small in magnitude or undetectable, even when considered in combination with timber harvesting. High frequency of low intensity fire did reduce the soil N pool in the absence of timber harvesting, which induced a rise in the C:N to levels known to cause nutrient immobilisation (Soong and Cotrufo, 2015). This may reduce productivity leading to a net reduction in both aboveground biomass and the soil C pool. The only substantial effect of fire regimes on soil C pools was a substantial increase in % C_Tot for the unlikely scenario of a long absence of fire after a wildfire (i.e. TSF > 30). This is a difficult management outcome to plan for and achieve in the context of fire-prone forests with relatively short fire return intervals (i.e., 15 - 20 years: Bradstock et al., 2012a) and would be contrary to the likely longer term response of fire to climate change. Overall, the evidence supports the contention that the soil C of these forests is strongly buffered against the effects of all but the most extreme changes in fire regime.

Given the functional importance of the high C:N identified in Chapter 5, I have utilised previously unpresented data on soil N associated with the study described in Chapter 4 to outline possible trends in C:N across DSF of the Sydney region (Table 6.1a). C:N was estimated to show little response to fire frequency with the peak occurring at intermediate frequencies (i.e., two or three fires in 40 years). There was greater variation in mean C:N between the climate regions. The highest C:N of ca. 60 occurred in the Wet Cold region (WC), while the Wet Warm (WW) region had a C:N ca. 49 and the Intermediate Warm (IW) region a C:N of ca. 37 (Tables 6.1a and b).

Table 6.1(a): Summary C:N estimates by climate region and fire frequency. (b) Summary C:N statistics by climate region. Climate regions: wet, cold (WC), annual precipitation 1200mm and annual average temperature 12°C; wet, warm (WW) annual
precipitation 1200mm, annual average temperature 16°C; intermediate precipitation, warm (IW) annual precipitation 1000mm, annual average temperature 16°C. Fire frequencies: one, two, three and four fires in 40 years, collected at standardized TSF of ca. 10 years. The mean values and upper and lower 95% confidence bounds of observed data for top 5cm of soil are presented. Total C and total N were determined by dry combustion spectrometry (LECO) and the ratios fitted using Generalised Additive Models (GAM).

<table>
<thead>
<tr>
<th>Climate Region</th>
<th>Fire Frequency</th>
<th>Lower 95%</th>
<th>Mean</th>
<th>Upper 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IW</td>
<td>1</td>
<td>28.78</td>
<td>34.85</td>
<td>38.27</td>
</tr>
<tr>
<td>IW</td>
<td>2</td>
<td>29.44</td>
<td>38.34</td>
<td>50.14</td>
</tr>
<tr>
<td>IW</td>
<td>3</td>
<td>29.30</td>
<td>41.58</td>
<td>65.44</td>
</tr>
<tr>
<td>IW</td>
<td>4</td>
<td>27.38</td>
<td>34.62</td>
<td>40.75</td>
</tr>
<tr>
<td>WC</td>
<td>1</td>
<td>44.60</td>
<td>57.81</td>
<td>70.69</td>
</tr>
<tr>
<td>WC</td>
<td>2</td>
<td>51.29</td>
<td>63.80</td>
<td>84.14</td>
</tr>
<tr>
<td>WC</td>
<td>3</td>
<td>45.06</td>
<td>58.28</td>
<td>76.76</td>
</tr>
<tr>
<td>WC</td>
<td>4</td>
<td>49.55</td>
<td>59.63</td>
<td>75.79</td>
</tr>
<tr>
<td>WW</td>
<td>1</td>
<td>35.53</td>
<td>46.62</td>
<td>58.24</td>
</tr>
<tr>
<td>WW</td>
<td>2</td>
<td>42.27</td>
<td>52.99</td>
<td>63.31</td>
</tr>
<tr>
<td>WW</td>
<td>3</td>
<td>34.72</td>
<td>48.53</td>
<td>82.80</td>
</tr>
<tr>
<td>WW</td>
<td>4</td>
<td>33.18</td>
<td>47.00</td>
<td>57.78</td>
</tr>
</tbody>
</table>

These C:N estimates reflect the trends in estimated mean soil total C between the regions, implying that estimated soil N was relatively constant across the three regions at TSF 10 to 12.5 years. Possible explanations for the estimated lack of N response to either climate or fire frequency in the Sydney region estimates, compared with the responses in Chapter 5, may be related to differences in TSF and the likely intensities of the fires experienced in the two studies.
The Chapter 4 study held TSF relatively constant at a point where aboveground fuel loads were known to be approaching steady state (i.e., aboveground flux to soil via decomposition at a maximum) for these forest types (Denham et al., 2009, Conroy, 1993, Van Loon, 1977, cited in, Watson and Hulley, 2011). The Chapter 5 study relied on timber harvesting and prescribed fire treatments subsequent to a prior wildfire. This gave some variability in TSF with the shortest TSF being associated with the highest frequency treatments. The Chapter 4 study was based on wildfires with inferred higher average fire intensities contrasting to the very low intensity and patchy prescribed burns associated with the Chapter 5 study. As established in the evaluation of fire type effects in Chapter 3, the different strengths of stimulation to NPP and the quality of litter may also account for a lack of N response to fire frequency and climate in Chapter 4. In the Chapter 5 study there was an accumulative negative effect on soil N from repeated low intensity burning while C remained relatively constant, most likely driven by the lower volatilization temperature of N relative to C compounds. Across the climate regions in Chapter 4, the C:N variation may have been related to temperature and precipitation limitations on decomposition and soil respiration rates (see discussion on C storage climate scenarios, Section 6.2.3).

In this thesis, the RPC response to fire frequency was estimated to be relatively constant in relation to wildfires with a maximum frequency of four fires in 40 years. By contrast, Hobley et al. (2017) reported rapid assimilation of PyC into mineral soil following a low intensity, prescribed fire. In Chapter 5 of this thesis the estimated C content of soils was relatively constant despite very high frequency low intensity prescribed fires (i.e., up to 7 fires in 22 years). It is possible that the total soil C pool was being maintained by PyC addition, increasing the proportion of RPC in the total C pool. Therefore, it would be prudent to consider future research on the RPC response to high frequency of low intensity fire treatments, using the stored material from the Chapter 5 study.

