Storage and stability of soil organic carbon down the profiles under native woodland, native pastures and cultivation

Soil core removed from a native pasture paddock near Uralla NSW Australia.
Photographer: Christine Walela 2012

CHRISTINE KAINYU WALELA

Mphil. Soil Science, Moi University, Kenya

Bsc. Forestry, Moi University, Kenya

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Declaration

I certify that the substance of thesis has not already been submitted for any degree and is currently not being submitted for any other degree or qualification.

I also certify that any help received in preparing thesis, and all sources used, have been fully acknowledged in this thesis.

[redacted]

Christine Kainyu Walela

Prologue

The thesis is written in journal article format and parts of the literature review might be repeated in subsequent chapters. Formatting of each experimental chapter follows the editorial style of the relevant journal. Formatting of introduction, literature review and conclusions chapter follows the editorial style of Soil Research.

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Publications arising from this thesis

The contents of some of the chapters of this thesis have been submitted to journals and are awaiting publication or have been presented in national and international conferences.

Journal Articles

Walela C, Daniel H, Wilson BR, Lockwood, Cowie A, McCorkell B (2012) Soil organic carbon stocks down the profiles under native woodland, native pasture and cultivation in northern New South Wales, Australia. (To be submitted to Soil Use and Management journal)

Walela C, Daniel H, Wilson B R, Lockwood, Cowie A, Tucker D, McCorkell B (2012) Land use effects on dissolved organic carbon concentrations down the profiles and determination of amounts and composition of water soluble carbon extracted from different litter types. (To be submitted to Soil Use and Management journal)

Walela C, Daniel H, Wilson B R, Lockwood, Cowie A, McCorkell B (2012) Soil organic carbon storage and quality among soil particle size fractions down the profiles under native woodland, native pastures and cultivation in northern NSW Australia. Soil Research (To be submitted to Soil Use and Management journal)

Walela C, Daniel H, Wilson B R, Lockwood, Cowie A, Harden S (2012) Soil organic carbon mineralization: determination of carbon pools and turnover kinetics down the profiles under native woodland, native pastures and cultivation in New South Wales Australia. (To be submitted to Soil Biology and Biochemistry journal)

Walela C, Daniel H, Wilson B R, Lockwood, Cowie A, Harden S (2012) Biochemical composition of above and below ground plant litter from native woodland, native pastures and cultivation and its influence on C mineralization kinetics. (To be submitted to Soil Biology and Biochemistry Journal)

Walela C, Daniel H, Wilson B R, Lockwood, Cowie A, Harden S (2012) Litter quality effects on nitrogen mineralization and immobilization: A laboratory study (to be submitted to Biology and Fertility of Soils)

Conference proceedings and presentations

Walela C, Daniel H, Wilson B R, Lockwood, Cowie A (2011) Quantification and distribution of dissolved organic carbon concentrations in subsoils. International symposium on soil organic matter. Leuven, Belgium. (Oral presentation, see session on subsoil C properties and dynamics

Walela C, Daniel H, Wilson B R, Lockwood, Cowie A (2011) Sub-soil organic carbon storage under contrasting land uses on a duplex soil in northern NSW: Contribution from dissolved organic carbon and root carbon. (Oral presentation at a Rural Climate Change Solutions Symposium 2011, UNE, Armidale, NSW Australia).

Walela C, Daniel H, Wilson B R, Lockwood and Cowie A (2010) Profile stratification of carbon and nitrogen in a native pasture in northern New South Wales, Australia. Geophysical Research abstracts. Vol 12, EGU 2010-3845-3, 2010 (Presented at the European Geosciences Union, General Assembly 2010. Vienna, Austria, 2-7 May, 2010).

Abstract

Soils are widely recognized as having potential to sequester significant amounts of carbon (C). There is much speculation that deeper soil layers can store significant amounts of C in a relatively stable form and that land use can influence soil organic carbon (SOC) stocks at depth. Our understanding of the potential for subsoils to store C in the long term is however limited here in Australia, as little work has been done to quantify C stocks, and the available studies on C dynamics have been mainly focused in surface soils (30 cm or less), and conducted from a narrow range of management options. The main aim of this research was to examine SOC stocks and stability down texturally contrasted soil layers (up to 0.80 m) under three major land uses, namely native woodland, native pastures and cultivation, in the Northern Tablelands of New South Wales, Australia. Specifically, this study examined the effects of land use on a) the quantity of total SOC stocks down the profiles; b) dissolved organic carbon (DOC) concentrations down the profiles; c) the amounts and composition of dissolved organic matter (DOM) extracted from different litter types; b) quantity and quality of SOC among soil particle size fractions; c) SOC mineralization dynamics including C pools and turnover kinetics; and d) C and nitrogen (N) mineralization dynamics of decomposing plant litter and interactions with initial biochemical composition of litter. Understanding C stocks and organic matter stability in soils taking into account deeper soil layers as affected by land use will identify the most effective land use for carbon sequestration and thereby inform land use decisions in the northern region of NSW Australia.

Strong differences in SOC stocks between land uses were only apparent in the surface 20 cm, with native pastures and cultivation which were statistically similar to each other containing on average 9 and 11 t/ha less C than native woodland. Significantly larger SOC stocks in surface soils under native woodland may be partly attributed to more lignin-rich recalcitrant above ground litter inputs compared with the other two land uses. The combined subsoil (20 to 80 cm) layers contained 40 % of the total profile SOC stocks across all land uses, demonstrating that substantial amounts of SOC stocks reside in deeper layers and so the importance of preserving it.

The relative proportion of aromatic C in dissolved organic matter (DOM) extracted from litter was highest under native woodland, followed by native pasture then cultivation indicating qualitative differences in DOM fractions which may in turn influence

biodegradability of DOM among litter types. DOC represented between 0.01 and 0.1 % of total SOC down the soil profiles and across all land uses showing that the contribution of DOC to C stocks was relatively small.

Native woodland soils were associated with consistently wider C:N ratios in the particulate organic matter (POM) fraction throughout the soil profile compared with native pastures and cultivation which differed between each other in the top 50 cm. The result indicated differences in the quality of organic matter inputs (litter) entering the POM fraction with native woodland soils associated with less easily decomposed inputs due to their inherent chemical composition. Consequently, native woodland showed the least decline in particulate organic carbon (POC) with soil depth compared with the other two land uses. The proportion of MOC to the total SOC increased with soil depth indicating that subsurface C was more protected than surface C probably due to mineral association with clayey subsoils. Compared with native pastures and cultivation which were largely similar, native woodland soils contained significantly larger amounts of MOC in all soil depths suggesting that C was more physically protected from microbial attack.

SOC mineralization kinetics over 419 days was well described by decomposition of a single pool. Compared with native pastures and cultivation, native woodland had larger amounts of the active C pools in all soil depths which were mainly related to larger amounts of labile substrates mainly DOC and POC down the profiles. The decomposition rate of the active C pool, measured by laboratory incubations, was strongly dependent on soil depth with turnover of 66 and 47 days in surface and subsoils respectively. Shorter turnover of active C pool in subsoils compared with surface soils may be linked to destabilization of active C stores when environmental constraints on decomposition which might inhibit decomposition in the undisturbed profile are removed following incubation under similar conditions in the laboratory. Consequently, it is important that the current C stores remain undisturbed.

For all litter types, the active C pool whose decay rate constants ranged from 0.072 d^{-1} to 0.805 d^{-1}, initially constituted < 20 % of the litter mass, while the slow C pool with decay rates of between 0.002 d^{-1} to 0.019 d^{-1} comprised > 80 % of the litter mass. The decomposition rate of the slow C pool in litter was strongly and negatively correlated with the initial lignin:N ratio of plant litter suggesting that the interaction between these two litter quality variables had important controls over litter decomposition. Compared

with other litter types, above and below ground litter from native woodland had higher initial lignin:N ratio and were associated with more stable slow C pools with longer half lives of 109 and 446 days respectively.

The above and below ground litter components had distinct N mineralization patterns during the early stages of incubation as influenced by the initial biochemical composition of plant litter namely C:N ratio, % lignin and % water soluble carbon (WSC). Our results suggest that the biochemically recalcitrant lignin influenced the susceptibility of substrates to microbial attack and thereafter a demand for N by microbial decomposers. However, the subsequent release of N from substrates depended on the C:N ratio.

The results of this thesis are relevant to landholders, natural resource managers and policy makers as they inform that native woodland soil has an important role in storing a) larger SOC stocks in surface (20 cm) layers compared with native pastures and cultivation which were generally similar, b) relatively less decomposable C down the soil profiles as larger amounts of C are associated with the mineral fraction, and c) more slowly decomposing organic matter inputs which were characterized by relatively stable slow C pools in litter compared with native pastures and cultivation in the northern region of NSW Australia.

Key future research areas arising from this thesis include to: a) investigate land use effects on total SOC stocks in soil profiles using increased intensity of soil sampling in order to represent spatial variation and increase capacity to detect differences in total SOC especially where differences in C might be small such as in non-wooded land uses, b) compare land use effect on DOC storage at various depths during crop/pasture growing season as the living plant biomass may influence DOC concentrations, c) determine the stability of DOC derived from both soils and litter in order to determine its long term dynamics in soils, d) determine the long term stability of soil slow C pool and investigate the mechanisms by which C might be stabilized including the contribution of char C at various depths under the three land uses, e) determine the mechanism by which lignin and N interact to influence decomposability of litter, and f) understand the mechanism of SOC and N stabilization.

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Chapter 1. Introduction

Historically, an estimated 60 to 90 Pg carbon (C) has been lost from the global soil pool, with at least 50 Pg resulting from land use change such as deforestation followed by cultivation (Raich and Potter 1995; Schlesinger and Andrews 2000; Lal 2004; IPCC 2007). This large C loss has contributed to increasing atmospheric carbon dioxide (CO_2) concentrations and therefore to climate change (IPCC 2007). The threat of global climate change has led to an international response through commitments (such as the Kyoto Protocol) by most nations including Australia, to significantly reduce their CO_2 and other greenhouse gas (GHG) emissions (Sanderman *et al.* 2010). Soils have been widely recognized as having the potential to sequester significant amounts of C through more judicious land use or management practices, especially where prior C depletion has occurred, and so may provide an opportunity to offset large amounts of C emissions (Lal 2004).

For much of the Australian continent, including New South Wales (NSW), information on the C storage potential of soils is lacking, as only a limited amount of work has been done to quantify C stocks (Sanderman *et al.* 2010; Wilson *et al.* 2011). Moreover, nearly all soil C studies have been conducted on a narrow range of land uses and management systems (mainly agricultural and grazing practices) and have focused on the top 30 cm of soil or less (Young *et al.* 2009; Wilson *et al.* 2011). Subsoil layers have however been recognized as having a great potential to store substantial amounts of C with studies showing that > 50 % of the total C stored within the soil profile may be found in subsoils (Batjes 1996). Moreover, there is accumulating evidence that compared with surface soils, C in deep soil layers is more stable as evidenced by increase in radiocarbon age at depth (Trumbore 2009). While the processes that lead to high stability are not entirely clear, the speculation has been, subsoil layers represent a potential sink for organic C as they may not yet be saturated with C (especially those with low C concentrations) (Rumpel and Knabner 2011). To this end, Sanderman *et al.* (2010) observed that in many of Australia's well weathered regions, subsoils present the greatest potential for long term C stabilization. It has been proposed that land use may influence soil organic carbon (SOC) storage in subsoil layers (Jobbagy and Jackson 2000). However, firm conclusions have been hampered given that few studies have been conducted below surface soil layers (Lorenz and Lal 2005).

There are substantial gaps in our understanding of SOC dynamics and its implications especially in northern NSW Australia. Specifically, the relative quantities of C that can be stored in soils under a broad range of land uses is largely unknown and need to be determined before accurate and reliable predictions of soil C change resulting from land use change can be made. In doing so, it is important to take into account subsoil layers and a) quantify the amounts of C that can be stored at different soil depths; and b) understand processes that may be important in translocating C in subsoil layers. To further understand the long term dynamics/stability of C in soil profiles information is needed on the a) mechanisms by which C is stabilized, b) distribution of organic C among SOC pools and turnover kinetics; c) mineralization of C and nitrogen (N) from decomposing plant litter and their interaction with initial biochemical composition of litter in order to understand their transformations and interactions in soils which will inform their ability to influence formation of stable SOC pools. These key research areas were identified in the current study and were subsequently examined.

1.1 Thesis aims and research questions

This thesis was aimed at examining the stocks and stability of SOC at different texturally contrasted soil layers (up to 0.80 m) under three major land uses, namely native woodland, native pastures and cultivation, in the Northern Tablelands of New South Wales, Australia. Based on the gaps that were identified in literature a series of questions were asked about: a) the effect of different land use on SOC stocks at different soil depths, b) the role of subsoil SOC in soil C storage and potential to sequester atmospheric CO_2-C in subsoil, c) the influence of different land use on dissolved organic carbon (DOC) storage down the soil profiles, d) the composition of dissolved organic matter (DOM) extracted from plant litter obtained from different land uses, e) the influence of land use on the storage and quality of C among particle size fractions down the soil profiles, f) the influence of land use on SOC mineralization including the size of SOC pools and turnover rates down the soil profiles, g) the influence of soil depth on turnover rate of C pools, h) the initial biochemical composition of above and below ground litter and its influence on litter C mineralization dynamics; and i) nitrogen (N) mineralization and immobilization from above and below ground litter.

This study was conducted near the township of Uralla in the Northern Tablelands of New South Wales, Australia. The soils of the area are largely derived from Permian granite and are classified as yellow Chromosols (Isbell, 2002) (Alfisols-Soil Taxonomy). The study was conducted under three common land uses namely, native pastures, native woodland and cultivation.

1.2 Thesis structure

Chapter 1. Introduction, scope and objectives

This chapter provides a brief project overview and scope of the study. It identifies the research gaps and presents the thesis aims and key research questions addressed in this thesis.

Chapter 2. Review of literature

This chapter presents a review of literature on the important principles, subject areas and studies related to the current study.

Chapter 3. Soil organic carbon stocks down the soil profiles under native woodland, native pasture and cultivation in northern New South Wales, Australia

This chapter addresses the research question about the effect of different land use on SOC stocks at different soil depths and the role of subsoils in organic C storage and potential to sequester atmospheric CO_2-C in subsoil. This chapter provides the effects of land use on SOC stocks at different soil depths (0-0.80 m).

Chapter 4. Land use effects on DOC concentrations down the soil profiles under different land uses and determination of amounts and composition of DOM extracted from different litter types

This chapter addresses the research questions about a) the influence of different land use on dissolved organic carbon (DOC) storage down the soil profiles; and b) the composition of dissolved organic matter (DOM) extracted from plant litter obtained from different land uses. This chapter provides the effects of land use on DOC concentrations and stocks at different soil depths (0-0.80 m). It further provides the effects of land use on the aromaticity of DOM extracted from different litter components.

Chapter 5. Soil organic carbon storage and distribution among soil particle size fractions in soil profiles under native woodland, native pastures and cultivation in northern NSW Australia

This chapter addresses the research questions about the influence of land use on the storage and quality of C among two particle size fractions namely a) particulate organic matter (POM); and b) mineral-associated organic matter (MOM) fraction at different soil depths under native woodland, native pastures and cultivation. It provides the effects of land use on the quantity and quality of organic C stored in the two fractions at different soil depths.

Chapter 6. Soil organic carbon mineralization: determination of carbon pools and turnover kinetics in soil profiles under native woodland, native pastures and cultivation in New South Wales Australia

This chapter addresses the research questions about a) the influence of land use on SOC mineralization including the size of SOC pools and turnover rates down the soil profiles, and b) the influence of soil depth on turnover rate of C pools.

Chapter 7. Biochemical composition of above and below ground plant litter from native woodland, native pastures and cultivation and its influence on C mineralization kinetics

This chapter addresses the research questions about a) the initial biochemical composition of above and below ground litter, and b) its influence on litter C mineralization dynamics. It provides the relationships between the C mineralization kinetics and the initial biochemical composition of litter.

Chapter 8. Litter quality effects on nitrogen mineralization and immobilization: A laboratory study

This chapter addresses the research question nitrogen (N) mineralization and immobilization from above and below ground litter. It provides the relationship between the initial biochemical composition of litter and net N mineralization.

Chapter 9. General conclusions and future research

Provides a synthesis of the main findings and conclusions, and discusses management implications on the research. It provides the areas for future research which flow from this thesis.

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Chapter 2. Review of literature

2.1 Soil organic carbon

Soil organic carbon (SOC) is one of the most important terrestrial pools for carbon (C) storage and exchange with atmospheric carbon dioxide (CO_2). Globally, SOC is estimated to account for between 1200 and 1550 Pg C in the top 1m, which is twice the amount of atmospheric CO_2 pool and three times that in vegetation (Batjes 1996; IPCC 2000; Follett 2001).

Organic carbon (C) in soils is found in the form of organic compounds collectively referred to as soil organic matter (SOM) with amounts of C in SOM ranging from 40 to 60 % by mass (Sanderman *et al.* 2010). SOM refers to all living and nonliving organic material in the soil irrespective of origin or state of decomposition (Baldock *et al.* 1999) described as ranging in days for plant residues and root exudates to greater than a thousand years for the resistant humic substances (Trumbore *et al.* 1996).

The concentration of organic C in a soil sample is determined by either wet or dry oxidation (LECO) method. In wet oxidation method, oxidisable organic matter in the soil is converted to carbon CO_2 using an oxidant (usually dichromate) and the amount of oxidant used is then determined and equated to the amount of SOC originally present in the soil (Walkely and Black 1934). In dry oxidation method, finely ground soil samples ($\leq$ 0.5 mm) of known mass are sequentially introduced into an oxidation furnace and subjected to combustion from about 900 to 1050^{o}C in a stream of oxygen releasing CO_2, water and nitrogen (Rayment and Lyons 2011).

The wet oxidation method however is known to underestimate the amount of organic C in soil samples due to incomplete oxidation so correction factors, whose magnitude are known to vary across soil types are applied (Skjemstad *et al.* 2000). As a result, the more accurate method to quantitatively determine SOC is by dry combustion. Determination of C in soils containing carbonate (alkaline soils) and charcoal forms of C using the dry combustion procedure may however lead to an overestimation of C as this procedure measures the amount of total C in a soil sample. Hence a suitable acid pre-treatment of alkaline soils in the sample followed by a drying step eliminates the carbonate C prior to combustion (Kerven *et al.* 2000).

The amount of SOC that exists in any given soil is determined by many factors including climatic factors (temperature and moisture regime) and soil related factors (soil parent material, clay content, cation exchange capacity) (Dawson and Smith 2007). SOC stocks may also vary within a given soil type with the stocks being determined by the balance of net C inputs to the soil (as organic matter) and net losses from the soil (mainly CO_2 from microbial decomposition) (Lal 2004).

2.2 Land use change and soil organic carbon dynamics

The dynamics of C storage in soils are largely determined by land use through its direct effects on (i) quantity and quality of C cycled through the system, and (ii) the decomposition rate of C due to effects on soil physical and chemical microclimate (moisture, temperature, aeration, pH, nutrient availability) (Schimel 1995; Paustian *et al.* 1997; Houghton *et al.* 1999). With respect to quantity of C inputs to the soil, forest systems are widely recognized as having the largest input of C to the soil all year round and often this material is recalcitrant (less decomposable by microbes) (Smith 2008). Land use such as grasslands/pastures also tend to have larger inputs, however, the material is often less recalcitrant compared with forest litter. Compared with forest and pasture land use, cultivation often has the smallest input of C, having inputs only during crop growing seasons with further C input losses attributed to biomass removal during crop harvest. Additionally, organic material from cultivation is often the most labile (more easily decomposed) compared with forest and pastures litter (Smith 2008). Land use is also known to control soil erosion, aggregate formation and biological activity all of which have a significant impact on SOC accumulation and CO_2 evolution. Historically, approximately 26 Pg C has been lost from the global soil pool as a result of soil erosion (Lal, 2004b). A change in land use therefore, significantly affects SOC stocks and fluxes (Guo and Gifford 2002).

Globally, the long-term flux of C from soils associated with past and present changes in land use such as deforestation followed by cultivation is estimated at between 40 and 90 Pg C (Lal 2004; IPCC 2007). Lal (2004) reported that as much as 60 % and 75 % of SOC stocks have been lost from temperate and tropical regions respectively, following conversion of natural to agricultural ecosystems. In a comprehensive review of published data from 74 studies conducted in Australia, Brazil, New Zealand and USA, Guo and Gifford (2002), found that soil C stocks declined from surface soils after land use changes from pasture to plantation (-10 %), native forest to plantation (-13 %), native forest to crop (-42 %), and pasture to crop (-59). Another comprehensive review of published data of 385 studies conducted in the tropics found SOC stocks declined following conversion of primary forest into cropland (-25 %), primary forest to perennial crops (-30 %) and forest to grassland (-12 %) (Don *et al.* 2011). Generally all studies reported in literature show that a conversion of native forest into cultivation always leads to a significant decline in SOC stocks (Murty *et al.* 2002; Don *et al.* 2011). In contrast, the impact on SOC dynamics upon conversion of native forest to pasture is however inconsistent showing little or no change (Murty *et al.* 2002; Harms *et al.* 2005; Radford *et al.* 2007). A review by Murty *et al.* (2002) found that a conversion from native forest to pasture did not, on average, lead to a loss of soil C, although individual sites may lose or gain soil C, depending on site specific management such as frequent application of fertilizer or pasture systems that include legumes.

The magnitude of change in SOC resulting from changes in land use may have been inflated in many studies due to the confounding influences of bulk density changes. For example, Don *et al.* (2011) reported smaller relative changes in C than those reported in previous reviews which calculated higher global and tropical SOC changes after cultivation of forests (Detwiler 1986; Davidson and Ackerman 1993; Paustian *et al.* 1997; Amundson 2001; Guo and Gifford 2002). Disparities in the estimates of SOC decline following land use change among different reviews may be partly attributed to data quality especially, the application of a soil mass correction which accounts for changes in different land use types (Ellert and Bettany 1995). Mass correction of SOC stock estimates is crucial in order to estimate land use change effects since land use change is always accompanied by bulk density changes (Ellert and Bettany 1995; Don *et al.* 2011). In this respect, only few reviews (e.g. by Don *et al.* 2011) have provided a meta analysis on studies with soil-mass corrected estimates of SOC stocks changes due to land use change. The Intergovernmental Panel on Climate Change (IPCC) recognizes that there are disparities in reporting of SOC change due to land use change and has set default values of -31 % and -42 % of SOC loss for dry and wet tropical regions following cultivation respectively (IPCC 2007).