6.3 Climate change scenarios and the likely shift in soil C pools

The mineral soil C pool was strongly buffered against fire regime variations and other disturbances. It was not so strongly buffered against variations in climate. Thus, climatic change has the potential to affect NPP, litter decomposition rates or soil respiration and
resultant soil C over long periods of time. The extent to which shifts in climate will alter
the vulnerability of forest biomass to fires is dependent on the nature of their
adaptation to fire frequency and intensity (Backnäss et al., 2012). The forests under
consideration in my studies are dominated by resprouting species and are adapted to
survive and recover rapidly after irregular and frequent high intensity fires (Bassett et
al., 2015). As a result, the estimated aboveground biomass of these forests is relatively
insensitive to wide variations in fire frequency and severity (Gordon et al., 2018 in
review). Similarly, the soil C pool was estimated to be relatively unresponsive to such
fire regime variations (Chapters 3,4 & 5).

By contrast, climate was potentially a more potent influence on soil C stocks. Here I
outline the magnitude of climate as a determinant of overall soil C stocks investigated
by scaling up the available plot-scale estimates to account for the area of each climate
region. Once the stocks in each climate region had been estimated, the effects of a range
of probable climate change scenarios on soil C stocks were briefly examined and
discussed. Development of a more comprehensive modelling approach that can
accommodate the uncertainties of data associated with each of the variables is then
outlined.

6.3.1 Calculation of approximate soil C stocks by climate regions
The previously estimated mean % \( C_{tot} \) associated with climate region, was used to
estimate likely changes in the amount of C stored in the ridgetop soils of the Sydney
Basin Bioregion across a range of possible future climate scenarios. The area and
volume of ridgetop topsoils under DSF or woodland, within each of the identified
climate regions, was estimated to establish the current C stocks. The bounds of any
potential climatic shift were set by the range used in Chapter 4 i.e., up to 4 \( ^\circ \)C difference
in MAT and up to 200 mm difference in mean annual precipitation (MAP).

The approach for scaling up study site data to an estimate of the soil C stock in the
climate regions is described in Fig. 6.1. The first step in this process was to estimate the
proportion of study sites occupied by each micro-site type. It was assumed that the
three different micro-sites occupied equal proportions of the area of each sample site (based on the author’s unpublished data). A landscape C stock (Mg. ha⁻¹) estimate for each micro-site was then calculated from Equation 6.1, using the estimates of mean C concentrations (% CTot) for each microsite provided in Chapter 4, and the same with their 95% confidence bounds (Fig. 6.1).

\[
C_{\text{Mirg}} = \% C_{\text{Tot}} \cdot D \cdot B
\]

**Equation 6.1**

where: \( C_{\text{Mirg}} \) was the estimated stock of C in a given microsite across a particular climatic region (Mg. ha⁻¹); \( \% C_{\text{Tot}} \) was the mean soil C concentration for that microsite/region combination; D was soil depth (cm); B was bulk density (g. cm⁻³). A landscape average B = 0.9 g cm⁻³ was estimated (author’s unpublished data). Only the topsoil component was considered: \( i.e., D = 5 \)cm.

The resulting estimates of \( C_{\text{Mirg}} \) stock for each microsite region combination were then averaged to produce an overall estimate of average upper soil C stock for that combination (Fig. 6.1). The results of these calculations are presented in Table 6.2.
Figure 6.1: Flow diagram summarising the method for converting plot scale experimental data to estimates of site-level total C stock in each climate region of the Sydney Basin Bioregion. The schematic shows the three micro-site types: open patches largely between tree crowns (Open); adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth).
Table 6.2: Mineral soil C stocks, in the 0 – 5 cm layer, by climate region: wet, cold (WC), annual precipitation 1200mm and annual average temperature 12°C; wet, warm (WW) annual precipitation 1200mm, annual average temperature 16°C; intermediate precipitation, warm (IW) annual precipitation 1000mm, annual average temperature 16°C. Mean C stocks and their upper and lower 95% confidence bounds are expressed in Mg ha\(^{-1}\).

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean Mg ha(^{-1})</th>
<th>Lower 95% Mg ha(^{-1})</th>
<th>Upper 95% Mg ha(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>IW</td>
<td>13.4</td>
<td>6.57</td>
<td>24.2</td>
</tr>
<tr>
<td>WW</td>
<td>16.2</td>
<td>8.06</td>
<td>29.4</td>
</tr>
<tr>
<td>WC</td>
<td>20.7</td>
<td>10.1</td>
<td>37.8</td>
</tr>
</tbody>
</table>

The extent of DSF and its woodland forms using vegetation maps based on Keith (2004) was estimated for the bioregion. Because soil C concentrations were estimated on ridgetops, it was necessary to define the area of ridgetop within this forest type. While there are many possible approaches for identifying ridgetops from a digital elevation model (DEM) (see discussion in section 6.3.3) the Top Hat Transformation (THT: Dawson et al., 2016) was selected as a viable published methodology that provided sufficient accuracy. It was then necessary to estimate the area of the climate regions studied in Chapter 4 within the domain of DSF on ridgetops (Table 6.2). The simplest analogue was achieved by categorising on altitude. Ridgetops above 700 m corresponded to the WC region, ridgetops >400 m <700 m to IW region and those at <400 m were analogous to the WW region.
Table 6.3: Extent of studied landscape (MGA Zone 56): 190 - 400 km E, 6190 - 6365 km N (Approximately: south to Wollongong, north to Cessnock, west to Bathurst). Vegetation mapping based on dry sclerophyll forest/woodland (Keith, 2004) and ridgetops identified from digital elevation model by Top Hat Transformation (Dawson et al., 2016) were used to delineate the landscape element of interest. These areas were categorised by elevation class to approximate the three climate regions: wet, cold (WC), annual precipitation 1200mm and annual average temperature 12°C; wet, warm (WW) annual precipitation 1200mm, annual average temperature 16°C; intermediate precipitation, warm (IW) annual precipitation 1000mm, annual average temperature 16°C.