2.2.1 The Australian Context

Similar to other parts of the world, land use change has had a significant impact on SOC dynamics and therefore GHG emissions in Australia. To this end, Australia is among the 20 largest countries of emitters (including emissions from land-use change and forestry) which are responsible for more than 80 per cent of the global emissions (IPCC 2007). The National Carbon Accounting System (NCAS) which provides Australia's GHG emissions estimates showed that land use change from 1988 to 2003 was a major contributor to Australia's net GHG emissions contributing an estimated 22 % of the total anthropogenic GHG (AGO 2005). Land use activities which have been mostly linked with past C losses in Australian soils include native forest clearing, biomass burning, crop intensification, pasture cropping and technological revolution (mechanized farming) (AGO 2005).

The percentage loss of organic C from majority of Australian soils following conversion of native forest to cultivation has been estimated to range from 10 to 60 % over 10 to 80 years (Russell and Williams 1982; Dalal and Mayer 1986; Bridge and Bell 1994; Cogle *et al*. 1995; Chan 1997; Dalal and Chan 2001). The climate extremes of much of Australia, year to year variability in temperature and especially soil moisture has undoubtedly played a major role in influencing the amount of C inputs and decomposition rates and may explain the variability in SOC decline across Australian soils. Generally, temperature affects both the amount of C inputs from plant biomass into the soil and SOM decomposition. The large historic C losses and the related potential to return to pre-clearing SOC conditions are the major reasons that many researchers believe there is great potential for C depleted soils to sequester large amounts of atmospheric CO_2-C levels relative to the current SOC levels (Sanderman *et al*. 2010).

The threat of global climate change as a result of rising atmospheric CO_2-C concentrations is a major concern and has led to an international response through commitments (such as the Kyoto Protocol) by most nations including Australia, to significantly reduce their CO_2-C and other GHG emissions (Sanderman *et al*. 2010). The current annual CO_2-C emissions to the atmosphere is estimated at 6.3 ± 1.3 Pg C y^{-1} with projections that by 2100 C emissions could be up to four times greater (25 Pg C y^{-1}). As such it is critical that the issue of removing CO_2-C from the atmosphere is addressed. A potential offset that has been identified for the GHG emissions is by sequestering C in terrestrial ecosystem (IPCC 2000). Soils have been widely recognized as having the potential to sequester between 0.6 and 1.2 Pg C from the atmosphere through more judicious land use or management practices, especially where prior C depletion has occurred, and so may provide an opportunity to offset large amounts of C emissions (Lal 2004; Lorenz and Lal 2005). Although the contribution of soil C sequestration would contribute a maximum of only about 2-5 % towards reducing C emissions under the highest emission scenario, still soil C sequestration might play a central role in reducing

CO_2-C emissions at least in the first 20 years of implementation when C sequestration is most effective while new technologies are developed and implemented (Smith 2008).

Information quantifying the soil C storage potential is lacking in Australia as only limited work has been conducted to quantify C stocks (Sanderman *et al*. 2010; Wilson *et al*. 2011). Moreover, our present knowledge on C stocks in Australia including New South Wales (NSW) relies on studies that have been conducted within a narrow range of land use or management systems, mainly grazing management practices (Dalal and Chan 2001; Chan 2001; Lodge *et al*. 2003) and field agronomic trials that were designed to test best management practices and not C studies (Sanderman *et al*. 2010) with few studies conducted on a broad range of land uses (Young *et al*. 2005; Wilson *et al*. 2008, 2011). Knowledge of the relative quantities of SOC stocks that can be stored in soils needs to be determined as it is a prerequisite before accurate and reliable predictions of soil C change resulting from land use change can be made.

Below is a brief literature review on the impacts of cultivated systems, pasture systems and native forest on SOC storage in Australia and elsewhere.

2.2.2 Cultivation

The loss of soil C after disturbance, mainly through cultivation of previously untilled native land has been widely demonstrated by many studies (Fig 1.1) (Dalal and Mayer 1986; Davidson and Ackerman1993; Whitbread *et al*. 1998; Janzen *et al*. 1998; Kirschbaum 2000; Follett 2001). The reasons for SOC decline upon cultivations are well known and fall into two broad categories: a) reduced input of organic materials and increased export of nutrients through crop harvest, stubble burning, and b) increased SOC mineralization rates (Sanderman *et al*. 2010). During exploitive cultivation, plant residue inputs are low and soil C levels can be reduced to as little as 30 % of the C in the initial undisturbed surface horizon (Dalal and Mayer 1986). A study conducted in southern Queensland Australia by Dalal and Mayer (1986) reported a 70 % decline in initial SOC from a site where native vegetation had been cleared followed by cultivation for over 40 years which was attributed to the difference between cultivated soil and preceding forest in both the quantity and quality of residue inputs. Crops are bred and managed to maximise above ground growth and when biomass is removed from the site the amount of plant material available to build soil C is greatly diminished. Furthermore, tree residues are more resistant to decomposition (more lignified) than agricultural residues and may therefore persist in soils for longer eventually being incorporated into the SOM fraction thereby contributing to SOC build up compared with cultivated soils.

With respect to influence of cultivation on decomposition rates, intensive tillage affects decomposition process through the physical disturbance and mixing of soil thereby disrupting soil aggregates and exposing formerly occluded organic matter to microbial oxidation and accelerating its decomposition (Chan *et al.* 1992; Reicosky 1997). Cultivation/tillage also affects soil temperature, aeration (gaseous exchange between soil and atmosphere) and water relations by its impact on surface residue cover and soil structure. Chan *et al.* (1992) observed a close relationship between oxygen uptake and the level of soil disaggregation therefore proposing that this was the main mechanism for depletion of soil C through cultivation. Soil disaggregation creates a more favourable environment for microbial decomposition by influencing soil porosity attributes. In particular, the breaking of soil aggregates leads to increase in soil aeration and moisture availability exposing more soil surfaces to invasion by bacteria and fungi therefore decomposition Chan *et al.* (1992). Six *et al.* (1999) further observed that the rate of turnover of soil aggregates influences C stabilization. Soil disturbance through cultivation is further detrimental to soil structure by continually exposing new soil to wet-dry and freeze-thaw cycles, at the soil surface, thereby increasing the susceptibility of aggregates to disruption and availing physically-protected inter-aggregate organic material for microbial decomposition (Beare *et al.* 1994; Elliot and Coleman 1988). Additionally, cultivation especially when followed by fallow creates favourable conditions for both transport of organic matter off-site by wind or water (surface run-off) (Packer *et al.* 1992).

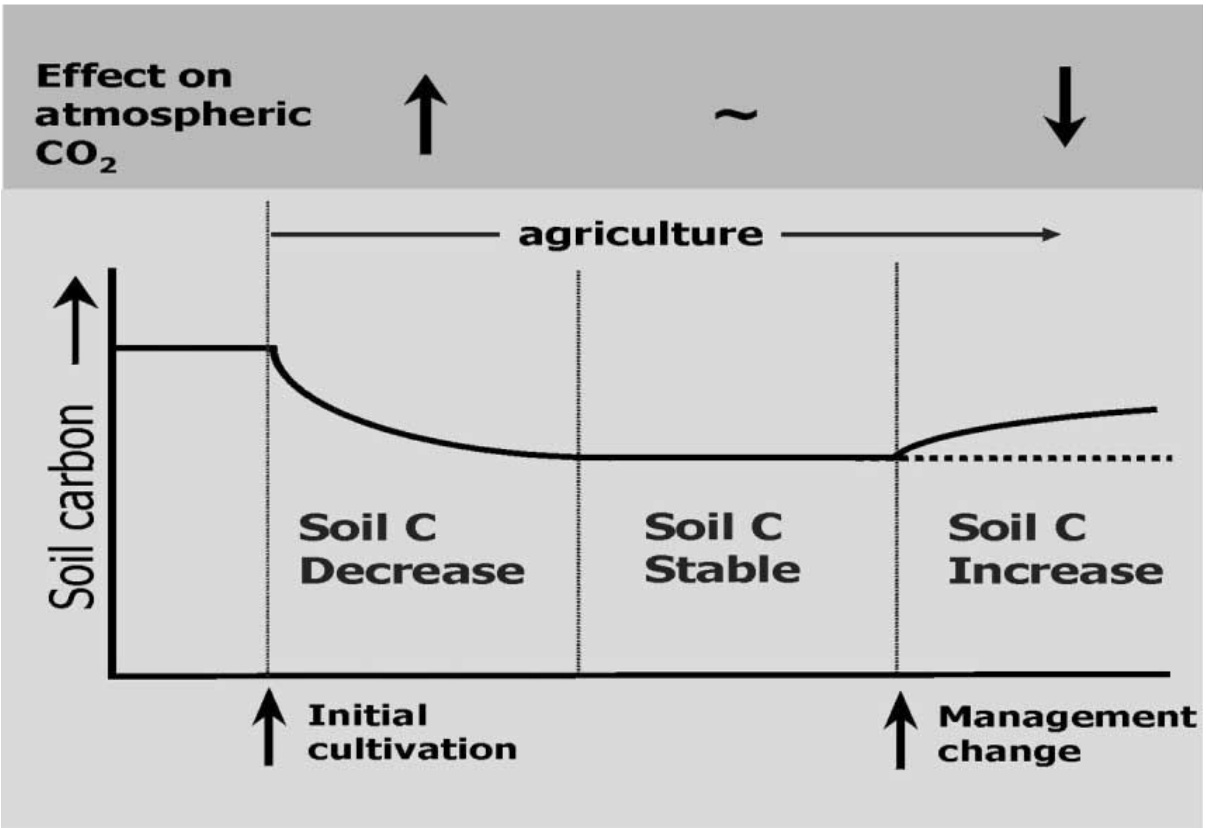

Fig 1.1 Changes in the long-term storage and release of soil carbon in soil and its release as carbon dioxide as a function of agriculture practices (adapted from Janzen *et al.* (1998). Source Follett (2001).

The C depletion in cultivated soils offers an opportunity for C sequestration as soils are believed to have capacity to store additional C relative to the current amounts, with altered management or land use change. However, without the information of the current C stocks in cultivated soils, it is not possible to make accurate and reliable predictions of C change following land use change especially in northern New South Wales Australia where such information is lacking. Understanding the impacts of cultivation on soil carbon pools and fluxes against other common land uses such as native pasture and native forest might allow for a better understanding of the potential of soil to sequester and release C.

2.2.3 Native pastures

Grazing lands cover a large area worldwide as a result of significant amounts of native forest, shrubland, and woodland conversions. In Australia, and particularly New South Wales extensive areas of native vegetation have been replaced by conventional livestock-based agriculture over the last 100 years (Mendham *et al.* 2002). Land conversion into grasslands are driven by the demand for forage production since significant portions of world milk and beef production occur on grasslands managed solely for those purposes (Mendham *et al.* 2002). This huge demand has subsequently led to grassland overuse/overgrazing and poor pasture management (Oldeman 1994) and large areas especially in Australia are recognised as either deteriorating or degraded (Tothill and Gillies 1992). Maintenance of SOM is therefore a key factor in the sustainability of grassland ecosystems.

Overall, areas maintained under well managed permanent/native grasslands, as pastures or rangelands, constitute potential C sinks (Conant *et al.* 2002) with a globally estimated SOC sequestration potential of 1.87 Pg C/yr at a mean SOC sequestration rate of 0.54 Mg C/ha/yr (Lal 2003). Previous studies have reported increasing soil C stock after forest clearing to well managed pastures (Fisher *et al.* 1994).When forest is cleared for pasture establishment, considerable aboveground C is lost but it is not necessarily that there be a decline in SOC (Post and Kwon 2000). A study by Neil *et al.* (1997) reported that eleven of fourteen pasture conversion sites studied in Brazil showed increases in SOC. For at least 10 years, all sites showed increases with rates as high as 74.0 g C m^{-2} y^{-1} over 20 years. However two sites studied in Costa Rica found a decline in SOC when native forest was cleared for pasture (Veldkamp 1994).

While the loss of C in cultivated land use is mainly associated with soil disturbance and lower organic matter inputs (Beare *et al.* 1994), larger C inputs and absence of tillage disturbance are the main reasons that perennial native pastures may sequester more C (Paustian 1997). Within established pastures, soil C can be increased by eliminating disturbances to the soil and by increasing primary production. To this effect, a variety of management techniques with the potential to increase SOM and increase forage production for livestock have been developed. This improved management includes fertilization (especially super-phosphate in Australia), irrigation, introduction of earthworms, improved grazing management and sowing favourable forage grasses and inclusion of legumes (Conant *et al.* 2001). Further, grasslands have high inherent SOM content that supplies plant nutrients, increases soil aggregation, limits soil erosion and also increases cation exchange and water holding capacities (Miller and Donahue 1990). Perennial grasses, in particular, are deeply rooted and have a high relative allocation of C belowground.

Studies have shown that the process of soil C depletion in cultivated land can be reversed by utilization of perennial native pastures (Guo and Gifford 2002; Murty *et al.* 2002; Six *et al*. 2002). However, without information on the current SOC stocks and stability of C under native pastures, it is difficult to inform on the SOC change in cultivated soils as a result of land use change. It is important that the current SOC socks in soils under native pastures be determined and SOC dynamics be understood especially in New South Wales Australia where information is lacking.

2.2.4 Native forest

The world's forests contain an estimated 80 % and 40 % of above and below ground terrestrial C respectively (Dixon *et al.* 1994). The size of the global forest C stocks has however been declining as a result of deforestation activities releasing an estimated 18 % of the world's greenhouse gas emissions (Stern 2006). Further, changes in forest land use have been estimated to produce a net C flux to the atmosphere of 0.4 to 2.5 Pg/y in 1980 mainly from mid-latitude forests (including Australia)(Houghton and Skole1990; Flint and Richards 1994). With land-use change such as forest clearing, C is immediately released to the atmosphere during biomass burning. In many instances, ground vegetation rather than trees is established after clearing, and depending on the management the decline in SOC stocks may continue indefinitely.

In the past, tree clearing in Australia has been a key component in the development of agriculture and was equated to good land stewardship (Sherren *et al*. 2008). As a result, native vegetation has been extensively cleared since European settlement and the lack of natural regeneration and gradual death/senescence of mature trees has left only a small portion of the original native forests (Manning 2006). In many areas particularly in eastern Australia, these native woodlands are largely restricted to strips along roads or as scattered trees within farms occurring at low density (Reid and Landsberg 2000). Reid and Landsberg (2000) reported that for temperate Australia, native woodland cover up to 20 million ha of farmland. The predominant perception of these native woodlands amongst land holders has been that trees provide good stock shelter. More recent studies by Barnes (2011) have however shown that the values of these native woodland patches are not just limited to stock shelter and aesthetics, but that these trees benefit the surrounding soil by increasing soil fertility. Moreover, the few studies that have been quantified SOC stocks under these native woodland in northern NSW Australia, have consistently reported larger SOC stocks in surface soil layers compared with native pastures and cultivation (Young *et al*. 2005; Wilson *et al*. 2008; 2010, 2011). Rural land systems such as native woodlands have significant potential to store or mitigate greenhouse gases and offset large amounts of Australia's emissions. However, only few studies have quantified SOC stocks under native woodland especially in northern NSW Australia and as such it is difficult to inform land holders, natural resource managers and policy makers of this huge potential. There is a great need to understand the impacts of these native woodlands on SOC stocks and fluxes as this might allow for a better understanding of SOC dynamics in this land use.

2.3. Distribution of soil organic carbon down the soil profiles

SOC is both spatially and vertically distributed down the soil profile. The estimate for organic C stocks in the 0-1 m of the world soils ranges from 1,200 to 1,550 Pg C while, the second and third meters are estimated at 491 and 351 Pg C respectively (Batjes 1996; Jobbagy and Jackson 2000). The biomes with the most SOC at 1–3 m depths are tropical evergreen forests (158 Pg C) and tropical grasslands/savannas (146 Pg C) (Jobbagy and Jackson 2000).

The overwhelming majority of studies on SOC dynamics conducted in Australia (Chan *et al*. 2003; Young *et al*. 2009; Wilson *et al*. 2007 a, b, 2008, 2010, and 2011) have focused in the surface soils (30 cm or less) with few studies conducted below these layers (Trumbore 1995; Jobbagy and Jackson 2000). Further, the existing guidelines for C accounting refer to the upper 30 cm only (IPPC, 1997). This fixed zone is intended to cover the actively changing soil C pool as the majority of roots, plant inputs, and microbial activity are in this zone. However, while the above holds, there is growing evidence that a significant proportion (> 50 %) of total profile soil C is found deeper than 30 cm and that this C may have escaped our attention (Batjes1996). Further, the estimation of SOC stocks when such a relatively shallow and fixed depth is used may be challenging given the changes in bulk density among land uses. Moreover, the selection of sampling depth is critical as calculation of total C in near-surface layers might overlook some significant differences in SOC among land uses as a result of variation in the partitioning of C down the soil profile.

There is a growing interest to understand the properties and dynamics of subsoil C particularly because in addition to the recent evidence that subsoils may store substantial amounts of C, a number of studies have also shown that compared with surface soils, subsoil C is more stable as evidenced by increase in radiocarbon age with depth (Rumpel *et al*. 2002; Jenkinson and Coleman 2008; Trumbore 2009). The mechanisms leading to more stable C in subsoils are however still largely unknown and the literature presented is somewhat inconsistent with conflicting conclusions reached on the potential for subsoils to release C into the atmosphere. For example, radiocarbon studies have also shown that a fast cycling active C pool continues as deep as plant roots extend (Trumbore *et al*. 1995; Richter *et al*. 1999; Trumbore 2000; Baisden *et al*. 2002). Integrating even small changes in concentrations of active C and microbial biomass (Fierer *et al*. 2003) over the large volumes of soil below 30 cm means that fluxes associated with these pools can be significant, particularly on decadal timescales (Trumbore 1995; Trumbore 2000).

The scarcity of SOC data below the routinely sampled top layers constrains estimates for deeper SOC pools and their respective decay kinetics. Belowground C is often difficult to measure and therefore, through modelling, mathematical functions that can be extrapolated to deeper layers, have mainly been applied to improve the understanding of SOC stocks at depth (Arrouays and Pelissier 1994). The modelling efforts have however sometimes resulted in unrealistic rates of SOC mineralization with SOC often treated as equally susceptible to mineralization throughout the soil profile. The kinetics of SOC mineralization should not however be assumed to be equal throughout the profile or extrapolated from surface soil layers because surface and subsoils differ with respect to a) contrasting environments - in terms of soil temperature, soil aeration and moisture conditions) (Gill *et al*. 1999), b) differences in supply of litter input both in quantity and quality (meaning organic matter is less decomposable with soil depth) (Rasse *et al*. 2000), c) differences in composition (Fierer *et al.* 2003) and activity (Taylor *et al*. 2002) of microbial decomposers. A number of studies have shown that microbial biomass and activity is considerably more variable in subsoils compared with surface soils (Lomander *et al*. 1998; Garcia-Pausas *et al*. 2008; Salomé *et al*. 2010) and d) differences in the nature of associations with soil mineral particles (Eusterhues *et al*. 2003).

The specificities of SOC dynamics in deeper soil layers are potentially of critical importance when evaluating the impact of land use as there is much speculation that land use may influence SOC stocks with depth (Jobbagy and Jackson 2000). Firm conclusions on land use effects on subsoil SOC have however been hampered given that few studies have been conducted below surface soil layers (Lorenz and Lal 2005). Sanderman et al. (2010) observed that in many of Australian’s well weathered regions, subsoils present the greatest potential for long term C stabilization. Knowledge on the effects of land use on

SOC stocks taking into account subsoil layers is important in order to fully understand the role of subsoils in C storage.

The main factors that influence the distribution of SOC in subsoils include; allocation of above and below ground net primary productivity, vertical leaching of dissolved organic carbon (DOC) and vertical soil mixing by microorganisms among others. These three factors are reviewed below.

2.4. Factors determining the distribution of soil organic carbon down the soil profiles

2.4.1 Allocation of fixed carbon

The distribution of SOC down the profiles in most soils is generally characterized by larger stocks near the surface layers followed by a decline in C stocks with increasing soil depth. This characteristic feature in distribution of C in soil profiles may be explained by the differences in allocation of above and below ground C inputs down the soil profiles. For example, the majority of above ground litter inputs are deposited in the surface soil layers thereby strongly contributing to SOC in the upper horizons (Lorenz and Lal 2005). Forest trees which generally contribute larger amounts of organic C inputs in the surface soils have been found to contain larger SOC stocks in surface soil layers compared with cultivation and native pastures soils (Wilson *et al*. 2011). Both cultivation and native pasture land uses are associated with reduced C inputs through biomass harvest and grazing respectively, hence the lower SOC stocks. On the other hand, deeper soil layers receive a higher proportion of root C contributions than from above ground residues through translocation of photosynthetically fixed C (Rasse *et al*. 2005). Cereal crops (e.g. wheat and barley) have been reported to translocate 20 to 30 % of photosynthetic C below ground (Kuzyakov 2002). About half of the translocated C was used for root growth, while a third was respired by the root or was readily decomposable root exudates, and the balance was incorporated into SOC or microbial biomass. Molina *et al*. (2001) estimated that 24 % of the net C fixed photosynthetically by corn became rhizodeposition. The more active fine roots are likely to contribute more to turnover and rhizodeposition than the larger roots mostly present in the surface horizon (Rasse *et al*. 2006). The source for young C in soils through rhizodeposition may contribute to organic C storage in subsoil layers (Rasse *et al*. 2006).

The suggestion that land use may influence SOC at depth is based on the premise that the differences in allocation of below ground C between vegetation types may affect the distribution of C down the soil profiles (Jobbagy and Jackson 2000). Consequently, differences in rooting patterns between plant functional types may influence the placement of C down the soil profiles. In a global review, Jobbagy and Jackson (2000) found that the relative distribution of SOC with depth had a slightly stronger association with vegetation than with climate and related the results to the root distribution patterns. Root distributions have been reported to be deepest under shrubs followed by trees and grasses (Jackson *et al*. 1996).

2.4.2 Vertical leaching of dissolved organic carbon down the soil profiles

Dissolved organic carbon (DOC) in the soil is a constituent of dissolved organic matter (DOM) which is comprised of a complex mixture of low and high molecular weight substances that pass through a filter of 0.45µm pore size (Kalbitz *et al*. 2000; Michalzik and Matzner 1999; Zsolnay 2003). The contribution of DOC to the total SOC has been estimated to be between 0.04 and 0.2 % (e.g. Zsolnay 1996). Despite its relatively small fraction compared to the total SOC, DOC plays an important role in physical and biological processes in the soil. DOC is the main form in which organic C is transported to subsoils, where it can be mineralized, stabilized or further leached into ground water (Don and Schulze 2008).

The origin, function and fate of DOC in soil is still only partially understood and it is not clear whether DOC originates primarily from recent litter or from relatively stable localized organic matter (Kalbitz *et al*. 2000; Neff and Asner 2001; Sanderman *et al*. 2008). Kalbitz *et al*. (2000) reported that recent litter and humus constitute the two most important sources of DOC in soils. Conversely, a study on isotopic and spectroscopic properties of DOC concluded that the source of much of DOC within the mineral soil is the local SOM (Sanderman *et al*. 2008). Further, spectroscopic studies show that, DOC at deeper soil layers is not simply a fraction of undecomposed surficial leachates but rather consists of a shift from fresh plant material to older, highly altered organic matter (Sanderman *et al*. 2008).

DOC fluxes for most ecosystems are estimated to range between 1 to 10 g C m^{-2} y^{-1} (Hope *et al*. 1994). Due to the small fluxes relative to the carbon fluxes associated with primary productivity or heterotrophic respiration in terrestrial ecosystems, DOC fluxes are generally not considered to be important components of ecosystem C balance (Neff and Asner 2001). However, the view that the contribution of DOC to total SOC is negligible has been questioned by Kalbitz and Kaiser (2008) who presented a comprehensive review of published data showing that DOC has the potential to contribute substantially (about 19-50 %) to the total SOC in the mineral soil. The review by Kalbitz and Kaiser (2008) showed that the stability of DOC to microbial degradation increased with advanced decomposition of the parent organic matter. Further, sorption of the DOC (particularly the stable aromatic compounds) to soil minerals resulted in stabilization. To this effect laboratory incubation showed the mean residence time (MRT) of DOC from the Oa horizon of a Haplic Podzol increased from <30 y in solution to >90 after sorption to subsoil (Kalbitz and Kaiser 2008).

DOC release has only typically been studied in temperate forest soils of the northern hemisphere under more humid conditions where release of DOC may be expected and as such the literature on DOC dynamics is largely-derived from such studies (Kalbitz *et al.* 2000). The fate of DOC in the soil profile with respect to its quantity, distribution and biodegradability under other land uses is largely unknown and this is especially true for Australia. The concentration and bioavailability of DOC in soils is controlled by different factors including land use through its effects on quantity and quality of organic matter inputs (Lorenz and Lal 2005). Different land uses will influence the dynamics of DOC through change of organic matter input, substrate quality and altering the rates, extent and pathways of microbial degradation (Cronan *et al.* 1992). Soil DOC is therefore strongly linked to the dominant vegetation or land use. The transport and retention of DOC in soil profiles on the other hand, is strongly influenced by soil physicochemical parameters such as soil texture, with transport reported to be stronger in profiles with higher soil permeability and retention controlled by clay content (Don and Schulze 2008). In a texturally contrasted soil, the transport of DOC to subsoils may be an important process that might lead to accumulation of C at depth. Further, the pathways of DOC movement in soils involve both preferential and matrix flow (Guggenberger and Kaiser 2003). Biofilms develop particularly at sites receiving high input of nutrients and organic substrates enhancing the heterotrophic activity in the biofilms (Guggenberger and Kaiser 2003). Determining the effects of land use on concentrations of DOC in soils may be important for identifying land uses with potential to contribute substantial amounts of soluble forms of C to subsoils especially in texturally contrasted soil profiles.

2.4.3 Vertical mixing of soil organic matter by organisms

Vertical soil mixing by organisms and earthworm bioturbation, have been identified in previous studies as processes that may lead to heterogeneous distribution of SOC down the soil profile (Lee 1985; Jègoue *et al*. 2000; Don *et al*. 2008). Bioturbation may affect directly as well as indirectly inputs of SOC down the profile (Lee 1985; Wolters 2000; Jègoue *et al*. 2000; Bossuyt *et al*. 2005). Direct inputs include disposal of waste from e.g. earthworms into the soil and sequestration of litter into burrows. Indirect inputs on the other hand may occur by earthworm activity such as burrow which serve as fast ways for fresh C transport into deep soil horizons (Don *et al*. 2008). Anecic earthworms which generally inhabit one single vertical burrow for their whole life have been shown to increase C stocks by 270 and 310 g m^{-2} in the vertical burrows (Don *et al*. 2008). Lee (1985) found that Anecic earthworms can go down to deeper soil layers (up to 5m) transporting with them fresh organic detritus from the soil surface into the burrows while mixing with the mineral soil. Land use activities such as soil inversion by mouldboard ploughing have also been reported to influence bioturbation by concentrating plant residues in subsoil layers where it is redistributed by earthworms (Allmaras *et al*. 1996). Along climatic gradients, bioturbation tends to increase with precipitation and often an intense and deeper biological mixing resulting from a more diverse set of organisms has been reported to contribute to deeper SOC profiles (Jègoue *et al*. 2000).

2.5 Soil organic carbon pools and turnover

To better understand the mechanisms by which organic C in soils is lost or stored, SOC is separated into various conceptual pools namely active, slow and passive C pools, with varying intrinsic decomposition and turnover rates (Parton *et al*. 1987; Trumbore 2009). The active C pool is comprised of simple sugars, organic acids, microbial biomass, and metabolic compounds of incorporated plant residues, the slow C pool consists of structural plant residues and physically stabilized C while the passive C pool is made up of lignin and chemically stabilized C (Parton *et al*. 1987; Cochran *et al*. 2007). The conceptual C pools are thought to typically decompose following first-order kinetics which assumes constant zero-order input with constant proportional mass loss per unit time (Parton *et al*. 1987). The turnover of C pools is described by the mean residence time (MRT) which refers to the average time C resides in a particular pool at a steady state (Parton *et al*. 1987; Six and Jastrow 2002). The active C pool is a rapidly cycling pool with turnover times of days while, the slow and passive C pools are associated with turnover times of decades and up to thousands of years respectively (Follett 2001; Trumbore 2009). The active C pool accounts for less than 5 % of the total SOC while the slow and passive C pools account for between 20 to 40 % and 60 to 70 % of total C in soils respectively (Follett 2001).

SOC pools and their respective turnover rates have been determined in many studies by quantifying the amount of CO_2-C respired from soils as a result of microbial activity during laboratory incubation followed by curve fitting of the CO_2-C evolved using first order decomposition models (Paul *et al.* 1999, 2001; Collins *et al.* 2000; Yang *et al.* 2007; Lopes de Gerenyu *et al.* 2008; Rey *et al.* 2008). The evolution of CO_2-C during laboratory incubation is directly related to substrate availability to microbial C mineralization and therefore the use of extended laboratory incubation in particular enables partitioning of cumulatively evolved CO_2-C into various pools of varying sizes and turnover (Paul *et al.* 1999). Other techniques that have been used to determine C pools sizes and turnover rates include ^{14}C dating technique, acid hydrolysis and ^{13}C natural abundance techniques, (Balesdent *et al.* 1987; Goh 1991; Paul *et al.* 1997; Paul *et al.* 1999). While tools such as ^{14}C dating are able to present estimates of SOC turnover on decadal to millennial scales (Trumbore 2009), carbon dating is relatively expensive and often unavailable (Paul *et al.* 2001). In light of such shortcomings curve fitting procedures using data from laboratory incubations has been used widely to determine C pool sizes and turnover rates.

Land use or management type has been shown to impact on the size of SOC pools and turnover as a result of change in quantity of litter inputs, redistribution of litter inputs in soils (Paul *et al.* 1999; Collins *et al.* 2000), litter quality (Yang *et al.* 2007; Rey *et al.* 2008) and duration of soil restoration (Lopez de Gerenyu *et al.* 2008) among others. Collins *et al.* (2000) reported larger amounts of SOC pools with slow turnover in soils under no till compared with continuously tilled soils with no residue application. Differences in litter quality, especially lignin and lignin/N ratio between conifer and broadleaved forests have been shown to influence SOC turnover, with slower turnover rates reported in soils under conifer forests which was attributed to larger amounts of recalcitrant lignin contents in litter (Yang *et al.* 2007; Rey *et al.* 2008).

A number of studies that have been conducted in Australia on SOC pool dynamics have used a fractionation procedure where SOM is fractionated into various components and assigned to the active, slow and passive C pool (Baldock and Skjemstad 1999). In this fractionation procedure, the active C pool is determined as the C present as particulate organic matter (POM) (>53µm) (Cambardella and Elliott 1993) or the light fraction < 2 Mg m^{-3} (Dalal and Mayer 1986). On the other hand, the amount of charcoal has been used to describe the amount of C in the passive pool, while the slow C pool is calculated by subtracting the sum of the active and slow C pool from the total organic C in soil. Studies on C pools using the above operational scheme have reported that the amounts of the active C pool in Australian soils vary widely depending on land use/management, climate and soil characteristics (Dalal and Mayer 1986; Chan 1997; Golchin *et al*. 1994). Significant influence of land use on the size of passive C pool such as char C has also been reported in Australia. Biomass burning which has been an integral part of contemporary or traditional land management practiced since the European settlement has significantly contributed to this pool (Skjemstad *et al*. 1996, 2002; Clough and Skjemstad 2000; Lehman *et al*. 2008). The results of Skjemstad *et al*. (1996) indicate that char C can account for up to 60 % of the total organic C in Australian soils. Consequently, Australian soils have been associated with relatively stable SOC pools (Lehman *et al*., 2008).

The challenge of using the above fractionation procedure to derive SOC pools is that while active C pools can be easily quantified, quantification of the stable slow C pool is more difficult by means of physical and chemical methods since the latter is composed of chemically recalcitrant substances (inherent stability) as well as physically protected C (Krull *et al*. 2003). Moreover it's difficult to determine the turnover of the physically determined pools (Krull *et al*. 2003). The use of conceptualised pools with varying turnover rates is a much simpler and direct approach of studying SOC mineralization dynamics in depth profiles to provide information on the amounts of the different C pools and their rates of turnover.

The rate of C turnover is further dependent on factors such as a) primary production (the rate of organic matter input) and soil microbial activity (the rate of SOM transformations and decay) b) climate, especially temperature and precipitation as it influences both production and decomposition of SOM, c) soil type, through its influence on stabilization of organic C. Association of SOM with soil mineral particles has been shown to slow C decomposition hence turnover rate, d) disturbance or management practices also exert considerable influence on destabilizing older carbon and hence SOM turnover via direct effects on inputs and outputs and through indirect effects on the factors controlling these fluxes.

In northern NSW Australia, majority of work on the dynamics of SOC with respect to land use has been focused mainly on quantifying the amounts of total SOC that can be stored in soils with studies conducted mainly on the top 30 cm only (Chan *et al*. 2003; Young *et al*. 2009; Wilson *et al*. 2007 a, b, 2008, 2010, and 2011). Information on the amounts of SOC pools and respective turnover rates is limited or not available. The potential for subsoils to act as net C sinks still remains poorly understood as the decomposability of SOC in subsoils remain less explored, despite the increasing evidence that substantial amounts of C resides in subsoils (Batjes 1996). Knowledge on the quantities and depth distribution of SOC pools and their respective decay kinetics is important for assessing the stability of SOC and will assist in understanding the long term dynamics of C down the soil profiles of common land uses in NSW Australia.

2.6 Soil organic carbon mineralization

SOC mineralization is a key process for soil-carbon efflux or CO_2 evolution to the atmosphere and is defined as the release of CO_2 as a metabolic by-product of organic matter decomposition by soil microbes and fauna (Raich *et al*. 1995; Hakkenberg 2008). The rate of SOC mineralization is largely dependent upon soil temperature, soil aeration and moisture conditions (Sanderman *et al*. 2010). Water is an important regulator of SOC mineralization as it dissolves organic carbon as well as oxygen and by diffusion, controls the access rate of these substances to the cell. Hence, water facilitates the availability of organic C and energy, while, at the same time, it restricts access to oxygen. The optimal water content in the soil for mineralization is thought to be 50-70 % of the soils' water holding capacity (Collins *et al*. 2000).

Other factors potentially influencing rates of soil respiration in situ include the bioavailability of C substrates for microorganisms (Amundson 2001), plant root densities and activities (Bundt *et al*. 2001; Hinsinger *et al*. 2009), soil organism population levels, soil physicochemical properties, soil drainage (Schimel *et al*. 1985; Dalal and Mayer 1986; Baldock and Skjemstad 2000) and chemical quality of the substrates (Collins *et al*. 2000). Moreover, the rate at which CO_2 moves from the soil to the atmosphere is controlled by the rate of CO_2 production in the soil (the true soil respiration rate), the strength of CO_2 concentration gradient between the soil and the atmosphere, and properties such as pore size, air temperature, and wind speed that influence the movement of CO_2 through and out of the soil (Raich *et al*. 1995).

Across major world biomes, the flux of CO_2 from the soils to the atmosphere is closely related to net primary productivity (NPP) which supplies organic residues to decomposers (Schlesinger and Andrews 2000). The global flux of CO_2 from the soils to the atmosphere is estimated to be 60-80 Pg C/yr while estimates of global terrestrial net productivity and litter fall is estimated at between 50-60 Pg C/yr (Lal 2004). The greatest rates of SOC mineralization are found in the tropics, where plant growth is luxuriant and the conditions are ideal for decomposers. Land use on the other hand is an important determinant of SOC mineralization through its influence on soil microclimate and structure and the quantity and quality of detritus. To this end, cultivated land use systems disrupts soil aggregates and exposes stable and adsorbed organic matter to decomposition (Elliott 1986; Six *et al*. 1998).

2.7 Litter decomposition dynamics

Plant litter has been recognized as the primary source of SOC (Kögel-Knabner 2002). Plant litter enters the SOM where it is subject to decomposition which involves physical consummation by fauna, leaching, and microbial utilisation of C for energy sources and of nutrients for biomass production (Berg and McClaugherty 2008). The decomposition of litter involves a complex set of stages in material loss and each stage is thought to be associated with a particular set of micro-organisms, fauna and a set of substrate conditions. During decomposition, a part of plant fixed C is respired mainly as CO_2-C and also lost in soluble forms (e.g. DOC); at the same time, the more recalcitrant forms of C (e.g. lignin) increase and substantial portions enter the mineral soil becoming precursors for humus formation (Guggenberger and Zech 1994). The rate of litter decomposition is determined by climatic factors (e.g. actual evapotranspiration, temperature and soil water availability) and biochemical composition of litter (Cotrufo *et al*. 2010).Under similar climatic conditions, litter chemical composition controls litter decomposition (Berg 2000; Cotrufo *et al*. 2010).

Following numerous studies, litter decomposition models have been developed to reflect different decomposition stages with different properties, before the litter eventually turns into humus (Fig 1.2) (Berg 2000). The early stages of litter decomposition are characterised by a quick loss of water-soluble substances (e.g. DOC) and a fast loss of C (e.g. carbohydrates) which is not encrusted by lignins before reaching relatively similar and stable levels (Berg and McClaugherty 2008). Carbohydrates are normally the more labile components as they can be readily broken down by a wide range of organisms and serve as the initial energy and carbon source for biological life (Krull *et al.* 2003). Hence, mass loss during this stage may be substantial if there is a high content of easily decomposable substances such as carbohydrates (Krull *et al.* 2003).

During the second stage, decomposed or resynthesised sugars (such as mannose) which were produced during the initial stages of decomposition are utilised by decomposer organisms (Berg and Matzner 1997). Berg and Matzner (1997) suggested that the second stage of decomposition is characterised by evolution of CO_2 at a reduced rate as the soluble fractions of C and other elements have already been lost or undergone transformations. Further, the C:N ratio decreases to 12 or less and there is a decrease in cellulose content, whereas the lignin concentration of the remaining material increases and becomes fairly stable (Melillo *et al.* 1989). By the end of the second stage, the C fractions become very similar for litter types with distinctly different initial fractions of lignin, cellulose and extractives. The remaining non-lignin C is shielded by lignin, which must be degraded before the non-lignin C can be accessed. Consequently, lignin concentrations have been reported to show negative relationships to mass loss rates during this stage (Berg *et al.* 2000). In addition to being influenced by the lignin content, decomposition at this stage may also be negatively related to the amount of N present in litter (Berg and Matzner 1997).

The late stages of litter decomposition are mainly characterized by organic matter in stable forms (mainly recalcitrant lignins) as the lack of soluble C for microbial activity is the limiting factor affecting decomposition. As a result the production of CO_2 is low and decomposition rates approach to a final limit value which can be described by an asymptotic function (Berg and McClaugherty 2008). Berg and Matzner (1997) postulated that the concentration of N in litter during the late stage is negatively related to decomposition rates. Nitrogen in the form of low-molecular weight compounds may inhibit the formation of ligno-lytic enzymes, or form recalcitrant complexes with the products of lignin-degradation (Berg *et al.* 2000). Therefore, the amount of N available at the beginning of this stage of litter decomposition may determine how much of the original organic matter will become stable and thus contribute to soil humus formation (Melillo *et al.* 1989). To this end, Berg *et al.* (2000) reported that between 0 to 55 % of litter mass remained in highly stabilized forms by the end of the third stage of decomposition.

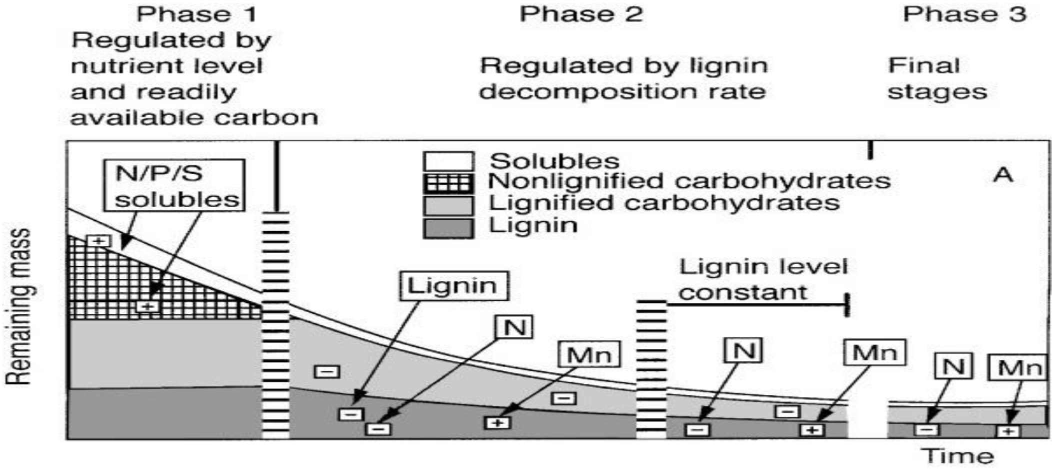

Fig1.2 Model for chemical changes and rate-regulating factors during decomposition. Modified from Berg and Matzner (1997). Source Berg (2000)

The initial biochemical composition of litter is reported to differ substantially between above and below ground plant litter inputs (Kögel-Knabner 2002) with generally larger concentrations of biochemically recalcitrant material in the latter than the former (Lorenz and Lal 2005). As a result, litter inputs are expected to influence C storage and cycling differently within the soil profile due to the obvious reasons that both litter types contributes differently to different layers of the soil profile. For example, the majority of above ground litter inputs are deposited on the soil surface thereby strongly contributing to SOC in the upper horizons (Lorenz and Lal 2005) while root litter is the main source of C in the subsoils (Rasse *et al*. 2005). A review by Lorenz and Lal (2005) reported that the majority of research on the biochemical composition of litter and its effect on decay have mainly concentrated on the above ground inputs. However, below ground residues and root turnover have potential to contribute to SOC in subsoils as they represent direct inputs into the soil system (Sanderman *et al*. 2010). Moreover, Abiven *et al*. (2005) suggested the importance of separately considering different plant parts when studying the kinetics of plant residue decomposition. Understanding how the composition of both above and below ground litter and their respective decay kinetics compares with each other will enhance our understanding on the differential contribution of plant litter materials to SOC storage in soil profiles (Mafongoya *et al*. 1998; Johnson *et al*. 2007).

Litter decomposition kinetics may be studied through laboratory incubations where mineralization of C from litter residue due to microbial activity is assessed and measured as the respired CO_2-C (Zeng *et al.* 2010; Cotrufo *et al.* 2010). The mineralization of N from decomposing litter similarly need to be determined as N has a considerable influence on formation of stabilized SOC compounds that are less easily degraded by microbial decomposers and may in turn foster an increase in C sequestration (Knicker 2011; Hagedorn *et al.* 2003; Gill *et al.* 2006; Christopher and Lal 2007; Berg and McClaugherty 2008).

2.8 Stabilization of soil organic carbon

Stabilization of SOC is defined as any process which acts to slow down decomposition of soil organic carbon compounds (Krull *et al.* 2003; Sanderman *et al.* 2010). The mechanisms by which SOC is stabilized include biochemical recalcitrance and physical protection (Krull *et al.* 2003; Sanderman *et al.* 2010). Biochemical stabilization occurs due to the complex chemical composition of organic materials. This complex chemical composition can be an inherent property of the primary molecular structures e.g. aromatic ring structures (Krull *et al.* 2003). Additionally the complex chemical composition can be attained during decomposition through the condensation and complexation of decomposing residues, rendering them more resistant to subsequent decomposition (Six *et al.* 2002). With reference to conceptualized SOC pools, carbohydrates are considered the most labile components and are usually assigned to the active C pool which turns over in months or years (Krull *et al.* 2003). On the other hand, plant biochemical structures such as lignins are considered to be stable due to their aromatic ring structure which is more resistant to decomposition than carbohydrates. In addition, alkyl structures such as lipids (waxes, cutin, fatty acids), insoluble polyesters and macromolecules synthesized by micro-organisms are also considered resistant to microbial attack due to their highly aliphatic nature. Consequently, SOC containing alkyl and lignin-derived aromatic compounds have been reported to have turnover times ranging from decades to hundreds of years (Krull *et al.* 2003). Organic C in soils may further become biochemically stabilized by the presence of charcoal (black C) whose structure is highly aromatic in nature and is therefore considered as highly resistance to biological degradation (Krull *et al.* 2003). Most Australian soils have been reported as containing substantial amounts of charcoal C (up to 60 % of total SOC) associated with past land use practices such as biomass burning after land clearing (Skjemstad et al., 1998). With the turnover time or

MRT of charcoal estimated to range from 5000 to10 000 years, it is likely that majority of Australian soils have relatively stable SOC pools (Lehmann *et al*. 2008)

The adsorption of organic C onto soil minerals (clay and silt) and occlusion within aggregates are two mechanisms by which organic C may become physically protected from microbial decomposition (Cambardella and Elliot 1992; Six *et al*. 2000; Conant *et al*. 2004; Lützow *et al*. 2006). Adsorption of SOM onto soil minerals and both amorphous iron and amorphous aluminium colloids play a major role in preservation of SOM due to their large charged surface areas (Kiem and Kögel Knabner, 2002; Six *et al*. 2002). The intimate association between SOM and soil minerals renders SOM protection because the adsorption affinity to the mineral exceeds that of the enzyme active site. The fact that clay and silt fractions further combine to form soil aggregates enhances SOM protection (Martens *et al*., 2003). The physical protection of SOM however, is not considered to equate to permanent and complete removal of organic C from decomposition, but rather to a reduced decomposition rate relative to similar unprotected materials (Baldock and Skjemstad 2000).