<table>
<thead>
<tr>
<th>Climate region analog</th>
<th>Elevation</th>
<th>Area (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC</td>
<td>Area above 700m</td>
<td>20 707.1</td>
</tr>
<tr>
<td>IW</td>
<td>Area 400m - 700m</td>
<td>20 793.5</td>
</tr>
<tr>
<td>WW</td>
<td>Area below 400m</td>
<td>19 655.9</td>
</tr>
<tr>
<td><strong>Total landscape</strong></td>
<td><strong>All</strong></td>
<td><strong>61 156.6</strong></td>
</tr>
</tbody>
</table>

These new data were used to predict effects of possible climate change scenarios on soil C across the DSF ridgetops of the Sydney Basin Bioregion (see discussion in section 6.2.3). The data for each region when considered individually have valid 95% confidence bounds. The process of adding or subtracting the mean values of C for regions to estimate shifts across climate gradients, did not permit the simple addition or subtraction of the bounds. Therefore, it was not possible to present reliable bounds of the shifts in means between regions. A more sophisticated modelling approach, that will enable bounded estimates on the differences between climate regions and other environmental factors is proposed and outlined in section 6.3.3.

6.3.2 Selecting likely climate change scenarios

The general consensus for future climates in the study region is that MAT is most likely to rise while predictions for MAP are equivocal and determined by more localised factors (CSIRO and Bureau of Meteorology, 2015, Bradstock et al., 2014, Clarke et al., 2011). My study data provided comparison of MAT between climate regions of 4°C and a MAP difference of 200mm. The following sections are based on climate changes that are both likely and quantifiable within the limits of the MAT and MAP combinations studied.
6.3.2.1 Warmer, precipitation unchanged

Under this scenario NPP may remain limited by precipitation while soil respiration rates are likely to increase, thereby lowering total soil C stocks. If MAT rose by 4 °C in the 20 707 ha of ridgetop DSF currently in the WC region, the projected loss from soil C stocks would be \( \text{ca.} 4.5 \text{ Mg ha}^{-1} \) (Table 6.2. and Chapter 4). The estimated total loss from this region would be 93.18 Tg C from the soils, based on its area (Table 6.3).

6.3.2.2 Warmer, drier

A decline in MAP of 200 mm per annum (\( \text{i.e.} \) the range used in the study in Chapter 4) is likely to retard NPP slightly. The elevated MAT is unlikely to provide substantial stimulation to NPP when considered together with the retarding effect of lower moisture availability (Chapter 4 Discussion). Soil respiration is also most likely to be stimulated by warmer temperatures; but decomposition rates may well be diminished by the drying effect. Aboveground biomass is likely to increase slightly with potentially the greatest increase occurring in surface litter/fuel. The combined acceleration of soil respiration and slowing of C flux to the soil from decomposing litter will most likely reduce soil C stocks to a greater extent than any aboveground C gains. Potential C losses from the WC region ridgetop soils (\( \text{ca.} 20 707 \text{ ha} \)) was calculated from estimated differences in soil C stocks between the WC and IW regions (7.3 Mg ha\(^{-1}\), Table 6.2.2.1 and Chapter 4), yielding a potential loss of soil C in the order of 151.16 Tg.

6.3.2.3 Temperature unchanged, drier

Under this scenario NPP may be reduced through lower MAP while soil respiration rates would be little altered, although litter decomposition would be retarded by the drier conditions. The rate of inputs to the soil would therefore be lower, while output remained constant. Soil stocks would be lower, but much of the difference may be retained in the form of ground litter. This may potentially increase fuel availability and risk of fire. The difference in soil C stocks between the region with MAP of 1000 mm and MAT of 16 °C (IW) when compared to the region with MAP of 1200 mm and the same MAT (WW) was 2.8 Mg ha\(^{-1}\) (Table 6.2 and Chapter 4). This would yield a potential loss from the soil C pool of 55.04 Tg. It is not possible to extrapolate the results to estimate
the effect of drying on the WC region however some retardation of NPP and aboveground decomposition rates could be expected.

6.4 Implications, future directions and conclusions.

6.4.1 C sequestration potential
The C content in the soils of the fire-prone forests of the Sydney Basin Bioregion and Yambulla State Forest, were strongly buffered against shifts in fire regime over the short to median term (i.e., up to four decades). This resistance to change in all but the most extreme application of fire frequency indicates that there is little potential for enhancing soil C sequestration through manipulation of fire regimes. Variations in fire regimes did not appear to influence the concentration of PyC in the soils of these forests. Thus, any potential differences in the production of PyC under particular fire regimes may be masked by the transport of this material in the steep dissected landscapes of the region. There is a need to better track the movement of PyC through fluvial processes at catchment scales, through major watercourses and ultimately into oceanic sinks (Neris et al., 2014, Santín et al., 2016).

6.4.2 Where does the recalcitrant PyC go?
Hobley et al. (2017) reported the rapid assimilation of PyC into the mineral soil. This finding was not supported by findings presented in this thesis, for DSF on sandstone ridgetops of the Sydney Basin Bioregion, which focused on the most recalcitrant fraction of PyC (RPC). Only minor responses of estimated RPC to variations in fire regimes were found.

If PyC is being incorporated after being produced in fires, how recalcitrant is it? In Chapters 2, 3 & 4, the measure of PyC (% RPC) was estimated on the basis of the amount of total C that survived a robust, yet consistent, chemical digestion (1M HNO₃ and 35% H₂O₂). Prior to the adoption of this methodology, tests on standards that contained known amounts of charcoal, humic acid and sawdust, demonstrated near complete oxidation of both humic acid and sawdust. By contrast approximately 50% of the
charcoal C survived the digest (Chapter 2). This surviving charcoal was therefore considered to be highly recalcitrant material.

Santín et al. (2012) estimated that 5.9 t ha⁻¹ of PyC was deposited in ash following a severe wildfire in south east Australian mixed eucalypt forest. Assuming that 50% of the PyC material was in the same highly recalcitrant form defined as RPC in this thesis, RPC would have been added at an expected rate of ca. 2.9 t ha⁻¹ to the mineral soil after each wildfire. The fact that RPC stocks were relatively constant across wide variations in fire frequency and that the total amount of RPC in mineral soils was lower than the expected addition from a single fire (see Table 6.4) implies that a high throughput rate of PyC in the system must occur. The simplest explanation would be that very little of the RPC deposited in ash at the soil surface is actually incorporated into the mineral soil. The uncertainty of the fate of the missing PyC reinforces the need for further research in this area.

Table 6.4: Mineral soil recalcitrant pyrogenic carbon (RPC), in the 0 – 5 cm layer, stocks by climate region: wet, cold (WC), annual precipitation 1200mm and annual average temperature 12°C; wet, warm (WW) annual precipitation 1200mm, annual average temperature 16°C; intermediate precipitation, warm (IW) annual precipitation 1000mm, annual average temperature 16°C. Mean RPC stocks and their upper and lower 95% confidence bounds are expressed in t ha⁻¹.