Hassink (1997) however reported that compared to soils from other temperate and tropical regions, Australian soils have a much lower capacity to retain organic C on their mineral particles (clay and silt) and related this to a combination of low precipitation and high temperatures in the Australian continent, leading to low accumulation of organic C. In a study conducted in NSW Australia, Chan (2001) similarly reported a tendency for Alfisols to lose mineral-associated organic C and related this to the smaller capacity of the clay and silt fraction to protect C. Krull *et al*. (2003) however interpreted these findings as indicating that the saturation level of the protective sites in these Australian soils was not reached and therefore there is still ample capacity for protection. Notably, the soils studied by Hassink (1997) and Chan (2001) were taken from the surface 10 cm soil layers and therefore the dynamics of the association of C with mineral particles at depth was not explored. Eusterhues *et al*. (2003) observed that C is increasingly associated with soil minerals in subsoil layers thereby becoming increasingly stabilized. In soils where there is an abrupt increase in clay with soil depth such as Chromosols, the association of organic C with mineral particles may be an important mechanism for stabilizing C at depth. There is a lack of information regarding the association of organic C with soil minerals at depth as majority of studies have either been conducted in shallower soil depths (10 cm) e.g. Chan (2001) or have focused on quantifying organic C in whole soil without separating SOM into soil fractions (Young *et al*. 2005, 2009; Dalal and Chan 2001; Wilson *et al*. 2007, 2008, 2010, 2011; Chan *et al*. 2010). A study on the extent and degree to which the SOC is bound to soil mineral particles in soil profiles will inform on the stability of SOC especially in a duplex soil (Chromosol) in northern NSW Australia.

2.9 Conclusion

In the context of climate change and the need to identify land uses that can sequester significant amounts of C, there is a clear need for information on storage and stability of C in soils taking into account subsoil layers especially in northern NSW Australia where this information is lacking. The ability to accurately predict increases in soil C resulting from land use change requires firstly quantifying the current C stocks in major land uses and understanding the long term dynamics of these C stores. Soil C sequestration has potential to be an important component of a carbon offset scheme such as the Carbon Farming Initiative (CFI) currently being developed in Australia, as one of the tools aimed at reducing GHG emissions. However, due to a paucity of data on the dynamics of SOC such as its storage and stability under various management and land uses, it is difficult to make policies that can affect such an implementation. There is an urgent need in Australia, for research to provide information regarding soil C sequestration in the entire soil profile, in order fully understand the potential for soils to sequester C. While land use is proposed to influence SOC storage especially in subsoil layers (Jobbagy and Jackson, 2000), firm conclusions on these land use effects have been hampered by the small database of studies (in Australia and globally) conducted below surface soil layers (Murty *et al*. 2002; Lorenz and Lal 2005). Land use may indeed play an important role in influencing C storage at depth as it affects land resources that govern C sequestration. For example, land use alters the amount and quality of organic inputs entering the soil pool thereby influencing processes that affect net C sequestration (Sanderman *et al*. 2010). A high surface input of organic matter may favour the production DOC that can be transported to deeper soil layers which may then contribute to C storage in subsoils (Lorenz and Lal 2005). The supply of biochemically recalcitrant organic matter inputs into the soil pool may lead to accumulation of more stable C pools which may interact with mineral particles therefore becoming stabilized in soil (Sanderman *et al*. 2010). Land use has a major influence on the amount, rate of turnover and distribution of C among SOC pools through its influence on the amount and quality of organic matter inputs supplied in the soil. Finally, land use may influence the initial biochemical composition of litter, therefore affecting the C and N mineralization dynamics and ability to influence formation of stable C pools. Understanding the influence of land use on these processes will increase knowledge of the potential for soils to store C in the long term.

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Chapter 3. Soil organic carbon stocks down the profiles under native woodland, native pasture and cultivation in northern New South Wales, Australia

Christine Walela[1], Heiko Daniel[1], Brian Wilson[1, 2], Peter Lockwood[1], Annette Cowie[1, 3], Bruce McCorkell[4]

[1]School of Environmental and Rural Sciences, Agronomy and Soil Science, University of New England, NSW 2351, Australia (fax +612 6773 3238).

[2]NSW Office of Environment and Heritage, PO Box 0221, Armidale, NSW 2351, Australia.

[3]Rural Climate Solutions, University of New England, NSW 2351, Australia/ Department of Primary Industries, NSW Australia.

[4]Department of Primary Industries, Tamworth Agricultural Institute, NSW, Australia.

3.1 Abstract

There is a wide acceptance that soils have the potential to sequester carbon (C) to offset greenhouse gases (GHG) with much speculation that deeper soil layers can store significant amounts of this C. It has been proposed that land use can influence soil organic carbon (SOC) storage at depth but evidence is limited as majority of studies have been conducted on the surface (0-30 cm) soil layers or less. We quantified SOC stocks in soil profiles (0-80 cm) under three common land uses in New South Wales (NSW) Australia. 50 to 60 % of SOC was found in surface (0-20 cm) soil layers however, 40 % of the total profile SOC stocks were stored in subsoils from 20 to 80 cm across all land uses demonstrating the potential for SOC storage in deeper soil layers. SOC stocks in subsoil layers did not however differ between land uses. The effects of land use on SOC stocks were largely restricted to the surface (0 to 20 cm) soil layers with native woodland containing on average11 and 9 t/ha more SOC stocks than native pastures and cultivation respectively, which were statistically similar to one another. There were large quantities of SOC stocks at depth. Differences between land uses were however only apparent in surface soil layers. It will therefore be challenging to manage deep soil C to affect significant change. Perhaps the priority is to understand the SOC at depth, its form and processes of incorporation and ensure it remains undisturbed.

Keywords: Soil organic carbon, carbon stocks, depth distribution, land use, Australia.

3.2 Introduction

Soils play an important role in the global carbon (C) cycle acting as both sinks and sources of C. As sinks, soils represent the largest terrestrial reservoir of C estimated to account for between 1500 and 1600 Pg C in the top 1m, twice the atmospheric carbon dioxide (CO_2) pool and three times that in vegetation (Batjes 1996; Jobbagy and Jackson 2000; Follett 2001). Conversely, the annual loss of CO_2-C from soils has been estimated to account for 60 and 90 Pg C globally, mainly as a result of land use change, particularly conversion to agricultural management (IPCC 1995; Schimel 1995; Houghton 1999; Lal 1999). Global increases in atmospheric CO_2 have elicited huge concern, with overwhelming evidence that the earth's climate is changing and GHG emissions are contributing significantly to this change. Most countries including Australia have now focused considerable attention on land use practices that might contribute to GHG abatement. There is a wide acceptance that soils have the potential to sequester significant amounts of C, especially where C depletion has occurred, soils might provide an opportunity for GHG abatement through the application of more judicious land use or management practices. Globally this potential has been estimated to be between 0.6 and 1.2 Pg C (Lal 2004; Lorenz and Lal 2005).

Whilst the potential for soils to store C under various land uses has been reported in a number of studies in NSW Australia, much of the work conducted has focused on surface 30 cm of the soil or less e.g. Chan *et al.* (2003), Young *et al.* (2009), Wilson *et al.* (2007 a, b, 2008, 2010, and 2011), however, fewer studies have been conducted below these layers. The rationale for limiting SOC studies to surface soils has been based on the premise that this zone covers the actively changing soil C pool where the majority of roots, plant litter inputs, and microbial activity reside. However, while the above holds, there is growing evidence that a significant proportion (> 50 %) of total profile soil C is found deeper than 30 cm and that this C may have escaped our attention (Batjes 1998; Jobbagy and Jackson 2000). It has been proposed that land use may influence SOC storage in deeper soil layers (Jobbagy and Jackson, 2000). However, firm conclusions on the effects of land use on soil C stocks have been hampered by the small global database of studies conducted in deeper soil layers (Murty *et al.*, 2002; Lorenz and Lal, 2005).

Subsoils not only contain significant amounts of soil organic carbon (SOC) but this C is also reported to be relatively stable, characterized by long residence times of up to thousands of years compared to highly labile SOC in surface soils with a turnover rate of less than 10 years (Paul et al. 1997). A range of explanations have been put forward for the accumulation of stable SOC in deeper soils and include; (a) accretion of relatively recalcitrant organic matter inputs e.g. root C, whose decomposition has been shown to decrease with soil depth (Gill and Burke, 2002); (b) Interaction with soil minerals (clay and silt) and inclusion in soil aggregates whose importance has been shown to increase with soil depth as SOC becomes increasingly protected (Moni *et al*. 2010); (c) Scarcity of fresh plant inputs leading to an energy and N limitation for microbial biomass (Fontaine *et al*. 2007); and (d) contrasting environmental factors (e.g. soil moisture, aeration, oxygen supply) controlling SOC stability within surface and subsoils (Sanaullah *et al*. 2011).

There is therefore an urgent need for information on the amounts of SOC stocks stored through the whole profile under a range of common land use in order to make informed judgements regarding the influence of land uses on deep soil C storage potential. Our study therefore aimed to determine the quantities of SOC stored down the soil profiles (0-80 cm) under native woodland, native pastures and cultivation in northern NSW, Australia.

3.3 Materials and methods

3.3.1 Site description

The study was conducted on the Northern Tablelands of NSW, Australia (Fig. 3.1). Sampling sites were selected from an existing network of monitoring sites on the Bundarra Granite Soil Monitoring Unit (as defined in Chapman *et al*. 2009) adjacent to the township of Uralla. Bundarra Granites are of Permian age and soils derived from these are typically classified as Yellow Chromosols (Isbell 2002) equivalent to Alfisols (Soil Survey Staff 1999). Uralla lies at 30.64°S, 151.49°E and located at an altitude of 1,012 m and receives a mean annual rainfall of 807 mm and has a mean maximum and minimum temperature of 26.4°C and 12.5°C respectively (1901-2012; Bureau of Meteorology 2012).

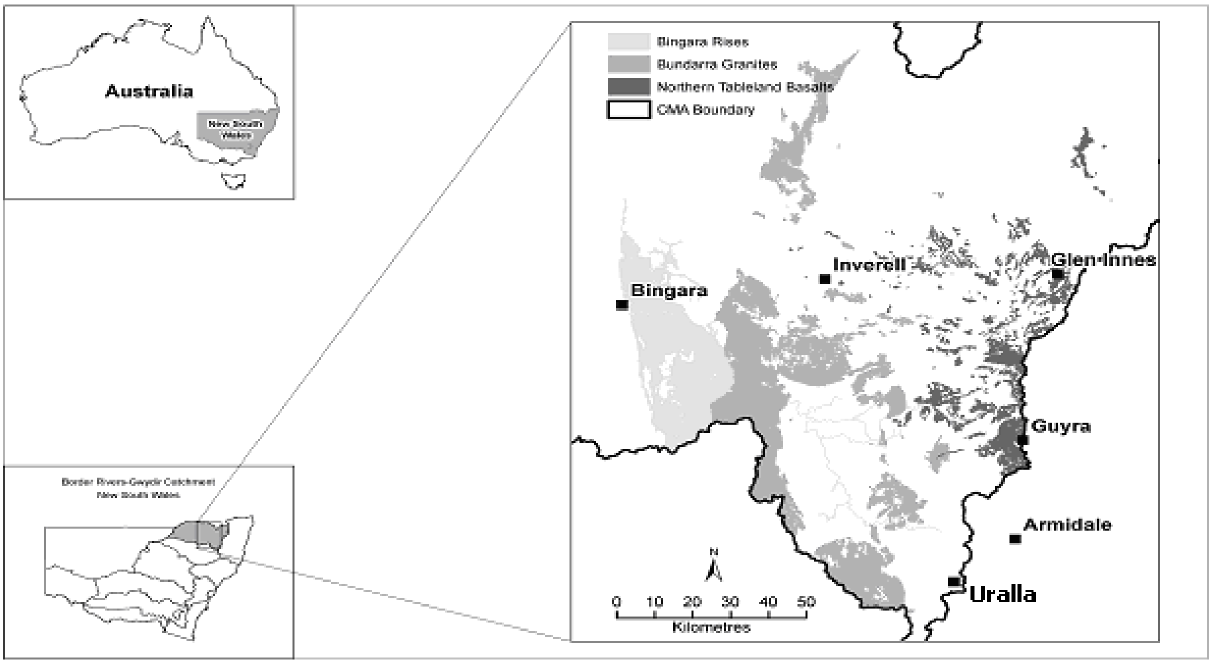

Fig. 3. 1 Location map of the study sites in NSW Australia

3.3.2 Land use sampling approach

The study was carried out using a sampling approach similar to that used by Wilson *et al.* (2008, 2010, and 2011). Site clusters containing land uses namely; native pastures, native woodland and cultivation were randomly selected at each site and replicated at three sites. Different land uses were separated by <500 m and were on similar soil type. Additionally, at each site and upon interviewing the land owners, it was confirmed that the land uses selected had been under current management for >20 years. It was therefore assumed that each site was at equilibrium for the respective land uses. Native woodland sites consisted of mixed, grassy woodlands of *Eucalyptus* species including Yellow box (*E. melliodora*) and Blakely's Red Gum (*E. blakelyi*) with a range of native grasses and shrubs in the understorey. These sites had never been cleared and had received minimal management and only periodic light grazing. Native pastures were typically minimally fertilized (superphosphate) and were dominated by native grasses such as *Microlaena stipoedes* with limited oversown legumes (e.g. sub-clover). These pasture sites have historically been grazed, mainly with sheep, although cattle have become more common in recent years. The cultivated paddocks had been intermittently cultivated with forage oats and pasture for at least 30 years. The cultivated paddocks were not cropped at the time of soil sampling but were under patchy volunteer pastures of native grasses.

3.3.3 Soil sampling

Soil sampling was conducted following protocols similar to those described by Wilson *et al*. (2011). A stratified random sampling design was used for each cluster, land use and sample site. A 25 x 25 m sample plot was randomly located within each cluster, land use and sample site. Nine soil cores were randomly collected from each 25 x 25 m sample plot. At each sampling point, litter and plant materials were cleared from the soil surface and a truck-mounted hydraulically driven soil corer of 50 mm internal diameter was used to sample to 0.80 m (to weathering rock). The soil core was then placed on a graduated Polyvinyl Chloride (PVC) tube that had been halved lengthwise and labelled in 0-5, 5-10 and subsequent 10 cm depth increments. Using a sharp cutting knife, each core was accurately divided into nine depth increments of 0-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70 and 70-80 cm. Samples were placed into plastic bags, sealed, stored in cooler boxes and transported to the laboratory and stored under cool conditions (<4 ^{o}C) until they could be processed (typically <48 h).

3.3.4 Soil sample preparation and analysis

Soil samples were initially weighed wet then dried at 40^{o}C for 48 h or until fully dried (i.e. weight stabilized). Samples were then re-weighed to determine sample dry weight Wilson *et al*. (2009). The soil bulk density was calculated for each individual sample as the dry weight of a soil divided by its volume. Soil samples were then crushed to pass a 2 mm sieve. Each sample was analysed for the following soil properties: total C and nitrogen (N) using LECO CN-1000 autoanalyser (LECO Corporation, St. Joseph, Mich); pH (1:5 $CaCl_2$) using electrode method (Rayment and Higginson 1992), particle size distribution determined using Andreasen pipette method (Green 1981). Identification of clay minerals was conducted on soil samples obtained from 30-50 cm soil layers, which represented a zone with higher clay accumulation within the soil profiles across all land uses. A qualitative X-ray diffraction (XRD) spectrometry method was used to identify clay minerals. Prior to identification, samples were subjected to a pretreatment by saturating with Mg and glycolation following methods described by Tan (2005).

3.3.5 Calculations of soil organic carbon stocks on equivalent soil mass basis

SOC stock per unit area was calculated as the product of concentration of C in the soil; bulk density and thickness of soil layer (Wilson *et al*. 2011). The concentration of C was obtained directly from chemical analyses (section 3.3.4). Since bulk density is significantly influenced by management, soil C stocks were calculated on ‘equivalent soil mass’ basis (after Ellert and Bettany 1995). The mass of soil at each depth increment was determined from the cultivated soil at each site and this was used as a reference for recalculation of C stocks for the other two land uses to an equivalent mass. This protocol is well established in literature where the mass of the heaviest soil layer (in our case cultivated soils) and most susceptible to the influence of land use change is designated as the ‘equivalent’ mass (Ellert and Bettany 1995, Chan *et al.* 2010; Wilson *et al.* 2011).

The following equations were used in the calculations of ‘equivalent mass’ (after Ellert and Bettany 1995):

$M_{element} = conc.P_b.T.10\,000\ m^2\ ha^{-1}.0001\ Mg\ kg^{-1}$ Equation 1

Where:

$M_{element}$ = Element (in this case carbon) mass per unit area (Mg ha^{-1})

Conc = element concentration (kg Mg^{-1})

ρ_b = field bulk density (Mg m^{-3})

T = thickness of soil layer (m)

The additional soil thickness required to attain this equivalent mass in lighter soil layers was calculated as follows:

$T_{add} = \frac{M_{soil,\ equiv} - M_{soil,\ surf}).0.0001\ ha\ m^{-2}}{\rho_{b\ subsurface}}$ Equation 2

Where:

T_{add} = additional thickness of subsurface layer required to attain the equivalent soil mass (m)

$M_{soil,\ equiv}$ = equivalent soil mass = mass of heaviest horizon (Mg ha^{-1})

$M_{soil,\ surf}$ = sum of soil mass in surface layer (s) (Mg ha^{-1})

$\rho_{b\ subsurface}$ = bulk density of subsurface layer (Mg m^{-3})

The mass of the element per unit area in an equivalent soil mass was calculated by summing the elements in surface layers plus those in the additional thickness of subsurface layer required to attain the equivalent soil mass:

$M_{element,\ equiv} = M_{element,\ surf} + M_{element,\ T_{add}}$ Equation 3

Where:

$M_{element,\ equiv}$ =element mass per unit area in equivalent soil mass (Mg ha^{-1})

$M_{element,surf}$ =sum of element mass in surface layer (s) (Mg ha^{-1})
$M_{element, Tadd}$ =element mass in the additional subsurface layer (Mg ha^{-1})

3.3.6 Statistical analyses

Statistical analysis was undertaken using ASREML software (Gilmour *et al*. 2006). Fixed factors were land use and soil depth while site clusters and soil cores within land uses were random factors. The analysis of C %, N %, C to N ratio, SOC stocks, soil pH and bulk density was performed using a generalised linear model (GLM) that incorporated cubic smoothing splines. The cubic smoothing splines absorbed the serial correlations associated with depth measurements within the same cores. A correlation structure that modelled the correlation between regression parameters for the linear component of the splines was added to the splines to account for the variation between cores ensuring that comparisons between cores were less affected by isolated spatial effects. Data were transformed, as appropriate, to compensate for heteroscedascity and lack of normality. Confidence bands (95 %) were fitted along the splines to test for significant differences between land uses at various depths. A correlation matrix was used to show significant correlations between soil attributes.

3.4 Results

3.4.1 Soil depth and land use effects on soil carbon, nitrogen and C:N ratio

There were strong and significant ($P<0.001$) land use and depth main effects on C concentrations, suggesting a strong and consistent change in C concentrations with land use and soil depth. The distribution of C in the soil profile was characterized by large concentrations in surface soils followed by a rapid decline with increasing soil depth, a trend that was similar for all land uses (Fig 3.2). Significant differences in C concentrations between land uses were limited to the surface (0 to 20 cm) soil layers (Fig 3.2) with native woodland soils shown to contain significantly larger C concentrations than the two other land uses, which were generally statistically similar to each other. C concentration in subsoils (20-80 cm) was largely similar between land uses. N concentrations were not influenced by land use, but were shown to change significantly ($P<0.001$) with depth following a similar pattern to that of C concentrations. No significant land use and soil depth main effects or interactions were found for C:N ratio.

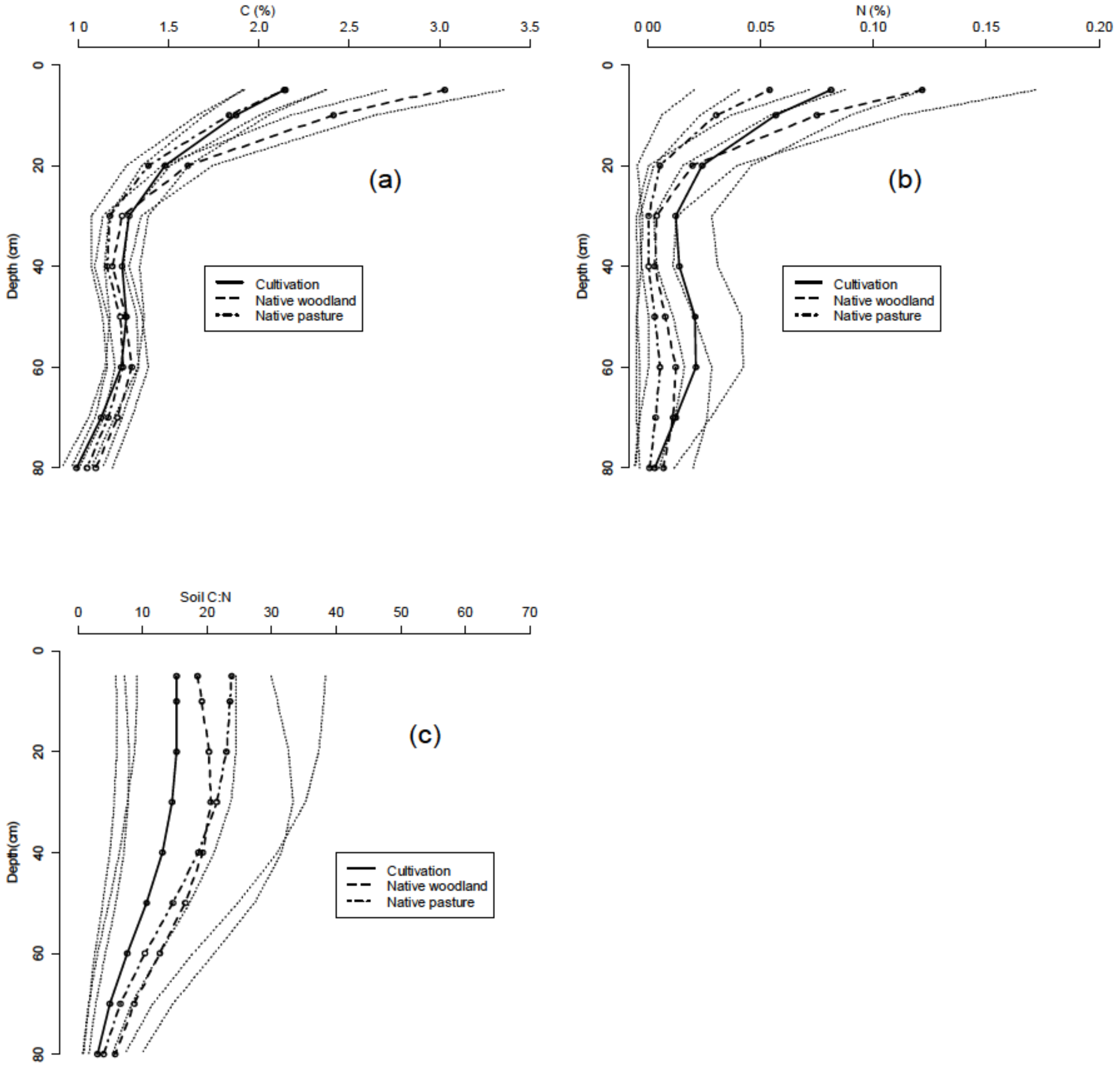

Fig. 3. 2 Soil carbon (a), nitrogen concentrations (b), and C:N ratios (c) in soil profiles under native woodland, native pastures and cultivation. 95 % confidence bands are fitted along the splines to test for significant differences between land uses at various soil depths.