<table>
<thead>
<tr>
<th>Region</th>
<th>RPC t ha⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>WW</td>
<td>1.85</td>
</tr>
<tr>
<td>WC</td>
<td>2.39</td>
</tr>
<tr>
<td>IW</td>
<td>1.53</td>
</tr>
</tbody>
</table>

6.4.3 Proposed model for estimating carbon at the landscape scale

The studies undertaken in this current thesis provided broad estimates of the total amount of soil C, and its potential shifts, associated with different climate change scenarios and fire regime outcomes. There is a need for a landscape scale model for the C stocks above and belowground in response to these influences. A more robust and
sophisticated modelling approach that builds on the foundations laid in this thesis is currently being developed (C. Gordon et al. pers. Comm). This involves a Bayesian hierarchical modelling approach to extend measurements of soil C from ridgetop field sites to bounded estimates for ridgetops at the landscape scale, while considering sources of variation at site and landscape levels. This approach builds on the existing analyses of field measurements, which examined the influence of site-level variables: soil depth, micro-site (sample from an open area or near a rough or smooth barked tree), fire history and broad-scale climatic effects. A summary of the model’s development to date is presented here.

Estimation at the whole site level will use field counts of rough and smooth barked trees at each site to infer the distribution of micro-site proportions overall and at the individual site level, with the tendency for overlapping influence between trees at different densities modelled as a ‘balls in bins’ process (Raab and Steger, 1998). Similarly, the distribution of surface soil versus rock proportions overall and at the individual site level will be inferred from transect counts of rock frequency. The resulting distributions will then be combined into for the surface soil layer within each micro-site at a new (unobserved) ridgetop site. Then, drawing on previous analyses, the distribution of site-level soil C will be estimated.

Extending site-level estimates to the landscape level requires mapping ridgetops. Many algorithms have been proposed for identifying ridges and other landforms from a digital elevation model (DEM: Zámolyi, 2006, Summerrell et al., 2005, Dawson et al., 2016). Different algorithms typically produce somewhat different results and it will be important to account for this uncertainty when estimating soil C at the landscape level. To accomplish this, ridgetop pixels will be identified from the DEM in a probabilistic manner rather than deterministically. The general approach will be to use field site locations (with additional, manually identified ridgetop locations) as training points for a statistical model, which relates the occurrence of a ridgetop to spatial layers derived from the DEM (e.g. slope; curvature; view azimuth; flowpath length). The results will then be expressed as credible distributions of ridgetop area with climatic or other
subsets of the study area. Finally, these components of within-site, between-site and landscape level variation will be brought together as a Bayesian hierarchical model, to estimate bounded stocks of soil C and to predict differences in relation to fire and climatic variables.

6.5 Future directions

The key generalisations to emerge from the work presented in this thesis are:

- a substantial negative impact on soil C stocks in response to the climate shifts is likely to occur in response to future climate change;
- there was little direct soil C response to fire frequency or fire severity;
- accordingly, there is little potential for the use of fire to manipulate soil C pools for positive sequestration in fire-prone forests.

The climate driven losses in soil C stocks may result from shifts in aboveground productivity and decomposition rates changing the potential flux to soil and the rate of release through belowground respiration i.e. flux to atmosphere. Any tendency for these forests to accumulate more litter may only exacerbate their attendant fire risk. Using prescribed fire to mitigate these fuels may then release the C to the atmosphere. This scenario applies in the short term but as aboveground productivity mass of C declines under a warmer, drier climate then eventually soil C stocks will follow. Both the short and longer-term scenarios potentially accelerate climate change.

Conversely, under current climate conditions the use of frequent prescribed fire in these landscapes is unlikely to negatively impact on soil C stocks in the short to medium term. The total soil N losses induced by the same fire management strategy may have longer-term implications on ecosystem productivity, decomposability of litter and resultant soil C pools. Enhancing soil C stocks by managing the fire regime would require long-term fire exclusion (>30yrs) subsequent to wildfire events. Given that these landscapes currently experience much shorter average fire return intervals such an outcome is improbable.
There is considerable scope for building further understanding by development of the model outlined in section 6.4.3. Current and ongoing collaboration on this project aims to achieve a fully integrated above and belowground C stock estimation in relation to climate and fire regime variables. There is the possibility that this model could be integrated with fire spread, catchment and hydrological models to track the fate of PyC (including RPC) from its terrestrial origins through to oceanic sinks. These steps would help reveal the sources and sinks of terrestrial C stocks, including PyC, relative to disturbance regimes and climate shifts.
7 References


SCHOENFELD, C. & MICHEL, T. 2006. An Open-Vessel Microwave Digestion System for Preparing Large Samples of Volatile or Heterogeneous Materials. American Laboratory, 38, 14.


# Appendices

## Appendix A Chapter 3 Study sites

Table 1. Time since fire chronosequence site locations datum GDA 94 zone 56 map grid of Australia (mga) coordinates. The type of the last fire to affect the site identified as wildfire (WF) and prescribed fire (PF) and the time since last fire (years, unless otherwise specified).

<table>
<thead>
<tr>
<th>Site ID</th>
<th>site name</th>
<th>E_mga</th>
<th>N_mga</th>
<th>FireType</th>
<th>years since fire to collection (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ro 20W2</td>
<td>321520</td>
<td>6219728</td>
<td>WF</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Scol 5W</td>
<td>277729</td>
<td>6275994</td>
<td>WF</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Fal 10pb</td>
<td>273263</td>
<td>6272953</td>
<td>PF</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>Red 15W</td>
<td>262624</td>
<td>6254816</td>
<td>WF</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>Bw 10w2</td>
<td>340644</td>
<td>6288138</td>
<td>WF</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Scol 10pb</td>
<td>277817</td>
<td>6276122</td>
<td>PF</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>Kt 30+</td>
<td>258477</td>
<td>6259505</td>
<td>WF</td>
<td>36</td>
</tr>
<tr>
<td>8</td>
<td>ING 2WPB</td>
<td>260424</td>
<td>6260962</td>
<td>PF</td>
<td>2 weeks</td>
</tr>
<tr>
<td>9</td>
<td>Boo 20 W</td>
<td>281219</td>
<td>6273180</td>
<td>WF</td>
<td>19</td>
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<tr>
<td>10</td>
<td>Red 5W</td>
<td>262345</td>
<td>6254907</td>
<td>WF</td>
<td>4</td>
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<tr>
<td>11</td>
<td>Vic 5WHS</td>
<td>248112</td>
<td>6282403</td>
<td>WF</td>
<td>7 months</td>
</tr>
<tr>
<td>12</td>
<td>Vic 5 WLS</td>
<td>248072</td>
<td>6282367</td>
<td>WF</td>
<td>7 months</td>
</tr>
<tr>
<td>13</td>
<td>AND 5W</td>
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<td>6256293</td>
<td>WF</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>Permo 5W</td>
<td>253288</td>
<td>6279065</td>
<td>WF</td>
<td>7 months</td>
</tr>
<tr>
<td>15</td>
<td>BO 5 PB</td>
<td>281314</td>
<td>6273202</td>
<td>PF</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>Red 30+</td>
<td>257062</td>
<td>6260133</td>
<td>WF</td>
<td>36</td>
</tr>
<tr>
<td>17</td>
<td>And 5p</td>
<td>261752</td>
<td>6256355</td>
<td>PF</td>
<td>7 months</td>
</tr>
<tr>
<td>18</td>
<td>Ro5P1</td>
<td>321156</td>
<td>6230515</td>
<td>PF</td>
<td>5 months</td>
</tr>
<tr>
<td>19</td>
<td>SHA 5pb</td>
<td>278944</td>
<td>6272893</td>
<td>WF</td>
<td>7 months</td>
</tr>
<tr>
<td>20</td>
<td>FAL 5PB</td>
<td>274399</td>
<td>6274869</td>
<td>PF</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>RO20W1</td>
<td>319411</td>
<td>6216647</td>
<td>WF</td>
<td>20</td>
</tr>
<tr>
<td>22</td>
<td>GreyPt2</td>
<td>321967</td>
<td>6229971</td>
<td>PF</td>
<td>1</td>
</tr>
<tr>
<td>23</td>
<td>WOM30W</td>
<td>306321</td>
<td>6312593</td>
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<td>24</td>
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<td>6262010</td>
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<td>25</td>
<td>WOM 20P</td>
<td>308148</td>
<td>6311563</td>
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<tr>
<td>26</td>
<td>Bun&lt;15P</td>
<td>328238</td>
<td>6225749</td>
<td>PF</td>
<td>11</td>
</tr>
<tr>
<td>27</td>
<td>PUT 10W</td>
<td>297220</td>
<td>6297558</td>
<td>WF</td>
<td>10</td>
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<tr>
<td>28</td>
<td>WOM30P</td>
<td>306877</td>
<td>6314176</td>
<td>PF</td>
<td>24</td>
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<td>29</td>
<td>GN=30W</td>
<td>313560</td>
<td>6307175</td>
<td>WF</td>
<td>38</td>
</tr>
<tr>
<td>30</td>
<td>GRD&lt;10W</td>
<td>246353</td>
<td>6258710</td>
<td>WF</td>
<td>10</td>
</tr>
<tr>
<td>31</td>
<td>BUN U2</td>
<td>329099</td>
<td>6225579</td>
<td>PF</td>
<td>11</td>
</tr>
<tr>
<td>32</td>
<td>WOM 10P</td>
<td>308413</td>
<td>6314084</td>
<td>PF</td>
<td>8</td>
</tr>
<tr>
<td>33</td>
<td>GRD&gt;30P</td>
<td>246542</td>
<td>6259919</td>
<td>PF</td>
<td>43</td>
</tr>
</tbody>
</table>

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8.2 Appendix B Chapter 3 Regressions

Regression tables presented for the analyses of % $C_{Tot}$ (Table 1) and % RPC (Table 2), the full R scripts and original data are available from the corresponding author.

**Table 1**: The analysis of % $C_{Tot}$ response to TSF via GAM smooth terms for interactions (Table 1a) and parametric regression of the categorical variables: two fire types, wildfire (WF) and prescribed fire (PF); two soil depths, 0 - 5 cm and 6 - 15 cm; and three microsites, Open, Rough and Smooth (Table 1b). Reference categories were: wildfire (WF); soil depth 0 - 5 cm; microsite Open.

**Table 1a**: Approximate significance of GAM smooth terms

<table>
<thead>
<tr>
<th>Smooth terms</th>
<th>edf</th>
<th>Ref.df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interaction: TSF, WF, 0 - 5 cm soil depth</td>
<td>1.908</td>
<td>1.973</td>
<td>5.310</td>
<td>0.00915**</td>
</tr>
<tr>
<td>Interaction: TSF, PF, 0 - 5 cm soil depth</td>
<td>1.511</td>
<td>1.823</td>
<td>0.392</td>
<td>0.56527</td>
</tr>
<tr>
<td>Interaction: TSF, WF, 6 - 15 cm soil depth</td>
<td>5.438</td>
<td>6.334</td>
<td>2.831</td>
<td>0.00799**</td>
</tr>
<tr>
<td>Interaction: TSF, PF, 6 - 15 cm soil depth</td>
<td>1.000</td>
<td>1.000</td>
<td>0.180</td>
<td>0.67195</td>
</tr>
<tr>
<td>Interaction: Site, flag, site</td>
<td>26.446</td>
<td>29.000</td>
<td>14.772</td>
<td>&lt;2e-16***</td>
</tr>
</tbody>
</table>

* P<0.05; ** P<0.01; *** P<0.001

**Table 1b**: Parametric coefficients

| Categorical variable         | Reference category | Estimate | Std. Error | T value | Pr(>|t|) |
|------------------------------|---------------------|----------|------------|---------|---------|
| (Intercept)                  |                     | 1.33574  | 0.06953    | 19.210  | <2e-16*** |
| fire type: Prescribed (PF)   | Wild (WF)           | -0.19773 | 0.09916    | -1.994  | 0.0467*  |
| soil depth: 6 - 15 cm        | 0 - 5 cm            | -0.56593 | 0.02402    | -23.561 | <2e-16*** |
| microsite: Rough             | Open                | 0.29559  | 0.02711    | 10.905  | <2e-16*** |
| microsite: Smooth            | Open                | 0.27876  | 0.02845    | 9.800   | <2e-16*** |

* P<0.05; ** P<0.01; *** P<0.001
Table 2: The analysis of % RPC response to TSF adjusted for % $C_{Tot}$ via GAM smooth terms for interactions (Table 2a) and parametric regression of the categorical variables: two fire types, wildfire (WF) and prescribed fire (PF); two soil depths, 0 - 5 cm and 6 - 15 cm; and three microsites, Open, Rough and Smooth (Table 2b).