3.4.2 Land use effects on SOC stocks down the soil profiles

There were strong and significant ($P<0.001$) land use and depth main effects on SOC stocks (t/ha), indicating a significant change in SOC stocks with land use and soil depth. Strong differences in SOC stocks were found in surface (0 to 20 cm) layers with native woodland soils containing on average 11 and 9 t/ha more SOC stocks than native pastures and cultivation respectively, which were again shown to be statistically similar to each other. Similar to SOC concentration, no land use differences were found for SOC stocks in the subsoil (20 to 80 cm) layers. When the entire soil profile (0-80 cm) was considered, native woodland soils had larger (51 t/ha) SOC stocks than soils under cultivation (40 t/ha) and native pastures soils (38 t/ha) which were similar between each other. SOC stocks decreased with increasing soil depth under all land uses, with surface 0 to 20 cm soils containing 57, 49 and 47 % of the total profile SOC stocks under native woodland, cultivation and native pastures respectively (Table 3.1).

Table 3. 1 Mean soil organic carbon stocks (t/ha) (±SE, n =9) (calculated and expressed as equivalent mass) down the profiles under different land uses. Different letters indicate significant differences (p=0.05) among similar depths between different land uses.

Soil depth (cm)	Native woodland (t/ha)	Native pastures (t/ha)	Cultivation (t/ha)
0-5	$12.82^{a}\pm1.3$	$7.66^{b}\pm1.0$	$7.77^{b}\pm1.0$
5-10	$10.28^{a}\pm1.0$	$6.28^{b}\pm0.8$	$6.86^{c}\pm0.8$
10-20	$6.29^{a}\pm0.8$	$4.21^{b}\pm0.6$	$5.39^{c}\pm0.7$
20-30	$4.31^{a}\pm0.7$	$4.10^{a}\pm0.6$	$4.61^{a}\pm0.7$
30-40	$4.00^{a}\pm0.6$	$3.49^{a}\pm0.6$	$3.90^{a}\pm0.7$
40-50	$4.28^{a}\pm0.7$	$3.90^{a}\pm0.6$	$4.36^{a}\pm0.7$
50-60	$4.09^{a}\pm0.7$	$3.81^{a}\pm0.6$	$3.61^{a}\pm0.6$
60-70	$3.14^{a}\pm0.6$	$3.00^{a}\pm0.5$	$2.65^{a}\pm0.4$
70-80	$2.00^{a}\pm0.6$	$1.50^{a}\pm0.4$	$1.49^{a}\pm0.2$
Overall SED	0.2601		
LSD (P < 0.05)	0.5123		

3.4.3 Soil physical and chemical properties down the soil depth

Soil profiles under all land uses exhibited a strong texture contrast between surface and subsoil layers with surface soils characterized by >80 % sand, <10 % clay and <10 % silt content whereas, subsoils showed abrupt increases in clay (20 to 40 %) content (Table 3.2). The abrupt increase in clay content occurred at a depth of 20 to 40 cm under cultivation, 30 to 40 cm under native pastures and 40 to 50 cm under native woodland soils. The clay content decreased in the deepest (70-80 cm) soil layers as the weathering granitic rock was encountered. Results on identification of clay minerals showed a XRD pattern characterized by a first-order diffraction at an angle of $2\ \theta = 12.4^{o}$ and a second-order diffraction at $2\ \theta = 25.0^{o}$ (Fig 3.3). This characteristic is exhibited by Kaolinite (1:1) clay minerals, and this pattern was observed from samples obtained from all land uses.

There were strong ($P<0.001$) and significant land use and depth main effects on bulk density. Land use influences on bulk density were found to exist in the 0 to 60 cm soil layers. Bulk density in these soil layers was calculated separately and was found to differ in all layers in this range. Cultivated and native pastures soils had larger and statistically similar bulk density compared to native woodland soils in the 0 to 60 cm soil layers; however, these differences diminished in subsequent soil depths (Fig 3.4). Generally, bulk density increased with soil depth and this increase was consistent for all land uses.

Soil pH $CaCl_2$ (1:5) showed significant ($P<0.001$) interactions between land use and soil depth. Native woodland soils had slightly higher soil pH at the surface (0 to 20 cm) soil layers compared with native pastures and cultivation which were statistically similar to each other. These latter land uses exhibited increasing soil pH with soil depth but the magnitude of increase was less for native woodland and therefore at depth it was more acidic than the other two land uses (Fig 3.4).

Table 3. 2 Soil texture distribution down the profiles under three land uses in NSW, Australia (*n*=3 sites).

	Native Woodland			Native Pasture			Cultivation		
Depth (cm)	Clay (%)	Silt (%)	Sand (%)	Clay (%)	Silt (%)	Sand (%)	Clay (%)	Silt (%)	Sand (%)
0-5	5	8.4	86.6	4.0	9.8	86.2	5.9	9.2	84.9
5-10	5.6	8.9	85.4	4.3	10.0	85.6	6.3	9.4	84.3
10-20	6.1	8.8	85.1	4.6	9.5	85.9	6.7	8.9	84.4
20-30	6.0	12.5	81.6	4.6	9.3	86.1	14.2	6.7	79.1
30-40	7.2	6.6	86.2	17.5	8.3	74.2	35.7	6.8	57.5
40-50	24.8	6.1	69.1	35.5	7.1	57.5	40.9	6.0	53.1
50-60	43.5	6.7	49.8	34.6	8.1	57.3	30.9	5.6	63.5
60-70	40.1	6.9	53.0	35.4	6.8	57.8	21.4	4.8	73.8
70-80	32.9	6.6	60.5	27.7	7.8	64.5	16.6	4.4	79.1

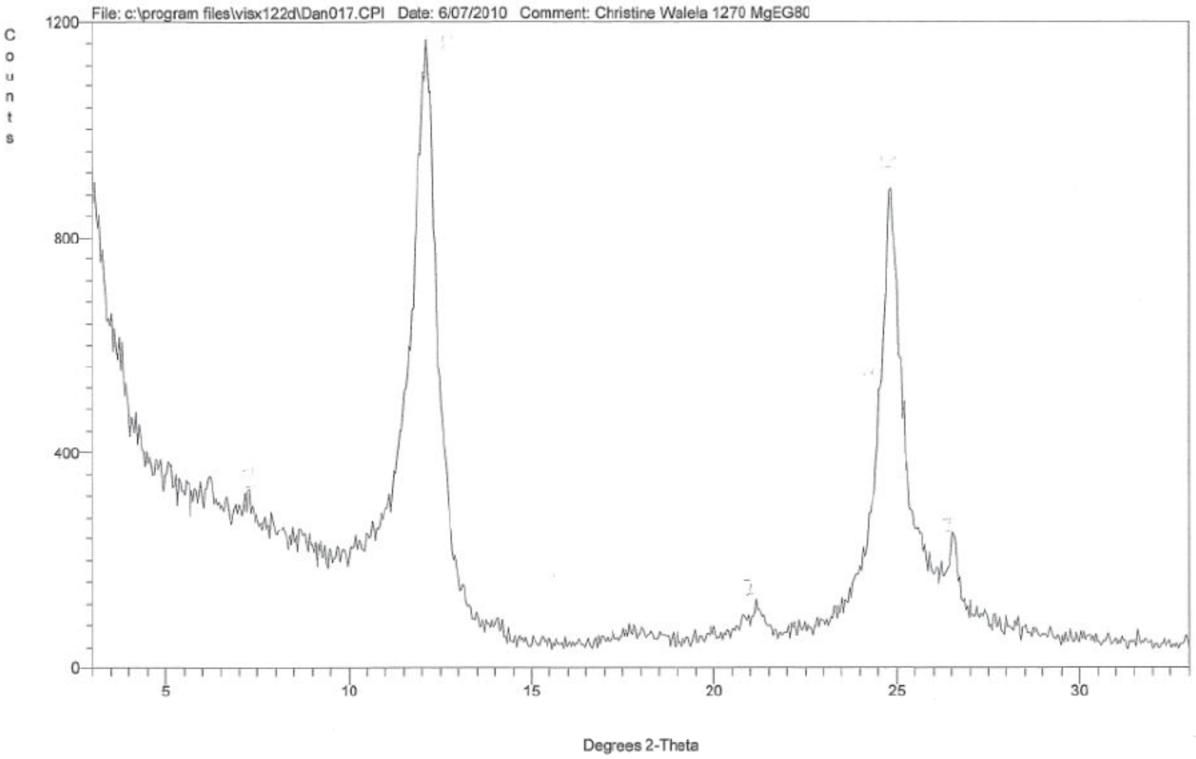

Fig. 3.3 Diffractogram (representative of all sites) identifying kaolinite clay mineral using its characteristic XRD patterns ($2\theta = 12.4^{\circ}$ and $2\theta = 25.0^{\circ}$)

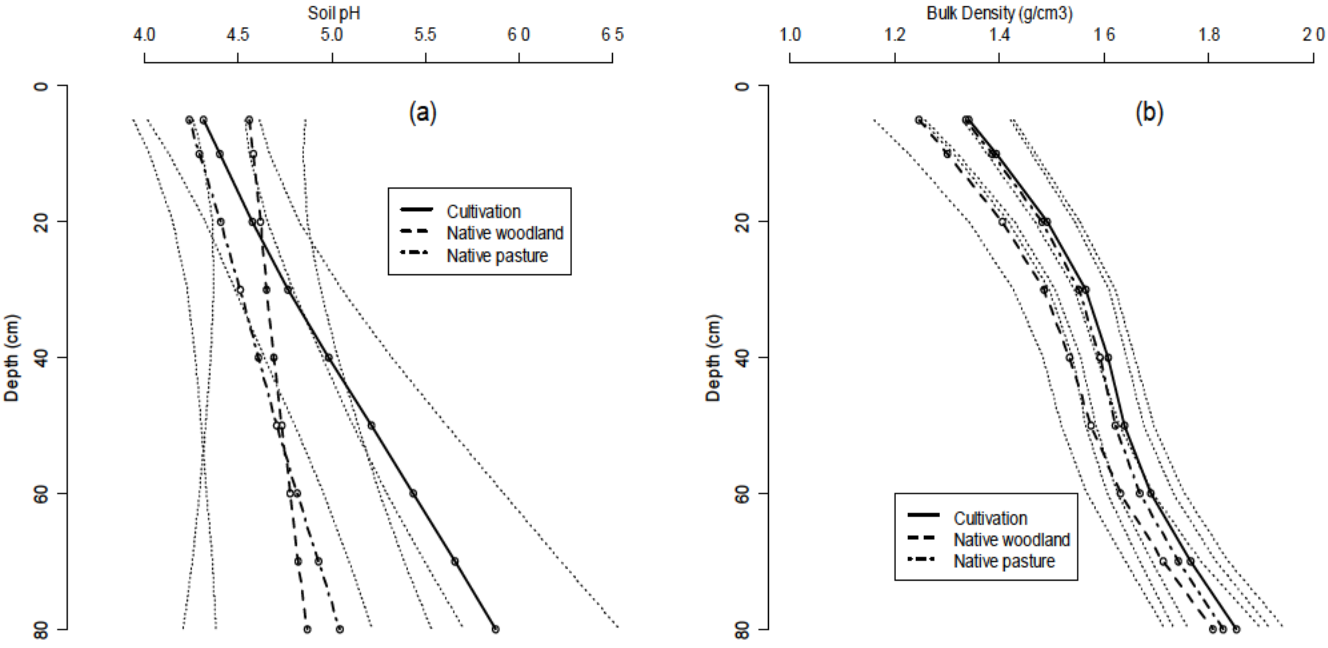

Fig. 3.4 Soil pH (a) and Bulk density (b) in soil profiles under three land uses. 95% confidence bands are fitted along the splines to test for significant differences between land uses at various depths.

3.5 Discussion

3.5.1 Soil depth and land use effects on soil carbon and nitrogen concentrations

The magnitude of soil C and N concentrations reported in this study compares well with results reported under similar land uses on granite derived soils in Northern Tablelands of NSW Australia (Wilson *et al*. 2011). Strong land use effects on SOC concentrations were limited to surface soils as was observed by Young *et al*. (2005) and Wilson *et al.* (2008, 2010, 2011) in studies conducted in NSW, Australia and elsewhere by Lal and Puget (2005). In contrast to subsoils, the near surface soils consist of an actively changing zone that is strongly influenced by key biological processes that govern SOC storage (primary production and soil organic matter mineralization) and change with land use (Lorenz and Lal 2005). Low SOC concentrations under native pastures and cultivated soils were probably related to reduced organic residue inputs as a result of intensive grazing, continued C decomposition and export of nutrients through harvesting of forage oats respectively. Higher SOC concentrations in surface soils under native woodland have been related to continued large inputs of low quality litter (Wilson *et al*. 2011). Further, the above and below ground litter residues under native woodland have been found to be of lower quality containing higher C to N ratio and larger concentrations of lignin and tannin compared with native pastures and cultivation (chapter 7).

3.5.2 Land use effects on SOC stocks down the soil profiles

The effects of land use on the quantity of SOC stocks were restricted to the surface (0-20 cm) soil layers with native woodland soils found to contain 11 and 9 t/ha more SOC stock than native pastures and cultivation respectively. Assuming that the SOC stocks presented in this study represent equilibria for the respective land uses, the above results indicate that the amounts of C in either native pastures or cultivation soils represent 38 % and 31 % losses in soil C respectively, in the surface soils as a result of conversion from native woodland. These results suggest that there is a potential to store C in these depleted soils (Smith *et al*. 2010). The results from this study are comparable with studies conducted in similar environments by Murphy *et al*. (2002) and Wilson *et al*. (2011). Wilson *et al*. (2011) reported that native woodland contained 10 and 13 t/ha more SOC in surface soils compared with native pastures and cultivation respectively. The results by Wilson *et al*. (2011) similarly showed that a conversion from native woodland to either native pastures or cultivation represented a 23 % and 28 % loss of SOC in surface soils respectively. While the losses of SOC resulting from land use change especially conversion from unmanaged (e.g. native woodland) to managed systems (pastures, cultivation) are well documented (Dalal and Chan 2001; Murty *et al*. 2002), these results provide an estimate of the magnitude of such losses at the soil surface especially for the temperate landscapes of NSW, Australia and demonstrate that such changes are restricted to these depths.

Over 40 % of the total profile organic carbon stocks were stored in subsoil (20 to 80 cm) layers across the three land uses, demonstrating the potential for SOC storage in deeper soil layers. The effects of land uses on stocks of SOC in deeper soil layers have not been previously reported in the study area and are consistent with reports that significant amounts of SOC stocks are contained in deeper soil layers (Batjes 1998; Jobbagy and Jackson 2000). Although there were large quantities of SOC at depth, differences between land uses were only apparent in surface soil layers suggesting that it will be challenging to manage deep soil carbon to affect significant change. Perhaps the priority is to understand the SOC at depth with respect to: a) its form, b) processes of incorporation and c) stability at depth and if this differs across land uses.

3.5.3 Soil physical-chemical properties down the soil profiles

The slightly higher soil pH values observed in surface soils under native woodland compared with other land uses is well documented and has been attributed to ‘a biological pumping process’ which results from trees drawing alkalinity from deeper soil layers and redepositing this in surface layers (Graham et al., 2004; Noble et al., 1996; McHenry et al., 2006; Wilson et al., 2011). Bulk density was significantly influenced by land use and since bulk density needs to be considered during calculation of SOC stocks, our results emphasize the importance of using equivalent mass of soil in order to reliably compare effects of land use change on SOC stocks, as similarly reported by Ellert and Bettany (1995).

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	Author's Name (please print clearly)	**% of contribution**
Candidate	Christine Walela	80
Other Authors	Heiko Daniel	4
	Brian Wilson	4
	Peter Lockwood	4
	Annette Cowie	4
	Bruce McCorkell	4

Name of Candidate: Christine Walela

Name/title of Principal Supervisor: Prof. Heiko Daniel

[redacted]

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Candidate

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[redacted]

____

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[redacted]

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Chapter 4. Land use effects on dissolved organic carbon down the profiles and determination of amounts and composition of water soluble carbon extracted from different litter types

Christine Walela[1], Heiko Daniel[1], Peter Lockwood[1], Brian Wilson[1], Annette Cowie[2], David Tucker[3] and Bruce McCorkell[4]

([1]) School of Environmental and Rural Sciences, Agronomy and Soil Science, University of New England, NSW, Australia ,

([2]) Rural Climate Solutions, University of New England, ([3]) NSW Department of Primary Industries , Beef Industry Centre,

NSW, Australia ([4]) NSW Department of Primary Industries, Tamworth Agricultural Institute, NSW, Australia

4.1 Abstract

Leaching of dissolved organic carbon (DOC) in soils is an important process that transports carbon (C) from surface to subsoil layers where it may accumulate and contribute to C storage. The amounts and fate of DOC in the soil is largely dependent on the quantity and quality of organic matter inputs. We quantified the amounts of DOC in texturally contrasted soil profiles (0-0.8m) under three major land uses namely native woodland, native pastures and cultivation.The amount and composition of dissolved organic matter (DOM) extracted from above and below ground litter components was determined. The relative proportion of aromatic C in DOM extracted from litter was highest in native woodland, followed by native pasture then cultivation indicating qualitative differences in DOM fractions which may in turn influence biodegradability of DOM among litter types. Land use influenced the concentration of DOC down the soil profiles with native woodland soils shown to contain significantly larger amounts of DOC in all soil depth increments compared with native pastures and cultivation which differed between each other in the 40 cm only. Larger DOC concentrations down the soil profiles under native woodland may be explained by larger organic matter inputs. DOC represented between 0.01 and 0.1 % of total SOC down the soil profiles and across all land uses showing that the contribution of DOC was relatively small. DOC concentrations decreased with increasing soil depth with the rate of decline shown to diminish in subsoil (> 20 cm) layers which may be related to adsorption to clay minerals particles which increased with soil depth. Although the contribution of DOC to total SOC was small, the results show that the sandy textured surface soils may play an important role in rapidly transporting DOC to subsoil clayey layers where it may be sorbed. A key management strategy would be to increase surface litter inputs which may favour the production of DOC that may be transported to deeper soil layers. The stability of DOC derived from both soils and litter will require further testing in order to determine its long term dynamics in soils.

Key words: Dissolved organic carbon, Land use, Plant litter, Aromaticity, soil texture

4.2 Introduction

Dissolved organic carbon (DOC) in the soil is a constituent of dissolved organic matter (DOM) which is comprised of a complex mixture of low and high molecular weight substances that pass through a filter of 0.45µm pore size (Kalbitz *et al*. 2000; Michalzik and Matzner 1999; Zsolnay 2003). DOC comprises a small fraction of soil organic carbon (SOC) (0.04 to 0.2 %) however due to its highly mobile nature, the leaching of DOC in soils plays a significant role in transporting organic carbon (C) from surface soils to subsoil layers where it can be mineralized, stabilized or further leached into ground water (Kalbitz and Kaiser, 2008; Sanderman and Amundson, 2009). The concentration and bioavailability of DOC in soils is affected by different factors including land use through its effects on quantity and quality of organic matter inputs (Lorenz and Lal, 2005). On the other hand, the transport and retention of DOC in soil profiles has been reported to be strongly influenced by soil physicochemical parameters such as soil texture, with transport reported to be stronger in profiles with higher soil permeability and retention controlled by clay content (Don and Schulze, 2008). In a texturally contrasted soil, the transport of DOC to subsoils may be an important process that might lead to accumulation of C at depth. Determining the effects of land use on concentrations of DOC in soils may be important for identifying land uses with potential to contribute substantial amounts of soluble forms of C to subsoils especially in texturally contrasted soil profiles. Further, the amounts and composition of DOC extracted from litter may suggest the amounts of DOC available for the immediate soil environment and biodegradability of DOC respectively.

The knowledge on DOC has to a great extent been based on forest soils, mainly in the northern hemisphere under more humid conditions where release of DOC may be expected and as such the literature on DOC dynamics is largely-derived from such studies which have been extensively reviewed by (Milchalzik *et al*., 2001). In a comprehensive review of published studies (about 42 studies) which were mainly carried out in North America and Europe, Milchalzik *et al*. (2001) observed that overall DOC represented a substantial part of the C cycle in forest ecosystems and especially in forest soils as indicated by high rates of input from the forest floor and strong retention of DOC in mineral soils. Kalbitz and Kaiser (2008) similarly presented findings from a comprehensive review of published studies showing that DOC contributed between 19 and 50 % to the total soil carbon in forest mineral soil. In studies where measurements of DOC have been conducted in contrasting land uses, different conclusions have been reached regarding the land use associated with the largest amounts of DOC in soils (Boyer and Groffman, 1996; Chan, 2001; Ghani *et al*., 2007). Boyer and Groffman (1996) reported higher concentrations of DOC under agricultural (cornfield) soils compared with forest soils and attributed their findings to an increase in the soluble humic fraction in the agricultural soils. The results of Boyer and Groffman (1996) suggested that while it is clear that agricultural activities such as harvest and tillage lead to reductions in total SOC, the relatively high litter quality (inherent biodegradability) of agricultural crop residues may increase concentrations of DOC in soils above those found under forest vegetation. In a study conducted in New Zealand, Ghani *et al.* (2007) reported that DOC concentrations tended to be comparatively greater in pasture soils than in intensively cropped agricultural land and natural systems/native forests. A study in southeastern Australia by Xiaoqi *et al*. (2012) reported that compared to nil-crop treatment, soils with cover crop treatments (wheat and oat) had significantly larger amounts of soluble C and attributed the findings to microbial decomposition of large quantities of crop residues applied as cover crops. A study conducted in New South Wales Australia by Chan (2001) reported that DOC concentrations contributed between 4.4 and 18.8 % of the total SOC under different land uses namely native vegetation, pastures and cropping on two soil types (Alfisols and Vertisols). The study by Chan (2001) found larger DOC concentrations under pastures sampled from an Alfisol compared to the other land uses.

Our present knowledge on the quantities of DOC in soils under major land uses in Australia and particularly in NSW region is however limited. There is little site specific information on the effects of land use on DOC concentrations as the majority of studies conducted in this region on soil C under different land uses have focused mainly on quantifying total SOC stocks (Chan *et al*.,2003; Young *et al*., 2009; Wilson *et al*., 2011). The contribution of DOC to total SOC in soil profiles has rarely been accounted for.