Table 2a: Approximate significance of GAM smooth terms

<table>
<thead>
<tr>
<th>Smooth terms</th>
<th>edf</th>
<th>Ref.df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interaction: TSF, WF, 0 - 5 cm soil depth</td>
<td>3.139</td>
<td>3.678</td>
<td>9.795</td>
<td>8.24e-07 ***</td>
</tr>
<tr>
<td>Interaction: TSF, PF, 0 - 5 cm soil depth</td>
<td>1.000</td>
<td>1.001</td>
<td>0.117</td>
<td>0.732</td>
</tr>
<tr>
<td>Interaction: TSF, WF, 6 - 15 cm soil depth</td>
<td>1.000</td>
<td>1.000</td>
<td>0.786</td>
<td>0.376</td>
</tr>
<tr>
<td>Interaction: TSF, PF, 6 - 15 cm soil depth</td>
<td>1.375</td>
<td>1.621</td>
<td>0.500</td>
<td>0.437</td>
</tr>
<tr>
<td>Log %$C_{Tot}$</td>
<td>2.718</td>
<td>3.493</td>
<td>89.033</td>
<td>&lt; 2e-16 ***</td>
</tr>
<tr>
<td>Interaction: Siteld, flag.site</td>
<td>25.251</td>
<td>29.000</td>
<td>7.252</td>
<td>&lt; 2e-16 ***</td>
</tr>
</tbody>
</table>

* P<0.05; ** P<0.01; *** P<0.001

Table 2b: Parametric coefficients

| Categorical variable     | Reference category | Estimate | Std. Error | T value | Pr(>|t|)   |
|--------------------------|--------------------|----------|------------|---------|------------|
| (Intercept)              |                    | -0.71224 | 0.05967    | -11.936 | <2e-16***  |
| fire type: Prescribed (PF)| Wild (WF)          | -0.03538 | 0.08123    | -0.436  | 0.6633     |
| soil depth: 6 - 15 cm    | 0 - 5 cm           | -0.06606 | 0.03917    | -1.686  | 0.0923     |
| microsite: Rough         | Open               | 0.04492  | 0.03561    | 1.261   | 0.2078     |
| microsite: Smooth        | Open               | -0.02328 | 0.03675    | -0.634  | 0.5266     |

* P<0.05; ** P<0.01; *** P<0.001
8.3 Appendix C Chapter 4 Study sites

Table 1: List of site locations used to assess the effect of 40 years of fire frequency on %C, %N and %recalcitrant pyrogenic C (RPC). The frequencies were 1 fire in 40 years, 2 fires in 40 years and 4 fires in 40 years. All sites were last burnt in either 2001/2002 or 2002/2003 fire years. The sites are divided into three climatic regions; wet and warm, wet and intermediate and wet and cold. Coordinate locations are given in MGS zone 56.

<table>
<thead>
<tr>
<th>Site label</th>
<th>Fire Frequency (fires/40years)</th>
<th>Climatic Region</th>
<th>MGA Zone 56</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Easting</td>
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<td>Wet and Warm</td>
<td>330416</td>
</tr>
<tr>
<td>Gosf1f2</td>
<td>1</td>
<td>Wet and Warm</td>
<td>329791</td>
</tr>
<tr>
<td>Gosf1f3</td>
<td>1</td>
<td>Wet and Warm</td>
<td>329506</td>
</tr>
<tr>
<td>Gosf2f1</td>
<td>2</td>
<td>Wet and Warm</td>
<td>337170</td>
</tr>
<tr>
<td>Gosf2f2 New</td>
<td>2</td>
<td>Wet and Warm</td>
<td>338437</td>
</tr>
<tr>
<td>Gosf4f3</td>
<td>2</td>
<td>Wet and Warm</td>
<td>337739</td>
</tr>
<tr>
<td>Gosf4f1 New</td>
<td>4</td>
<td>Wet and Warm</td>
<td>341096</td>
</tr>
<tr>
<td>Gosf4f2</td>
<td>4</td>
<td>Wet and Warm</td>
<td>340108</td>
</tr>
<tr>
<td>Gosf4f3 New</td>
<td>4</td>
<td>Wet and Warm</td>
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<td>Blac1f1</td>
<td>1</td>
<td>Wet and Cold</td>
<td>250699</td>
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<tr>
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<td>Wet and Cold</td>
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<td>Wet and Cold</td>
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<tr>
<td>Site label</td>
<td>Severity combination</td>
<td>MGA Zone 56 Easting</td>
<td>Northing</td>
</tr>
<tr>
<td>------------</td>
<td>----------------------</td>
<td>---------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Kuln 2F1</td>
<td>Low Low</td>
<td>315981</td>
<td>6322208</td>
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<td>Yengo LL2</td>
<td>Low Low</td>
<td>317396</td>
<td>6336732</td>
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<td>Yengo LL3</td>
<td>Low Low</td>
<td>317655</td>
<td>6340124</td>
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<td>Kuln 2F3</td>
<td>Low High</td>
<td>316883</td>
<td>6322210</td>
</tr>
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<td>Kuln 3F2</td>
<td>Low High</td>
<td>318893</td>
<td>6323625</td>
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<tr>
<td>Yengo LH3</td>
<td>Low High</td>
<td>317729</td>
<td>6340158</td>
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<tr>
<td>Yengo LH4</td>
<td>Low High</td>
<td>318246</td>
<td>6340028</td>
</tr>
<tr>
<td>Yengo LH5</td>
<td>Low High</td>
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<td>6340761</td>
</tr>
<tr>
<td>Kuln 2F2</td>
<td>High Low</td>
<td>318009</td>
<td>6323034</td>
</tr>
<tr>
<td>Kuln 3F2 New</td>
<td>High Low</td>
<td>322637</td>
<td>6325483</td>
</tr>
</tbody>
</table>

**Table 2:** List of site locations used to assess the effect of the severity pattern of successive fires in the 1993/1994 and 2001/2002 fire years. The severity combinations were; Low Low (LL), Low High (LH), High Low (HL) and High High (HH). The location coordinates are given in MGS zone 56.
<table>
<thead>
<tr>
<th>Name</th>
<th>Impact</th>
<th>Value1</th>
<th>Value2</th>
</tr>
</thead>
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<td>6323135</td>
</tr>
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<td>Kuln 3F1 New</td>
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<td>322675</td>
<td>6329359</td>
</tr>
<tr>
<td>Yengo HH2</td>
<td>High High</td>
<td>317163</td>
<td>6335978</td>
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<tr>
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<td>High High</td>
<td>317876</td>
<td>6337588</td>
</tr>
<tr>
<td>Yengo HH4</td>
<td>High High</td>
<td>318401</td>
<td>6339418</td>
</tr>
</tbody>
</table>
8.4 Appendix D: Chapter 4 Regressions

Table 1. Importance in ensemble modelling (% inclusion) of the predictors fire frequency, region and site characteristics and their interactions for predicting mean total % C. Fire frequency was represented as the number of fires (1,2,4) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200mm and annual average temperature 12°C; wet, warm (WW) annual precipitation 1200mm, annual average temperature 16°C; intermediate precipitation, warm (IW) annual precipitation 1000mm, annual average temperature 16°C. Soil depth was characterized by two layers (a,0-5cm; b,6-15cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open). Table includes the results of regression analysis, coefficient means, upper and lower bounds (95% confidence) and inclusion of zero or not within coefficient bounds (non zero).

<table>
<thead>
<tr>
<th>Effects</th>
<th>Variable</th>
<th>Percent Inclusion</th>
<th>Mean Included</th>
<th>Mean Overall</th>
<th>Lower</th>
<th>Upper</th>
<th>Non Zero</th>
</tr>
</thead>
<tbody>
<tr>
<td>main</td>
<td>Intercept</td>
<td>100.00</td>
<td>0.3360</td>
<td>0.3360</td>
<td>0.2613</td>
<td>0.4109</td>
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<td>main</td>
<td>regionCodeWC</td>
<td>100.00</td>
<td>0.1975</td>
<td>0.1975</td>
<td>0.0998</td>
<td>0.2998</td>
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</tr>
<tr>
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<td>depthb_6 to15</td>
<td>100.00</td>
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<td>-0.3223</td>
<td>-0.3935</td>
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<tr>
<td>main</td>
<td>barkR</td>
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<td>regionCodeWW</td>
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<td>firefreq2</td>
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<td>0.1755</td>
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<td>regionCodeWW:firefreq2</td>
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<tr>
<td>2 way</td>
<td>firefreq2:barkR</td>
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<td>0.0747</td>
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<td>firefreq4</td>
<td>79.18</td>
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<td>regionCodeWC:firefreq2</td>
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<td>-0.0826</td>
<td>-0.0616</td>
<td>-0.2229</td>
<td>0.0554</td>
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<tr>
<td>2 way</td>
<td>regionCodeWC:barkS</td>
<td>74.40</td>
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<td>0.0568</td>
<td>-0.0460</td>
<td>0.1959</td>
<td>0</td>
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<tr>
<td>2 way</td>
<td>firefreq2:barkS</td>
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<td>0.1015</td>
<td>0.0736</td>
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<td>2 way</td>
<td>regionCodeWC:firefreq4</td>
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<td>-0.0726</td>
<td>-0.2414</td>
<td>0.0195</td>
<td>0</td>
</tr>
<tr>
<td>2 way</td>
<td>regionCodeWC:barkR</td>
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<td>0.0323</td>
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<td>-0.0954</td>
<td>0.1592</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Region Code</td>
<td>Depth (m)</td>
<td>Fire Frequency 1</td>
<td>Fire Frequency 2</td>
<td>Fire Frequency 3</td>
<td>Fire Frequency 4</td>
<td>Fire Frequency 5</td>
</tr>
<tr>
<td>---</td>
<td>-------------</td>
<td>-----------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>2 way</td>
<td>depthb_6to15 : firefreq2</td>
<td>62.40</td>
<td>-0.0740</td>
<td>-0.0462</td>
<td>-0.1734</td>
<td>0.0224</td>
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<td>regionCodeW W:barkR</td>
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<td>-0.0559</td>
<td>0.1696</td>
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<tr>
<td>2 way</td>
<td>regionCodeW W:depthb_6to15</td>
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<td>-0.0271</td>
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Table 2. Importance in ensemble modelling (% inclusion) of the predictors fire intensity and site characteristics and their interactions for predicting mean total % C. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years, soil collected 10 years post fire, yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (a,0-5cm; b,6-15cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open). Table includes the results of regression analysis, coefficient means, upper and lower bounds (95% confidence) and inclusion of zero or not within coefficient bounds (non zero).

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Table 3. Importance in ensemble modelling (% inclusion) of the predictors fire frequency, region and site characteristics and their interactions for predicting mean % RPC. Fire frequency was represented as the number of fires (1,2,4) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200mm and annual average temperature 12°C; wet, warm (WW) annual precipitation 1200mm, annual average temperature 16°C; intermediate precipitation, warm (IW) annual precipitation 1000mm, annual average temperature 16°C. Soil depth was characterized by two layers (a,0-5cm; b,6-15cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open). Table includes the results of regression analysis, coefficient means, upper and lower bounds (95% confidence) and inclusion of zero or not within coefficient bounds (non zero).