Moreover, the few studies that have reported on DOC concentrations in soils have focused mainly on shallow depths of up to 10 cm (Chan, 2001; Xiaoqi, 2012) or less (<7.5 cm) (Ghani *et al.,* 2007). While some studies have reported that DOC is largely sourced from the local SOM in mineral soils (Sanderman *et al.*, 2008), the general literature suggests that DOC is mainly derived from litter layers in surface soils where it is transported in subsoil mineral layers which serve as major C sinks (Kalbitz and Kaiser, 2008). To better understand the potential for DOC to contribute substantial amounts of C in subsoil layers, quantitative information on the dynamics of DOC in soil profiles under major land uses is necessary especially in NSW Australia. Further, the dynamics of DOC in soils is largely determined by its quality which is largely dependent on its origin (Kalbitz *et al.*, 2000). The properties of DOC determine its biodegradability with DOC fractions rich in aromatic compounds and poor in carbohydrates shown to be less biodegraded by microbes compared with fractions rich in labile fractions e.g. carbohydrates (Kalbitz *et al.*, 2003). The leaching of root-derived DOC which was partly constituted by resistant aliphatic compounds was reported to contribute to humus formation in subsoils (Maie *et al.*, 1997). The composition of dissolved organic matter (DOM) extracted from above and below ground litter inputs could therefore provide insights on the fate of DOC in soils.

Our study therefore aimed to a) determine the quantities of DOC stored under native woodland, native pastures and cultivation in texturally contrasted soil profiles, and b) determine amount and the composition of DOM extracted from above and below ground litter sources from the three land uses.

4.3 Materials and methods

4.3.1 Site characteristics

This study was conducted near the township of Uralla in the Northern Tablelands of New South Wales, Australia. Uralla lies at 30.64^{o}S, 151.49^{o}E at an altitude of 1,012 m and receives a mean annual rainfall of 807 mm and has a mean maximum and minimum temperature of 26.4^{o}C and 12.5^{o}C respectively (1901-2012; Bureau of Meteorology, 2012). The soils of the area are largely derived from Permian granite and are classified as yellow Chromosols (Isbell, 2002) equivalent to Alfisols (Soil survey staff 1999).

4.3.2 Land use sampling approach and management history of sample sites

The study was carried out following a sampling approach similar to that used by Wilson *et al*. (2008, 2010, 2011). Site clusters containing three land uses namely, native pastures, native woodland and cultivation, were randomly selected at three separate locations on a similar soil type, giving 3 replicates of each land use, and a total of nine sites. Different land uses were separated by <500 m within each sample cluster. Land use type and management history is as described in Table 4.1.

Table 4. 1 Management history of native woodland, native pastures and cultivation land use selected for soil and litter sampling in Uralla northern NSW Australia

Land use	History
Native woodland	Had never been cleared (undisturbed) and was used as reference points against which changes in the managed landscape have been measured; and Consisted of a mixture of *Eucalyptus* species including Yellow box (*E. melliodora*) and Blakely's Red Gum (*E. blakelyi*) with a range of native grasses and shrubs growing beneath the trees.
Native pasture	Had been under current land use for >20 years; Minimally fertilized (superphosphate) with limited oversown legumes (e.g. sub-clover); Dominated by native grasses such as *Microlaena stipoedes;* Paddocks had been historically grazed under set-stocking mainly with sheep although cattle had become more common in recent years.
Cultivation	Forage oats was the dominant crop that had been historically cultivated in alternate years with pasture for > 30 years.

4.3.3 Field soil sampling

A stratified random sampling design was used for each land use and sample site. A 25 x 25 m sample plot was randomly located within each cluster, land use and site. Three sampling points were randomly selected within each plot. At each sampling point, litter and plant materials were cleared from the soil surface and a truck-mounted hydraulically driven soil corer of 50 mm internal diameter was used to sample to 0.80 m (to weathering rock). The soil core was then placed on a graduated Polyvinyl Chloride (PVC) tube that had been halved lengthwise and labelled. Using a sharp cutting knife, each core was accurately divided into 7 depth increments of 0-0.05, 0.05-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.40, 0.40-0.60, 0.60-0.80 m. Samples were placed into plastic bags, sealed, stored in cooler boxes and transported to the laboratory and stored at temperatures ranging from

0 to 4^{o}C. Field moist subsamples were sieved through a <6 mm sieve and mixed thoroughly and extracted for soil DOC analysis as described below.

4.3.4 Soil DOC extraction

Extraction of soil DOC followed procedures developed by (Zsolnay, 2003). Briefly, extraction procedures were performed on field-moist soils which had been stored overnight at temperatures ranging from 0 to 4^{o}C after field sampling. Soil subsamples of 15 g were weighed into 50 ml centrifuge tubes. 30 ml of 5 mM $CaCl_2$ solution was added to the mineral soil and gently stirred for 1 minute with a glass rod to make a homogeneous slurry. Stirring was done as gently as possible to avoid significant desorption of soluble materials. The slurry was centrifuged at 12,000 *g* for 10 minutes. The supernatant was then filtered through a vacuum or pressure filter unit equipped with <0.45 µm nylon membrane filters. The filtrate was transferred into glass vials and DOC concentration was analyzed using a Shimadzu TOC-5000 A analyzer.

4.4.5 Soil physicochemical analysis

Sub-samples of 100 g of soil from each soil depth were gently broken by hand and all litter fragments including roots were removed. Samples were dried at 40^{o}C for 48 h and soil bulk density was calculated following methods described by Wilson *et al.* (2009). Soil samples were sieved through a <0.5 mm and analyzed for total SOC using a LECO CN-1000 autoanalyser (LECO Corporation, St. Joseph, Mich). Soil texture determination was done following the Andreasson pipette method (Green, 1981).

4.4.6 Field sampling of above and below ground plant litter components

Plant litter consisting of above and below ground components were sampled from the site clusters containing the three land uses (Table 4.1) randomly selected at three separate locations. Briefly, above and below ground litter samples were collected from three randomly selected plots within each land use, giving a total number of 54 (3 sites x 3 land uses x 2 litter types x 3 reps) samples. The native pastures were dominated by native grasses such as *Microlaena stipoides.* Above ground biomass from this land use consisted of standing dead biomass and was sampled from quadrats measuring 50 x 50 cm randomly placed in the paddocks. Above ground biomass within each quadrat was cut just above the soil surface and collected into plastic bags. Thereafter, roots were collected by excavating soil from within quadrats from the upper 15 cm soil layer. Native woodland consisted of a mixture of *Eucalyptus* species including Yellow box (*E. melliodora*) and

Blakely's Red Gum (*E. blakelyi*). Above ground litter from this land use consisted of senescent leaves which were sampled from the ground within a randomly selected 25 x 25 m plot. Sampling for below ground root litter was done within the same plot by randomly selecting three trees, establishing a distance of 3 m from each tree base and excavating roots from 1.0 m soil depth.

The paddocks under cultivation had been predominantly cropped with forage oats (*Avena sativa*) for >20 years. At the time of sampling these paddocks were fallow and therefore forage oats was grown in glasshouse pots until physiological maturity (6 months) from which standing dead biomass and roots were harvested as above and below ground litter. The soil used for sowing forage oats was sampled from the field (from cultivation paddocks) within the plough layer (0-15 cm). At physiological maturity (after 6 month, above ground litter was sampled by first discarding the stems bearing grains and harvesting only standing dead biomass. In the laboratory, all above ground litter from all land uses was sorted to remove any green foliage and retain only brown litter. Roots were separated from soil by sieving through < 4mm and picked out with forceps. All plant above and below ground litter were oven-dried at 60°C to constant weight (~ 48 h). Subsamples were taken from each above ground litter type and ground through < 4 mm. Samples were subsequently extracted for water soluble C and subsequently conducted for water soluble C and ^{1}H-NMR spectroscopic analysis as described below.

4.4.7 Litter DOC extraction

Soluble organic matter was extracted following methods described by Don and Kalbitz (2005). Briefly, 0.2 g, of < 4 mm above and below ground litter was weighed into 130 ml specimen jars. Litter samples were soaked in 100 ml of ultra pure water for 24 h at 25°C. The mixture was then filtered using a < 45 µm nylon filter into 70 ml specimen jars. The samples were analyzed for water soluble carbon using a Shimadzu TOC-5000 A analyzer.

4.4.8 Solution-state ^{1}H-NMR spectroscopy

Extraction of litter DOM for ^{1}H-NMR spectroscopic analysis was conducted following methods described by Yano *et al*. (2005) with our laboratory modifications. Briefly, 10 g of <4 mm litter was weighed into 120 ml specimen jars and 100 ml of deionised water added. Jars were placed on a hot water bath at 60°C for 4 hours. The jars were then placed in an end over end tumbler and left to tumble overnight for 16 hours. Samples were then filtered through a vacuum or pressure filter unit equipped with nylon membrane filters <0.45 µm. At least 80 ml of sample was collected. The filtrate was evaporated to dryness using a Buchi rotavapor. The residues were redissolved in D_2O (Aldrich 99.9%-d, 5ml) to pre-exchange labile protons and each solution was then freeze dried for 18 h, dissolved in D_2O (100 mg / 0.75ml) and filtered prior to analysis by NMR spectroscopy. ^1H NMR spectra were measured on a Bruker Avance 300 NMR Spectrometer. 254 scans were acquired for each sample and an exponential multiplication of 0.3 Hz was applied to the data prior to Fourier transformation. The relative proportions of oxygenated compounds (mainly carbohydrates) and aromatic compounds were determined qualitatively at 3.0-4.8 ppm and 5.5-10.0 ppm respectively after Kalbitz *et al*. (2003).

4.4.9 Statistical analyses

Statistical analysis was undertaken using ASREML software (Gilmour *et al*., 2006). The analysis of soil DOC concentrations was performed using a generalised linear model (GLM) that incorporated cubic smoothing splines. The cubic smoothing splines absorbed the serial correlations associated with depth measurement within the same cores. A correlation structure, that modelled the correlation between regression parameters for the linear component of the splines, was added to the splines to account for the variation between cores ensuring that comparisons between cores were less affected by isolated spatial effects. Data were transformed, as appropriate, to compensate for heteroscedascity and lack of normality. Confidence bands were fitted along the splines at 95 % confidence intervals to test for significant differences between land uses at various depths. A Pearson correlation matrix was used to show significant correlations between soil attributes. Litter variables (i.e. DOM) were analyzed with a generalized linear model (GLM) that tested the effects of land use (i.e. cropping/woodland/pasture), litter type (i.e. above ground/below ground) and their interaction; with site and replicates within site as variance components.

4.5 Results

4.5.1 Land use effects on above and below ground litter DOC concentrations

Highly significant *($P < 0.001$)* land use and litter type interactions were found for litter derived DOC which indicated that the differences in DOC concentrations within and between land uses were dependent on litter type. Compared to other litter types, biomass from cultivation (oat straw) contained significantly larger DOC concentrations ($P < 0.05$). DOC concentrations ranged from 20 mg/kg in biomass from native pastures to 99 mg/kg in oat straw/biomass (Fig 4.1).

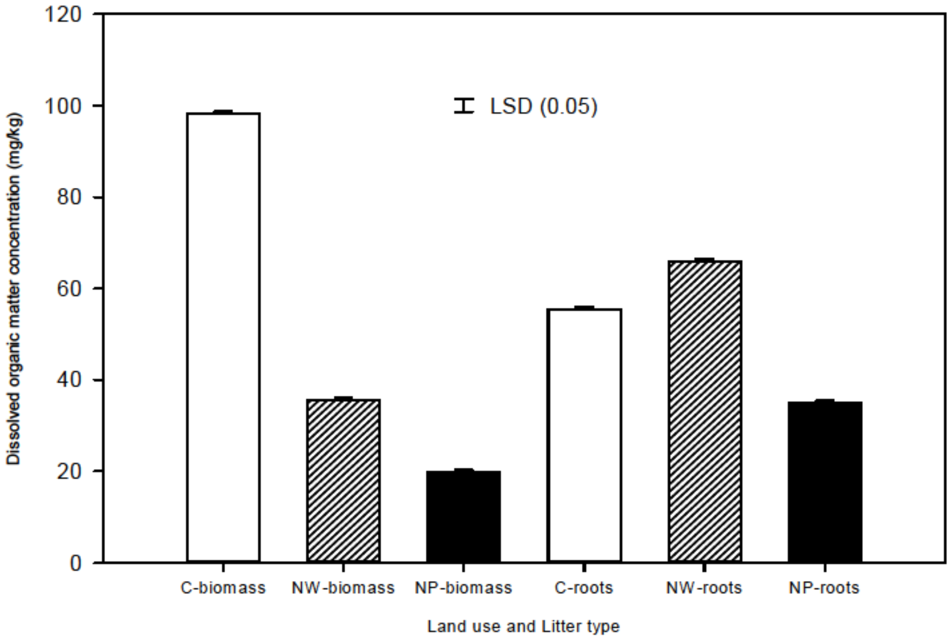

Fig 4. 1 Litter derived DOC concentrations in C-biomass (oat straw), NW-biomass (native woodland leaves), NP-biomass (native pastures biomass), C-roots (oat roots), NW-roots (native woodland roots) and NP-roots (native pastures roots)

4.5.2 Land use effects on soil DOC concentrations and density down the profiles

Both land use and depth had significant interaction effects on DOC concentrations ($P=0.034$) and density ($P=0.017$). Concentration of DOC down the soil profile decreased rapidly from surface layers to subsoil layers across all land uses. This rate of decline in DOC with depth diminished at 20-40 cm and further change was limited i.e. DOC content remaining constant with additional depth. Therefore, below 40 cm the form of the curves was similar across all land uses, although of different magnitude of DOC. Native woodland soils were found to contain significantly ($P < 0.05$) larger DOC concentrations in all soil depth increments compared with native pastures and cultivation which also significantly differed between each other in the 40 cm only (Fig 4.2a). Similar to DOC concentrations, the distribution of total SOC in soil profiles was characterized by large concentrations in the surface soils followed by a rapid decline with increasing soil depth across all land uses (chapter 3). The contribution of DOC to total SOC ranged from 0.01 to 0.1 % down soil profiles across all land uses (data not presented). The fraction of DOC in the total SOC in soil profiles was largely similar among the three land uses although, generally in native woodland soils, the contribution of DOC was slightly higher.

DOC density under the three land uses was calculated on an equivalent soil mass basis as the product of DOC concentration, bulk density (data not presented) and soil thickness.

Similar to DOC concentrations, surface soils contained larger DOC densities which declined with increasing soil depth. Native woodland soils had significantly larger DOC densities in all soil depth increments than the other two land uses which also differed between each other in 40 cm soil layers, with DOC densities remaining similar below these layers (Fig 4.2 b).

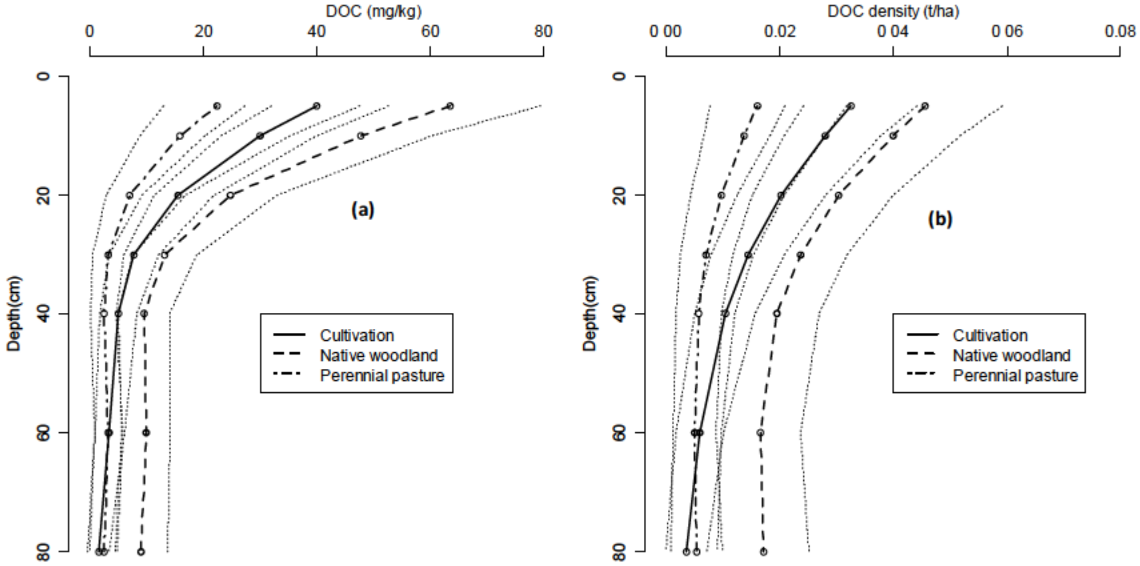

Fig 4. 2 Soil DOC; (a) concentrations; and (b) density, under native woodland, native pastures and cropping. Values are means pooled over three sites. Confidence bands in the splines have been fitted to ±2SE or 95% confidence interval.

4.5.3 Effect of soil particle size distribution on DOC concentrations and densities in soil profiles

A Pearson correlation matrix was constructed in order to establish the strength of relationships among particle size, DOC concentration and density. Results indicated that there is only a weak linear correlation between any of the soil particle components and DOC concentration and density (Table 4.2). Results from multiple linear regression analysis fitted with a model constant and model terms as depth, clay, sand, silt and land use with DOC concentration and density separately fitted as response variables showed that the model could account for 75.1 % and 55.1 % of the percentage variance. From the results, soil particle distribution components showed no significant effect on dissolved organic carbon except for clay at P =0.013 and P =0.020 with DOC concentration and density as response variables respectively.

Table 4. 2 Correlation matrix of soil particle size (clay, silt and sand), DOC concentration and density

	Depth	Clay	Silt	Sand	DOC concentration	DOC density
Depth	1	0.704	-0.46	-0.66	-0.56	-0.16
Clay	0.70	1	-0.40	-0.99	-0.33	0.02
Silt	-0.46	-0.40	1	0.24	0.13	-0.03
Sand	-0.66	-0.99	0.24	1	0.33	-0.01
DOC concentration	-0.56	-0.33	0.13	0.33	1	0.73
DOC density	-0.16	0.02	-0.03	-0.01	0.73	1

4.5.4 Aromaticity of litter derived DOM

4.5.4.1 ^{1}H-NMR spectra of litter derived from above ground components

Representative ^{1}H-NMR spectra of DOM fraction extracted from above and below ground litter derived from cropping, native pastures and native woodland are presented in (Fig 4.3). Interpretation of all the ^{1}H-NMR spectra results is mainly qualitative with an attempt to describe the relative proportions of oxygenated compounds (mainly carbohydrates) (3.0-4.8 ppm) and aromatic compounds (5.5-10.0 ppm) (Kaiser *et al.*, 2002; Kalbitz *et al.*, 2003). The major peak in all the above ground plant litter derived DOM samples was a series of overlapping broad resonances from 3 to 4 ppm. These broad peaks are assigned to carbohydrates, which may be in the form of simple sugars such as mono, di or oligosaccharides, or as water-soluble polysaccharides or combined with other organic molecules in glycosides. The resolution of the broad carbohydrate peaks was seen to vary considerably between different samples and may give an indication of the degree of complexity of the sugars, with monosaccharides and disaccharides expected to give spectra with better resolution and polysaccharides expected to have broad, poorly resolved resonances.

Biomass from oat/oat straw from all sites showed that the samples had very little non-carbohydrate material, with no evidence of phenolic compounds. In general the spectra were well resolved suggesting that the samples largely consisted of simple sugars.

Native pasture litter biomass DOM was also mainly simple sugars but there was a very small amount of phenolic material present and a relatively higher proportion of aliphatic resonances (2.5 to 0.7 ppm) than cropping litter. These peaks are most likely due to the presence of glycosidic material.

DOM samples derived from native woodland litter biomass showed a relatively large amount of phenolic material, with broad resonances in the spectral region from 5.8 to 7.5 ppm. The broadness of the peaks suggests that the phenolic material was polymeric in nature and most likely consists of tannins. On the other hand the carbohydrate resonances were well resolved, suggesting the presence of simple sugars, although a relatively larger amount of aliphatic resonances implies that some of the sugars may be present as glycosides as well.

4.5.4.2 ^{1}H-NMR spectra of litter derived from below ground components

The roots obtained from cultivation consisted of a DOM fraction containing mainly simple sugars. Additionally, aliphatic peaks were noted in the spectra. These aliphatic compounds are interpreted to be a complex mixture of glycosides due to the broadness of the peaks.

Spectra from pasture roots DOM fraction again showed a high percentage of simple free sugars such as mono or disaccharides but also a relatively high proportion of aliphatic peaks, implying the presence of glycosides, probably of terpenoid or steroidal compounds. A very small amount of phenolic compounds was present.

DOM samples from native woodland (eucalyptus roots) had a relatively high proportion of polymeric aromatic compounds. Two sharp resonances in the aromatic region of the spectrum (at 7.4 and 7.1 ppm) suggested the presence of simple phenolic compounds as well as polymeric compounds. The spectra also contained a substantial amount of aliphatic resonances, both broad and sharp. These are possibly due to a complex mixture of glycosidic compounds. The resonances in the carbohydrate region of the spectrum were quite sharp, again implying that the predominant components of the extract were simple sugars.