<table>
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<th>Mean Overall</th>
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<th>Upper</th>
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Table 4. Importance in ensemble modelling (% inclusion) of the predictors fire intensity and site characteristics and their interactions for predicting mean % RPC. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years, soil collected 10 years post fire, yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (a,0-5cm; b,6-15cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open). Table includes the results of regression analysis, coefficient means, upper and lower bounds (95% confidence) and inclusion of zero or not within coefficient bounds (non zero).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percent Inclusion</th>
<th>Mean Included</th>
<th>Mean Overall</th>
<th>Lower</th>
<th>Upper</th>
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8.5 Appendix E Chapter 4 RPC to total C ratio

Figure 1. Ratio of %RPC to \( %C_{Tot} \) in relation to fire frequency, region and site characteristics shown as mean values (points) with 95% credible intervals. Within each combination, mean %RPC was calculated for each field sample using a Bayesian procedure in which a heavy-tailed distribution (t-distribution with flexible shape parameter) was fitted to five replicate analytical values. This approach ensured that any outlying analytical values for a field sample would be down-weighted in a data-driven manner. Markov Chain Monte Carlo estimation resulted in a set of credible mean %RPC values for each field sample and a corresponding set of ratio values to \( %C_{Tot} \) from which the means and intervals shown here were derived. Fire frequency was represented as the number of fires \( (1,2,4) \) over 30 years prior to sampling 10 years post fire. Regions were characterized as: wet, cool (WC), annual precipitation 1200mm and annual average temperature 12\(^\circ\)C; wet, warm (WW) annual precipitation 1200mm, annual average temperature 16\(^\circ\)C; intermediate precipitation, warm (IW) annual precipitation 1000mm, annual average temperature 16\(^\circ\)C. Soil depth was characterized by two layers \((0-5\text{cm}; 6-15\text{cm})\). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).
The graph shows the ratio of %RPC to %C_tot for different micro-sites (IW, WC, WW) and fire frequencies (1, 2, 4) across different canopy conditions (open, rough, smooth). The x-axis represents fire frequency (number of fires), and the y-axis represents the ratio. Each data point is marked with symbols indicating the canopy condition: open (circle), rough (triangle), smooth (square).
Figure 2. Ratio of \%RPC to \%C_Tot in relation to fire intensity and site characteristics shown as mean values (points) with 95\% credible intervals. Within each combination, mean \%RPC was calculated for each field sample using a Bayesian procedure in which a heavy-tailed distribution (t-distribution with flexible shape parameter) was fitted to five replicate analytical values. This approach ensured that any outlying analytical values for a field sample would be down-weighted in a data-driven manner. Markov Chain Monte Carlo estimation resulted in a set of credible mean \%RPC values for each field sample and a corresponding set of ratio values to \%C_Tot from which the means and intervals shown here were derived. Fire intensity was rated low (L), tree crowns unaffected, or high (H), tree crowns affected. Sites selected from two successive fires separated by 8 years, soil collected 10 years post fire, yielding four intensity combinations (LL, LH, HL, HH). Soil depth was characterized by two layers (0-5cm; 6-15cm). Micro sites sampled were: adjacent to base of rough barked trees (Rough); adjacent to base of smooth barked trees (Smooth); open patches largely between tree crowns (Open).
### Appendix F: Chapter 5 Study sites

**Table 1**: Chapter 5 sites identifying the coupe number, whether sites were logged or not and their burn treatment: Logged (L) Unlogged(U)No Burn (N), Regular Burning (R), and Frequent Burning (F), number of actual fires affecting more than 20% of each site, Cumulative Burn Score, which is the number of times the total site area was affected by fire by adding the area affected in each fire, time since fire (TSF), their locations based on AMG Zone 56 map references and altitude.

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<th>TSF</th>
<th>AMG Easting</th>
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<th>Altitude (m)</th>
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</table>
8.7 Appendix G: Chapter 5 Regressions

From GAM models based on measurements (open circles) in 96 sites, divided between timber harvesting treatments (49 unharvested; 47 harvested) affected by a number of fires ranging from no fire to 7 fires, between 1986 and 2009. The data are presented for two microsites, Open (areas largely between tree crowns) and Tree (adjacent to the trunks of significant trees).

**Table 1.** Carbon (t ha⁻¹) R-sq.(adj) = 0.264
Deviance explained = 33.2%
GCV = 0.22836
Scale est. = 0.20685
n = 576

**Table 1a.** Approximate significance of GAM smooth terms

<table>
<thead>
<tr>
<th>Smooth terms</th>
<th>edf</th>
<th>Ref.df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
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<td>Interaction: n fires, unharvested</td>
<td>1.00</td>
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<td>Interaction: n fires, harvested</td>
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<td>49.25</td>
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</table>

**Table 1b.** Parametric coefficients

| Categorical variable | Reference category | Estimate | Std.Error | t value | Pr[>|t|] |
|----------------------|--------------------|----------|-----------|---------|----------|
| (Intercept)          |                    | 4.03775  | 0.03776   | 106.943 | <2e-16***|
| Timber harvest       | Unharvested        | -0.05353 | 0.04945   | -1.082  | 0.28     |
| Timber harvest       | Harvested          |          |           |         |          |
| Microsite: Tree      | Open               | 0.22912  | 0.03094   | 7.405   | 5.3e-13***|
| Microsite: Tree      | Tree               |          |           |         |          |
Table 2. Nitrogen (t ha⁻¹) R-sq.(adj) = 0.204
Deviance explained = 27.7%
GCV = 0.41811
Scale est. = 0.3795
n = 576

Table 2a. Approximate significance of GAM smooth terms

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<th>Ref.df</th>
<th>F</th>
<th>p-value</th>
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<td>1.000</td>
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Table 2b. Parametric coefficients

| Categorical variable      | Reference category | Estimate | Std. Error | t value | Pr(>|t|) |
|----------------------------|--------------------|----------|------------|---------|---------|
| (Intercept)                |                    | 0.12636  | 0.04985    | 2.535   | 0.0115* |
| Timber harvest:            | Unharvested        | 0.01217  | 0.06538    | 0.186   | 0.8524  |
| Harvested                  |                    |          |            |         |         |
| Microsite: Tree            | Open               | 0.10592  | 0.04191    | 2.527   | 0.0118* |

Table 3. Carbon:nitrogen R-sq.(adj) = 0.0309
Deviance explained = 35.1%
-REML = 201.79
Scale est. = 1
n = 576

Table 3a. Approximate significance of GAM smooth terms

<table>
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<tr>
<th>Smooth terms</th>
<th>edf</th>
<th>Ref.df</th>
<th>Chi.sq</th>
<th>p-value</th>
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</table>
Table 3b. Parametric coefficients

| Categorical variable | Reference category | Estimate | Std. Error | z.value | Pr(>|z|) |
|----------------------|--------------------|----------|------------|---------|----------|
| (Intercept)          |                    | 3.96636  | 0.03101    | 127.903 | <2e-16***|
| Timber harvest:      | Unharvested        | -0.06812 | 0.04250    | -1.603  | 0.109    |
| Harvested            |                    |          |            |         |          |
| Microsite: Tree      | Open               | 0.08453  | 0.01732    | 4.880   | 1.06e-06***|
|                      |                    |          |            |         |          |