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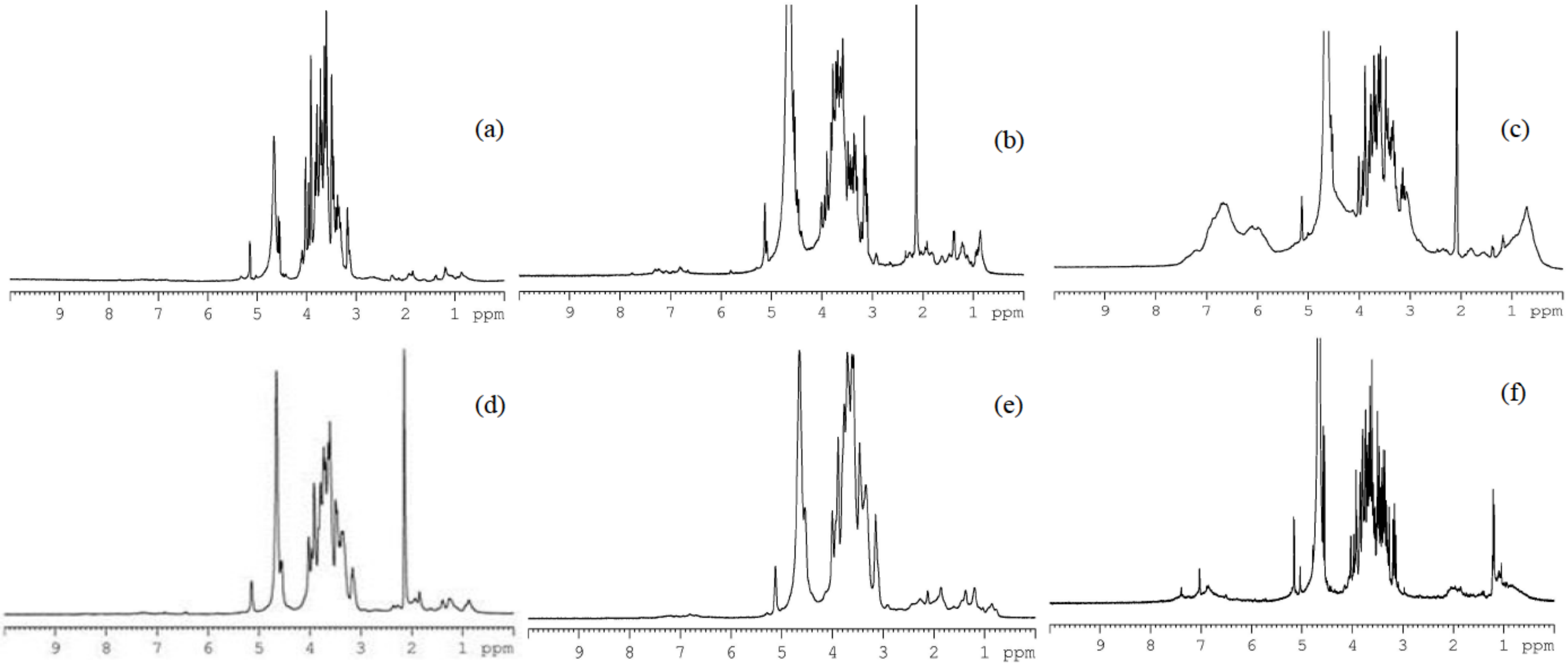

Fig 4. 2 Representative ^{1}H-NMR spectra for DOM fraction extracted from above ground litter under (a) cultivation, (b) native pastures, and (c) native woodland; below ground litter (d) cultivation, (e) native pastures and (f) native woodland.

4.6 Discussion

4.6.1 Land use effects on above and below ground litter DOC concentrations

Significantly larger DOC concentrations obtained from biomass/oat straw may be explained by the large proportion of labile compounds as observed from the ^{1}H-NMR spectra of this litter compared with the other litter types from other land uses. Our results of larger DOC concentrations from above ground litter derived from a cultivated land use are similar to those reported by Kalbitz *et al.* (2003). In their study, Kalbitz *et al.* (2003) reported significantly larger DOC concentrations from maize straw (819 mg/L) compared with litter from a natural forest (75.7 mg/L) and grassland (18.4 mg/L). Large DOC concentrations in oat litter are indicative of high quality litter with ability to mineralize faster and can be subsequently available to the soil environment (Boyer and Groffman, 1996).

4.6.2 Land use effects on DOC concentrations and density down the soil profiles

Significantly larger DOC concentration and density in all soil depth increments under native woodland soils compared to the other two land uses may be attributed to a continued large input of litter mainly in surface soil layers (Wilson *et al*., 2011) and supply of labile compounds released from live growing roots (Paul and Clark, 1996). Besides humus, litter is a major source of DOC in soils and a high surface litter input may favour the production of DOC which can be transported to subsoil layers (Lorenz and Lal, 2005). Conversely, native pastures and cultivation are both generally associated with reduced litter inputs and product removal and although litter which may explain the lower DOC concentrations. These results may however be partly biased against cultivation and native pastures as oats had been harvested at the time of sampling and native pastures paddocks comprised of biomass that was senescent. All in all, our results suggest that due to the associated benefits of large organic matter inputs in surface soils under native woodland, large amounts of DOC may be produced in surface layers which may be transported to subsoils and contribute to C storage. Moreover, trees are known to transfer C to considerably deeper soil layers because of their maximum rooting depths compared to many land uses (Canadell *et al*., 1996; Jobbagy and Jackson, 2000).

DOC represented between 0.01 and 0.1 % of total SOC down the soil profiles and across all land uses showing that the contribution of DOC to total SOC was relatively small.

4.6.3 Relationship between soil texture and DOC concentrations down the soil profiles

There was only a small linear correlation between any of the soil particle components and DOC concentration and density. These relationships are not regarded as causal relationships but may be due to an artefact of computation where soil texture effects were confounded due to strong depth effects as is the case with our duplex soil. Our study showed a rapid decrease in DOC concentrations from surface layers to subsoil layers across all land uses with the rate of decline in DOC shown to diminish at 20-40 cm where further change was limited and may be largely associated with the change in soil texture with soil depth which may have influenced the soils hydraulic properties. Soil hydraulic properties have been shown to influence the transmission and retention of soil fluid within the soil. Under sandy textured surfaces, DOC does not have sufficient time to interact with the particles and therefore stays in soil solution and hence is readily extracted from soil solution. On the other hand, clay particles have lower hydraulic conductivity giving DOC an ample time to interact with clay surfaces and thereby getting sorbed on the surfaces to form organo-mineral complexes. For this reason, DOC is removed from the soil solution which may explain the low DOC concentrations obtained in the clay-rich subsoil. Similar findings have been reported from a study conducted in south Australia by Stevens *et al.* (1999) who showed that the movement of DOC in pastoral catchments was largely influenced by soil texture. In their study, larger DOC movement was observed in surface sandy loam compared to B horizon with heavy clay and attributed their results to greater hydraulic conductivity in the A horizon compared with the heavy clay B horizon. Our results suggest that texturally contrasted soils especially where sandy loam overlay clay such as in our soils, DOC may be rapidly transported in subsoil layers where it may be sorbed in subsoils and may contribute to C storage in deeper layers.

4.6.4 Aromaticity of litter derived DOM

The relative proportion of aromatic C in DOM extracted from litter was highest in native woodland, followed by native pasture then cultivation indicating qualitative differences in DOM fractions which may in turn influence biodegradability of DOM among litter types. When the initial biochemical properties of the respective litter residues were determined, the order of lignin and tannin compounds was similarly in the order of native woodland > native pastures > cropping (chapter 7). It is possible therefore to link the aromatic compounds of DOM fractions with the respective initial chemical properties of litter residues. Biodegradability of litter DOM fraction is therefore expected to be highest in cropping litter and lowest in native woodland litter. We can therefore expect that the biodegradation of soil derived DOC will follow the same trend as that of litter derived DOM as litter is the major source of soil DOC fraction. The results indicate that litter derived DOM under native woodland partly constitutes aromatic compounds which suggests that is may be more resistant to microbial degradation compared with DOM from native pastures and cultivation which largely constitutes carbohydrates. As a result of this aromaticity DOM fraction derived from native woodland may persist in soil solution for a longer period and in turn they may be sorbed onto mineral surfaces as DOM percolates down the soil profile contribute to DOC storage in soil profiles (Kalbitz and Kaiser, 2008). Although this study showed that the contribution of DOC to total SOC was small, the results show that the sandy textured surface soils may play an important role in rapidly transporting DOC to subsoil clayey layers where it may be sorbed. A key management strategy would be to increase surface litter inputs which may favour the production of DOC that may be transported to deeper soil layers. The stability of DOC derived from both soils and litter will require further testing in order to determine its long term dynamics in soils.

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	Author's Name (please print clearly)	**% of contribution**
Candidate	Christine Walela	80
Other Authors	Heiko Daniel	3
	Brian Wilson	3
	Peter Lockwood	3
	Annette Cowie	3
	David Tucker	5
	Bruce McCorkell	3

Name of Candidate: Christine Kainyu Walela

Name/title of Principal Supervisor: Prof. Heiko Daniel

[redacted]

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Name of Candidate: Christine Kainyu Walela

Name/title of Principal Supervisor: Prof. Heiko Daniel

[redacted]

______________ 13/05/2013

Candidate Date

[redacted]

_______________ 13/05/2013

Principal Supervisor Date

Chapter 5. Soil organic carbon storage and quality among soil particle size fractions down the profiles under native woodland, native pastures and cultivation in northern New South Wales Australia

Christine Walela[1], Heiko Daniel[1], Brian Wilson[1, 2], Peter Lockwood[1], Annette Cowie[1, 3], Bruce McCorkell[4]

[1]School of Environmental and Rural Sciences, Agronomy and Soil Science, University of New England, NSW 2351, Australia (fax +612 6773 3238).

[2]NSW Office of Environment and Heritage, PO Box 0221, Armidale, NSW 2351, Australia.

[3]Rural Climate Solutions, Department of Primary Industries, NSW Australia.

[4]Department of Primary Industries, Tamworth Agricultural Institute, NSW, Australia.

5.1 Abstract

The extent and degree to which soil organic carbon is bound to soil mineral particles (clay and silt) has implications for long-term soil organic carbon (SOC) stability and storage. We isolated soil organic matter into two fractions namely particulate organic matter (POM) and mineral-associated organic matter (MOM) and determined the quantities of carbon and nitrogen associated with the two fractions in soil profiles (0-80 cm) of native woodland, native pastures and cultivation. The quantity and quality of SOC differed between the two fractions. Strong and significant land use by soil depth interactions were found for particulate organic carbon (POC) and mineral-associated organic carbon (MOC) concentrations. Native woodland soils were associated with consistently wider C:N ratios in the POM fraction throughout the soil profile compared with native pastures and cultivation which differed between each other in the 50 cm. The result indicated differences in the quality of organic matter inputs (litter) entering the POM fraction with native woodland soils associated with less easily decomposed inputs due to their inherent chemical composition. Consequently, native woodland showed the least decline in POC with soil depth compared with the other two land uses. The proportion of MOC to the total SOC increased with soil depth indicating that subsurface C was more protected than surface C probably due to mineral association with clayey subsoils. Compared with native pastures and cultivation which were largely similar, native woodland soils contained significantly larger amounts of MOC in all soil depths suggesting that C was more physically protected from microbial attack. As a result, C may be more slowly decomposed hence more stabilized which is key to long term C sequestration.

Keywords: Soil carbon sequestration, land use, particulate organic carbon, mineral-associated organic carbon, soil profile, Australia.

5.2 Introduction

Soil organic matter (SOM) is a dynamic and complex material comprising fractions that exhibit varying physical and chemical properties (Kölbi *et al.* 2005), degree of decomposability and turnover time (Whalen *et al.* 2000; Golchin *et al.* 1995; Yamashita *et al.* 2006) as well as different accessibility and interactions with soil mineral particles (Tan *et al.* 2007). The dynamics of SOM with change in land use has been studied by separating SOM into two or more fractions that are each functionally homogenous with respect to physicochemical properties and turnover rates using various fractionation techniques (Cambardella and Elliot 1992; Six *et al.* 2002; Degryze *et al.* 2004). For example, physical fractionation techniques have been used to separate SOM into particle size fractions, namely particulate organic matter (POM) (53-2000 μm) and mineral-associated organic matter (MOM) (<53 μm), and the soil organic carbon (SOC) associated with these fractions has been subsequently studied (Cambardella and Elliot 1992). Generally, POM is mostly composed of recently deposited and partially decomposed organic matter residue that is not bound to soil mineral particles, and the carbon (C) associated with this fraction i.e. particulate organic carbon (POC) is characterized by high turnover (Golchin *et al.* 1995; Kölbi *et al.* 2005; Gregorich *et al.* 2006). Conversely, MOM is largely biochemically recalcitrant organic matter that is closely associated with soil mineral particles and the C associated with this fraction i.e. mineral-associated organic carbon (MOC) is physically protected from microbial decomposition and hence represents a more stable C pool with slow turnover (Cambardella and Elliot 1992; Conant *et al.* 2004). Therefore, the extent and degree to which SOC is bound to soil mineral particles (clay and silt) has implications for long-term C stability and storage.

The location of SOM within the soil matrix has been shown to be a major factor determining its turnover. For example, Yamashita *et al.* (2006) found the mean age of MOC to be 102 yr compared with 10 yr for POC in the near surface soils at a site with maize cropping history. As a result of the energy rich, readily decomposable substrate, POC is inhabited by a substantial portion of soil microbial biomass and has been found to be strongly correlated with the rate of whole soil respiration (Alvarez and Alvarez 2000; Whalen *et al.* 2000). Hassink (1997) found the correlation between microbial biomass and mineralization to decrease with increasing stability of organic matter fractions as a result of greater protection of SOC in MOC. As such, POC has been shown to be more sensitive to management or land use induced changes than total SOC (Dalal and Mayer 1987; Janzen *et al*. 1992; Cambardella and Elliot 1992; Chan 2001; Chan *et al*. 2002).

POC is widely recognized for its role in soil aggregation, a mechanism central to C sequestration (Blanco-Canqui and Lal 2004) and its importance in short term nutrient cycling due to the highly labile nature of its constituents. These properties are however not ideal for long term C sequestration. It has been widely recognized that the adsorption of SOC to clay and silt particles is an important determinant of the stability of organic C as, C is physically protected from mineralization in soils and as such this fraction may play a major role in C sequestration in soils (Hassink 1997; Whalen *et al*. 2000). While the potential for land uses to sequester C has been a major focus in Australia, the majority of studies have focused on the dynamics of total SOC (Chan *et al*. 2003; Young *et al*. 2009; Wilson *et al*. 2011) with fewer studies on C storage in specific SOM fractions (Dalal and Mayer 1987; Golchin *et al*. 1994; Chan 2001; Chan *et al*. 2002). The dynamics of SOC among land uses will undoubtedly be better understood by studying the mechanisms in which SOC is stored down the soil profiles. Hence, knowledge on the amounts and quality of C associated with the POM and MOM fractions will enhance our understanding of the mechanisms of C sequestration; indicate stability of C and further our understanding of the potential for long-term C sequestration in soil profiles of different land uses. Our aim was therefore to determine the quantities and quality of SOC associated with POM and MOM fractions down the soil profiles under three common land uses in NSW Australia, namely native woodland, native pastures and cultivation.

5.3 Materials and methods

5.3.1 Site characteristics

The study was conducted on the Northern Tablelands of New South Wales, Australia. Sampling sites were selected from an existing network of monitoring sites on the Bundarra Granite Soil Monitoring Unit (as defined in Chapman *et al*. 2009) adjacent to the township of Uralla. Bundarra Granites are of Permian age and soils derived from these are typically classified as Yellow Chromosols (Isbell 2002) equivalent to Alfisols (Soil Survey Staff 1999). Uralla lies at 30.64°S, 151.49°E and is located at an altitude of 1,012 m. It receives a mean annual rainfall of 807 mm and has a mean maximum and minimum temperature of 26.4°C and 12.5°C respectively (1901-2012; Bureau of Meteorology 2012).

5.3.2 Land use sampling approach and management history of sample sites

The study was carried out following a sampling approach similar to that used by Wilson *et al*. (2008, 2010, 2011). Site clusters containing three land uses namely, native pastures, native woodland and cultivation, were randomly selected at three separate locations, giving 3 replicates of each land use, and a total of nine sites. Different land uses were separated by <500 m within each sample cluster. Land use type and management history is as described in Table 5.1.

Table 5. 1 History of three contrasting land uses selected for soil sampling in Uralla northern NSW Australia

Land use	History
Native woodland	Had never been cleared (undisturbed) and was used as reference points against which changes in the managed landscape have been measured; and Consisted of a mixture of *Eucalyptus* species including Yellow box (*E. melliodora*) and Blakely's Red Gum (*E. blakelyi*) with a range of native grasses and shrubs growing beneath the trees.
Native pasture	Had been under current land use for >20 years; Minimally fertilized (superphosphate) with limited oversown legumes (e.g. sub-clover); Dominated by native grasses such as *Microlaena stipoedes;* Paddocks had been historically grazed under set-stocking mainly with sheep although cattle had become more common in recent years.
Cultivation	Forage oats was the dominant crop that had been historically cultivated in alternate years with pasture for > 30 years.

5.3.3 Field soil sampling and sample preparation

A stratified random sampling design was used for each land use and sample site. A 25 x 25 m sample plot was randomly located within each cluster, land use and site. Three sampling points were randomly selected within each plot. At each sampling point, litter and plant materials were cleared from the soil surface and a truck-mounted hydraulically driven soil corer of 50 mm internal diameter was used to sample to 0.80 m (to weathering rock). The soil core was then placed on a graduated Polyvinyl Chloride (PVC) tube that had been halved lengthwise and labelled. Using a sharp cutting knife, each core was accurately divided into four depth increments of 0-0.1, 0.1-0.3, 0.3-0.5 and 0.5-0.8 m. Samples were placed into plastic bags, sealed, stored in cooler boxes and transported to the laboratory and stored under cool conditions (< 4°C) until they were processed (typically <48 h). Soil samples were dried at 40°C for 48 h, sieved to pass through a 2 mm sieve and analysed for total organic carbon (TOC) using a LECO CN-1000 autoanalyser (LECO Corporation, St. Joseph, Mich).

5.3.4 Determination of POC and MOC

POC and MOC were determined following methods described by Cambardella and Elliot (1992). Briefly, SOM was first separated into POM and MOM fractions using physical fractionation techniques on the basis of size into 53-2000 µm and <53 µm soil fractions respectively. Thirty grams of air-dried, 2 mm sieved soil samples were shaken overnight (16 h) on an end-over-end shaker in 135 ml of 5g l^{-1} sodium hexametaphosphate solution to disperse the soil. The dispersed slurry was passed through a 53 µm sieve on a Fritsch vibratory sieve shaker Analysette 3 Pro. The material that was retained on the sieve included POM (53 to 2000 µm) and sand while the material passing through the sieve was MOM (< 53 µm). The POM material was rinsed repeatedly to separate the organic matter from the sand. The material that passed through <53 µm was similarly collected into weighed beakers and together with the POM, oven dried (70°C) and reweighed. Both fractions were ground using a mortar and pestle to ensure all dried material was homogenous. Soil C and nitrogen (N) concentrations associated with the two fractions were determined as POC, particulate organic nitrogen (PON), MOC and mineral-associated organic nitrogen (MON) using LECO CN-1000 autoanalyser (LECO Corporation, St. Joseph, Mich).

5.3.5 Statistical analyses

Statistical analysis was undertaken using ASREML software (Gilmour *et al*. 2006). The analysis of TOC and C and N associated with POM and MOM fraction was performed using a generalised linear model (GLM) that incorporated cubic smoothing splines. The cubic smoothing splines absorbed the serial correlations associated with depth measurements within the same cores. A correlation structure, that modelled the correlation between regression parameters for the linear component of the splines, was added to the splines to account for the variation between cores ensuring that comparisons between cores were less affected by isolated spatial effects. Data were transformed, as appropriate, to compensate for heteroscedascity and lack of normality. Confidence bands

(95 %) were fitted along the splines to test for significant differences between land uses at various depths.

5.4 Results

5.4.1 Soil depth and land use effects on POC, PON and C:N ratio

Strong depth and land use ($P < 0.001$) interactions were found for POC concentrations suggesting that this property changed with depth in the soil but the nature of this change differed between the three land uses. The distribution of POC in the soil profile (0-80 cm) was characterized by large concentrations in surface soil layers followed by a rapid decline with increasing soil depth; this trend was similar for all land uses (Fig 5.1, a). Land use effects on POC were more strongly expressed in the surface (0-10 cm) soils and diminished with soil depth. The rate of decline/change in POC concentrations with soil depth differed between land uses, this observation was made when comparisons of slope parameters for the interaction terms (i.e. land use: depth) were made. Results from this analysis showed that the slope parameter of native woodland differed strongly and significantly from that of native pastures and cultivation which were generally statistically similar to each other. These results indicate that the POC under native woodland soils decreased with depth at a significantly lower rate than the POC under cultivation and native pasture which were similar to each other. The distribution of PON in the soil profiles (0-80 cm) across all land uses was similar to that of POC with large PON concentrations in surface soil layers followed by a rapid decline with increasing soil depth (Fig 5.1, b). No land use or soil depth main effects or their interactions were found for PON concentrations in any of the four depth increments.

Strong and significant ($P < 0.001$) land use and soil depth interactions were found for C:N ratio indicating that the differences in this property between land uses was depth dependent. C:N ratios declined with increasing soil depth, declining gradually under native woodland and rapidly under both native pastures and cultivation soils. Differences in C:N ratios between land uses were found at different depths, for example, significantly larger C:N ratios were found under native woodland in soil layers 0-10, 10-30, 30-50 and 50-80 cm than the other two land uses, which also significantly differed between each in the 50 cm soil layers (Fig 5.1, c). The C:N ratios in the deepest soil layers (50-80 cm) were found to be statistically similar between native pastures and cultivation.

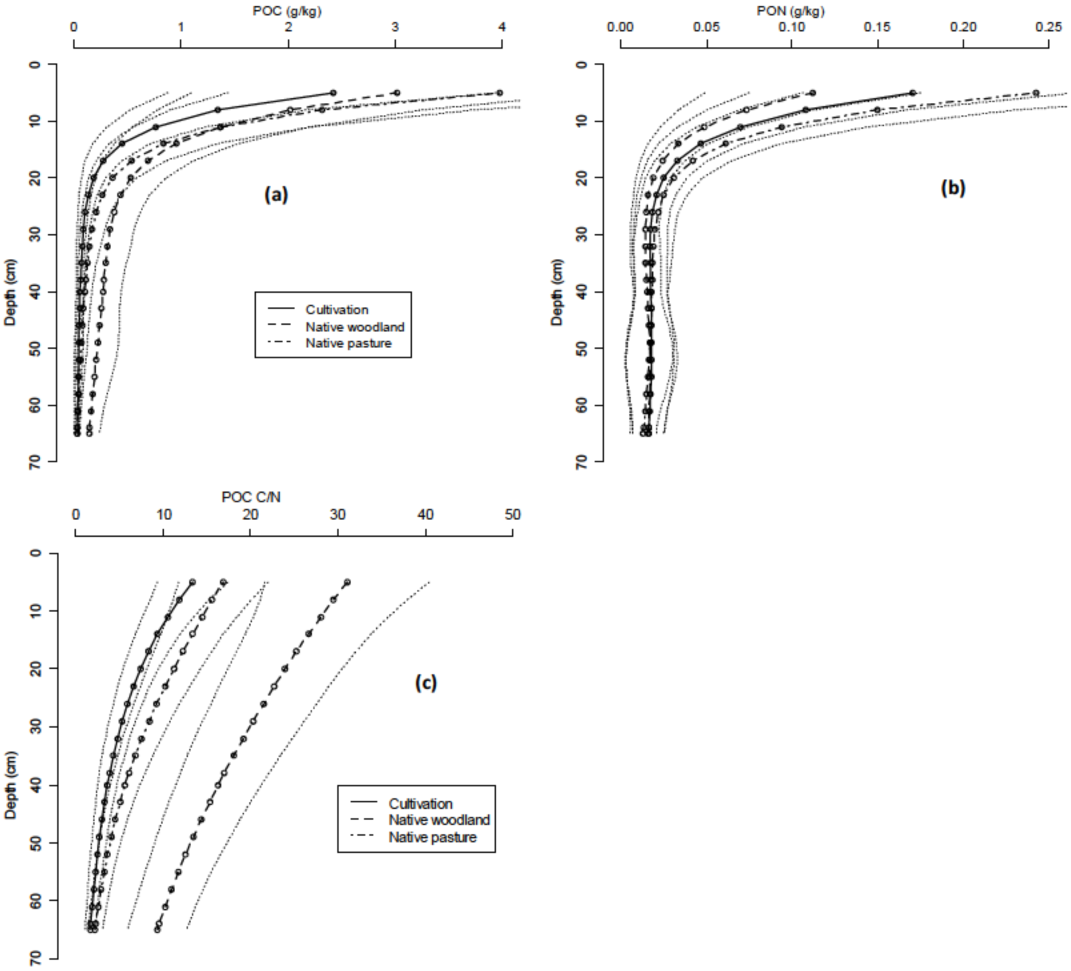

Fig 5. 1 Changes in (a) POC, (b) PON and (c) POC C/N ratio with soil depth under cultivation, native woodland and native pastures. 95% confidence bands are fitted along the splines to test for significant differences between land uses at various depths.

5.4.2 Soil depth and land use effects on MOC, MON and C:N ratio

Strong ($P < 0.001$) land use and depth interactions were found for MOC concentrations suggesting that differences between land uses were depth dependent. Larger MOC concentrations were found in surface soils and diminished with soil depth across the three land uses. MOC concentrations were significantly larger in native woodland soils in depths 0-10, 10-30, 30-50 and 50-80 cm than native pastures and cultivation which had statistically similar concentrations (Fig 5.2, a). MON concentrations showed strong land use and depth ($P < 0.001$) main effects but no interactions. The near surface soil layers had significantly larger MON concentrations which declined with increasing soil depth in all land uses. MON concentrations did not differ a great deal between the three land uses, and significant differences were detected in the 10 to 30 cm soil layers only with cultivated soils having the largest concentrations compared with the other land uses which were statistically similar to each other (Fig 5.2, b). Strong land use and depth main effects (P < 0.001) were found for C:N ratios. Significant land use differences in C:N ratios were limited to soil depth increments ranging from 0-10 and 10-30 cm where native woodland soils had significantly larger ratios than native pastures and cultivation which were largely similar to each other (Fig 5.2, c). Beyond these soil layers, C:N ratios between land uses remained statistically similar and the magnitude of these differences diminished with soil depth.

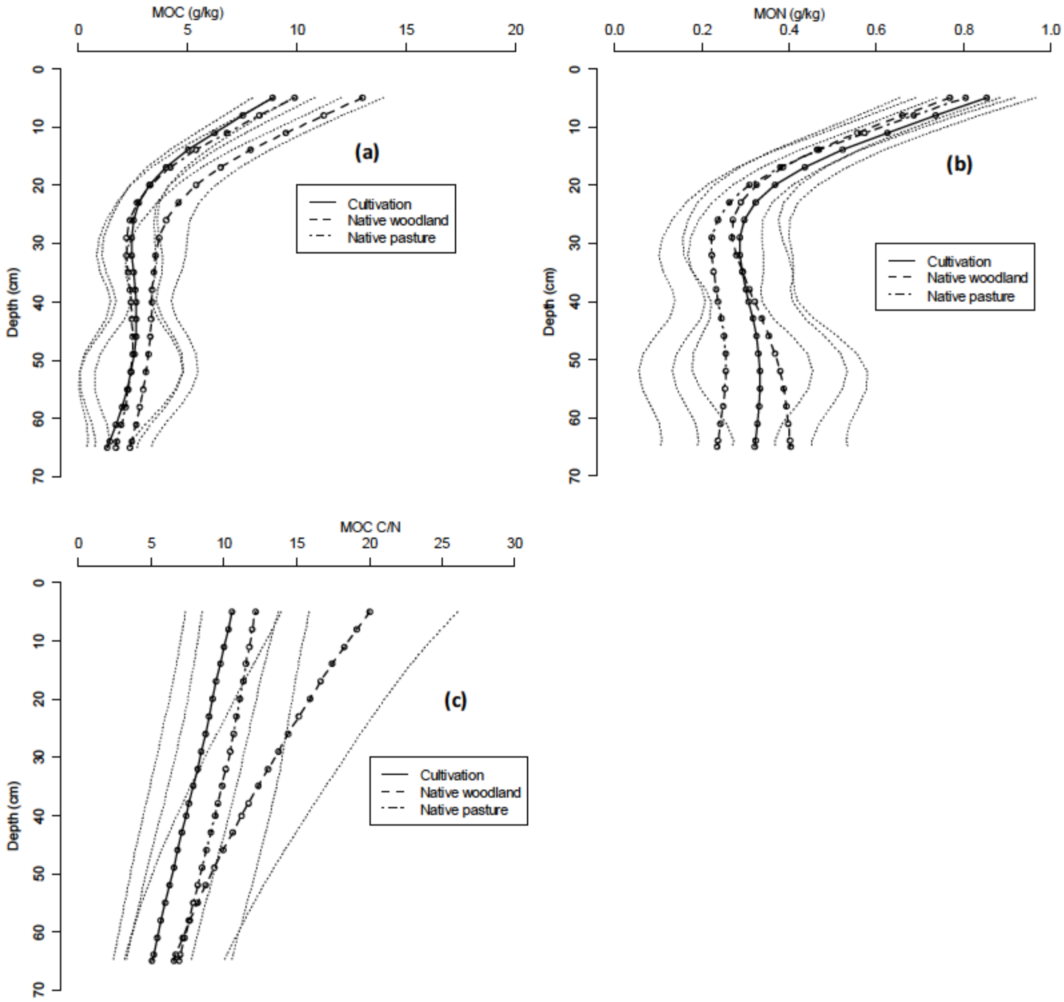

Fig 5. 2 Changes in (a) MOC, (b) MON, and (c) MOC C/N ratio with soil depth under cultivation, native woodland and native pastures. 95% confidence bands are fitted along the splines to test for significant differences between land uses at various depths.

5.4.3 The proportion of POC and MOC to TOC in soil profiles under different land uses

TOC was strongly (P<0.001) influenced by land use and depth main effects but no interactions were observed. TOC concentrations were typically larger in surface soils and declined with increasing soil depth (Fig 5.3). Differences in TOC concentrations between land uses were observed within the 0-10 and 10-30 cm soil increments only. Across all land uses, MOC made up 73 to 75 % of TOC within the 0-10 cm soil depths and this proportion increased gradually with soil depth reaching 95 and 99 % of TOC in the deepest soil increments (50-80 cm) (Table 5.2). Conversely, the proportion of POC in total SOC declined sharply with soil depth under all land uses comprising between 25 and 26 % of TOC in surface 0-10 cm soil depths and between 1 and 5 % in the deepest soil increments (50-80 cm). The largest decline in POC with soil depth was expressed under cultivated soils.

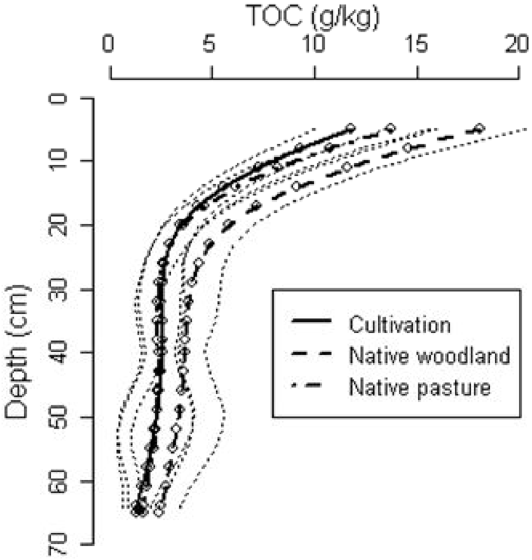

Fig 5.3 Changes of TOC with soil depth under three contrasting land uses

Table 5. 2 Fraction of particulate and mineral associated organic carbon in total SOC

Land use type	Soil depth (cm)	POC/TOC (%)	MOC/TOC (%)
Native woodland	0-10	26	74
	10-30	13	87
	30-50	11	89
	50-80	5	95
Native pastures	0-10	26	73
	10-30	15	85
	30-50	7	93
	50-80	1	99
Cultivation	0-10	25	75
	10-30	7	92
	30-50	3	96
	50-80	1	99

5.5 Discussion

5.5.1 Soil depth and land use effects on POC and C:N ratio

Our results of POC concentrations compare well with those reported in other studies e.g. Chan (2001) and Yamashita *et al*. (2006). The observed pattern of distribution in POC concentrations was mostly related to changes in the amounts of plant litter inputs with soil depth. POC is closely related to plant litter input (Golchin *et al*. 1994) and plant litter is mainly deposited in the surface soils therefore contributing more to SOM in the upper soil horizons than in deeper soil layers (Lorenz and Lal 2005; Yamashita *et al*. 2006). Land use effects on POC concentrations were strongest in surface soils, diminished with soil depth and may be related to differences in amounts of plant litter inputs among land uses with increasing soil depth. Our results seem to suggest that native woodland soils are associated with a steady supply of litter inputs from both above and below ground sources and, therefore, the slow rate of decline in POC concentrations with depth compared with the other two land uses. This result is not surprising as both native pastures and cultivation are associated with reduced above ground litter inputs resulting from product removal under cultivation and grazing under native pastures (Wilson *et al*. 2011) and may possibly explain the disparities.

The C:N ratios observed in the POM fraction are within the range of those reported elsewhere (Wagai *et al*. 2009). Wagai *et al*. (2009) compiled C:N ratios from several studies and found that on average C:N ratios of free particulate organic matter fraction, which is comparable to our POM fraction, were 29.2±14.4. The wider C:N ratios observed in our study are characteristic of the POM fraction (Golchin *et al*. 1994). Based on electron microscopy results, Golchin *et al*. (1994) found that the POM fraction consisted of recognizable plant debris which was free from mineral particles and microbial products. Golchin *et al*. (1994) and Kölbi and Kögel-Knabner (2004) have demonstrated that the spectra obtained for POM fraction indicated chemical shift regions similar to those of litter and plant material. Based on these results Golchin *et al*. (1994) argued that the chemical composition (in this case C:N ratio) of organic matter found in the POM fraction is controlled mainly by the organic structure and degree of decomposition of organic matter entering the soil. Basing our results on this argument, the strong differences in C:N ratio in POM fraction among land uses is indicative of differences in source and quality of litter among land uses (Golchin *et al*. 1994;

Yamashita *et al*. 2006). In our study, native woodland soils were associated with consistently larger C:N ratios throughout the entire soil profile (0-80 cm) compared to the other two land uses which also differed in C:N ratios in the depth increments of 0-10, 10-30 and 30-50 cm. Our results imply that both the above and below ground litter inputs entering the POM fraction under native woodland consisted of slowly decomposing compounds (i.e. low quality litter). Similarly, litter inputs under native pastures were of lower quality than those under cultivation. The larger C:N ratio in the POM fraction under native woodland might partly explain the slower rate of decline in POC concentration with soil depth. The decrease in C:N ratio with soil depth is probably related to an increase in the degree of decomposition.

5.5.2 Soil depth and land use effects on MOC and C:N ratio

Our results for mean concentrations of MOC in the combined soil layers of 0-10 and 10-30 cm under native woodland (17 g C kg^{-1}) and cultivation (11 g C kg^{-1}) compare well with those reported by Tan *et al*. (2007) as 20 and 10 g C kg^{-1} under the respective land uses in surface (0-20 cm) soil layers. Similarly, the MOC concentrations found in the surface (0-10 cm) soil layers under cultivation (8.8 g C kg^{-1}) and native pastures (9.8 g C kg^{-1}) in our study, exhibit the same magnitude as those reported by Chan (2001) for MOC under cultivation (3.5 to 8.8 g C kg $^{-1}$) and native pastures (8.3 g C kg^{-1}) in surface (0-10 cm) soil layers on a similar soil type (Alfisol) in NSW Australia.

Our study showed that the MOM fraction was associated with narrow C:N, which is consistent with other studies (Golchin *et al*. 1994; Yamashita *et al*. 2006; Wagai *et al*. 2009). On average most studies have shown that the C:N ratio of this fraction is about 13.5± 4.8 (Wagai *et al*. 2009). The organic component of the MOM fraction has been shown to be largely comprised of microbially processed materials (mainly microbial metabolites and biomass) strongly bound to soil minerals (Golchin *et al*. 1994). Hence, as SOM gets older and is repeatedly recycled by microorganisms its C:N tends to approach that of the microorganisms themselves which has been shown to range between 4 and 7 (Paul and Clark 1996). Differences in MOC concentrations among land uses may therefore be reflective of differences in: a) the supply of microbial metabolites and biomass into this fraction among land uses; and b) the amounts of structurally stabilized organic matter compounds among land uses (Golchin et al., 1994). Significantly larger MOC concentrations in native woodland soils therefore seem to suggest that these soils are associated with large supply of microbially stabilized products and biochemically recalcitrant compounds compared with the other two land uses which were statistically similar to each other. The native woodlands at these sites are generally associated with large supply of fresh litter inputs in soil layers (Barnes 2011), therefore, naturally becoming chemically attractive sites for high microbial activity. It's then assumed that with time, large amounts of microbial products and biomass together with biochemically stabilized compounds adsorb to soil mineral particles contributing to large amounts of MOC in soils under native woodland.

The significantly larger C:N ratios in the MOM fraction in the surface (0-10 and 10-30 cm) from native woodland soils compared with native pastures and cultivation soils which were statistically similar to each other is again probably related to differences in litter quality which in turn result in differences in the degree of microbial processing among land uses. According to our study, these probable litter compositional differences among land uses seem to have more effect on MOC concentrations in surface (0-10 and 10-30 cm) soils compared with combined deeper (30-80 cm) soil layers which were statistically similar to each other. The decline in C:N ratio with soil depth may be attributed to: a) an increase in the degree of microbial processing with depth (Yamashita *et al*. 2006); and b) an increase in association with soil mineral particles (clay and silt) with increasing soil depth (chapter 3). The mineral particles play a vital role by offering an extensive surface for adhesion by microbial products and biomass (Golchin *et al*. 1994). Our results seem to suggest that compared to MOC in surface soils, MOC in deeper soil layers is probably highly altered by soil microbes and strongly bound to soil minerals. This study was conducted in a texturally contrasted soil characterized by sandy loam texture overlying clay (chapter 3) and it is possible that the organic matter association increased with increasing soil depth thereby forming strong organo-mineral complexes and hence the low C:N ratio in deeper layers. We can therefore assume that MOC in deeper layers was highly stable due to a stronger interaction with the mineral particles compared to that in surface soils.

5.5.3 Dynamics of SOC associated with POM and MOC fractions in soil profiles under different land uses

We found that the relative contribution of organic carbon in the two fractions to TOC was greater in the MOM than in the POM fraction in the entire soil profiles among all land uses, again indicating the higher capacity of the former to preserve SOC by its association with clay and silt particles. Our results are consistent with the conclusion of several other studies that the bulk of the total SOC is associated with clay and silt particles (Christensen 1996; Post and Kwon 2000; Yamashita *et al*. 2006; Degryze *et al*. 2004; Gregorich *et al*. 2006). As POC is closely related to plant litter inputs (Golchin *et al*. 1994), the sharp decline in the relative proportion of POC to total SOC with soil depth may be interpreted to mean reduced litter inputs with soil depth and this was found to be highest under cultivation.

5.6 Conclusions

Our study was successful in isolating organic matter of different degrees of mineral association based on differences in particle size and in determining the influence of land use and soil depth on SOC storage in these fractions. Consistent with other studies, the quantity and quality of SOC differed between the two fractions. Strong and significant land use by soil depth interactions were found for POC and MOC concentrations. Native woodland soils were associated with consistently wider C:N ratios in the particulate organic matter (POM) fraction throughout the soil profile compared with native pastures and cultivation which differed between each other in the 50 cm. This result indicated differences in the quality of organic matter inputs (litter) entering the POM fraction with native woodland soils associated with less easily decomposed inputs due to their inherent chemical composition. Consequently, native woodland showed the least decline in particulate organic carbon (POC) with soil depth compared with the other two land uses. Significantly larger C:N ratio in surface (0-10 and 10-30 cm) soils under native woodland may be related to differences in litter quality which in turn result to differences in the degree of microbial processing among land uses. Across all land uses, MOC accounted for between 73 and 99 % of the total SOC indicating the strong capacity for soil minerals to adsorb organic C. The proportion of MOC to the total SOC increased with soil depth indicating that subsurface C was more protected than surface C probably due to mineral association with clayey subsoils. Compared with native pastures and cultivation which were largely similar, native woodland soils contained significantly larger amounts of MOC in all soil depths suggesting that C was more physically protected from microbial attack. As a result C may be more slowly decomposed hence more stabilized which is key to long term C sequestration.

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	Author's Name (please print clearly)	**% of contribution**
Candidate	Christine Walela	80
Other Authors	Heiko Daniel	6
	Brian Wilson	3
	Peter Lockwood	3
	Annette Cowie	3
	Bruce McCorkell	5

Name of Candidate: Christine Kainyu Walela

Name/title of Principal Supervisor: Prof. Heiko Daniel

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Name/title of Principal Supervisor: Prof. Heiko Daniel

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Chapter 6. Soil organic carbon mineralization: determination of carbon pools and turnover kinetics down the profiles under native woodland, native pastures and cultivation in New South Wales Australia

Christine Walela[1], Heiko Daniel[1], Brian Wilson[1], Peter Lockwood[1], Annette Cowie[1, 2], Steven Harden[3]

([1]) School of Environmental and Rural Sciences, Agronomy and Soil Science, University of New England, NSW, Australia (fax +612 6773 3238) , ([2]) National Centre for Rural Greenhouse Gas Research, Department of Primary Industries, NSW, Australia , ([3]) NSW Department of Primary Industries, Tamworth Agricultural Institute, NSW, Australia

6.1 Abstract

Knowledge on the distribution of organic carbon (C) among soil organic carbon (SOC) pools and the respective decomposition rates is important for assessing the stability of SOC and will assist in understanding the long term dynamics of C down the soil profiles of common land uses in NSW Australia. We sampled soils from 0-10, 10-30, 30-50 and 50-80 cm soil layers from three land uses namely, native woodland, native pastures and cultivation. Soils were adjusted to 60 % of their water holding capacity and incubated in the laboratory at 25°C for 419 days. The CO_2-C evolved was fitted to first order decomposition models in order to estimate SOC pool sizes and turnover rates. The cumulatively mineralized CO_2-C was related to physically measured SOC fractions namely; dissolved organic carbon (DOC), particulate organic carbon (POC) and mineral associated organic carbon (MOC) to better understand organic C dynamics in soil profiles. SOC mineralization kinetics over 419 days was well described by decomposition of a single pool; the active C pool. Strong land use and soil depth effects on the size of the active C pool were found and indicated differences in the supply and deposition down the soil profiles of labile C substrates such as POC and DOC between land uses. The decomposition rate of the active C pool was strongly dependent on soil depth with turnover of 66 and 47 days in surface and subsoils respectively. Shorter turnover of the active C pool in subsoils compared with surface soils may be linked to destabilization of active C stores when environmental constraints on decomposition which might inhibit decomposition in the undisturbed profile are removed following incubation under similar conditions in the laboratory. Although the amounts of CO_2-C evolved from the active C pool over 419 days accounted for < 10 % of the initial SOC, the results imply that the active C pools in subsoils may be vulnerable to destabilization and could become a significant source of CO_2 into the atmosphere if disturbed. The priority therefore is to

ensure this pool is not destabilized by factors related to changing environmental conditions especially in subsoils. The indication from the negligible amounts of CO_2-C evolved from the slow C pool during the 419 days laboratory incubation is that this pool may be relatively stable in the soils studied and may be important for long term C sequestration. Mechanisms by which C might be stabilized in the soils studied including the contribution of char C will however require further studies so as to establish the long term dynamics of this pool in soil profiles under the land uses studied in northern NSW Australia.

Key words: Soil organic carbon mineralization, soil organic carbon pools, turnover rate, land uses, Australia.

6.2 Introduction

SOC mineralization is a key process for soil-carbon efflux or CO_2 evolution to the atmosphere and is defined as the release of CO_2 as a metabolic by-product of organic matter decomposition by soil microbes and fauna (Raich et al., 1995; Hakkenberg 2008). Historically and in recent times, soils are estimated to have lost between 60-80 Pg/yr of their original carbon (C) to the atmosphere, with significant losses (50 Pg or more) associated with past and present land use changes such as deforestation followed by cultivation (Raich and Potter, 1995; Schlesinger and Andrews, 2000; Lal, 2004; Janzen, 2006; IPCC, 2007). Soils are therefore major sources of CO_2, an important green house gas associated with the current changes in the earth's climate with predictions for adverse global warming effects in the next decade (IPCC, 2007). Conversely, soils represent an important C sink with the potential to store significant amounts of C with the introduction of sustainable management practices/land uses (Lal, 2004). SOC storage depends on both the input of organic matter residues and its mineralization (CO_2 loss) (Trumbore, 2009). Critical to determining land uses that can act as net C sinks, is the identification of soil carbon pools that can accumulate C within a long period of time. The quantification of the amount of CO_2-C mineralized in soil profiles during laboratory incubation should provide us with information regarding the amount of substrate (SOC pools) available for microbial mineralization and the turnover of C in these pools (Paul et al., 1999, 2001; Collins et al., 2000; Yang et al., 2007; Lopes de Gerenyu et al., 2008; Rey et al., 2008).

SOC mineralization dynamics has been widely studied by separating SOC into various conceptual pools, namely active, slow and passive C pools thought to have varying intrinsic decomposition and turnover rates (Parton et al., 1987; Paul and Clark, 1996; Trumbore, 2000). These pools typically decompose following first-order kinetics and their turnover is described by the mean residence time (MRT) (Parton et al., 1987; Six and Jastrow 2002). The active C pool constitutes a small fraction (<10 %) of the total SOC and is characterized by a fast turnover (days) while; the slow and passive C pools account for between 20 and 40 % and 60 to 70 % of total C respectively (Follett 2001). The turnover of slow and passive C pools range from decades to thousands of years (Paul et al., 1999; Collins et al., 2000; Trumbore, 2000; Yang et al., 2007).

Land use or management type has been shown to impact on the size of SOC pools and turnover as a result of change in quantity of litter inputs, redistribution of litter input in soils (Paul et al., 1999; Collins et al., 2000), litter quality (Yang et al., 2007; Rey et al., 2008) and duration of soil restoration (Lopez de Gerenyu et al., 2008) among others. Collins et al. (2000) reported larger amounts of SOC pools with slow turnover in soils under no till compared with continuously tilled soils with no residue application. Differences in litter quality, especially lignin and lignin/N ratio between conifer and broadleaved forests have been shown to influence SOC turnover, with slower turnover rates reported in soils under conifer forests which was attributed to larger amounts of recalcitrant lignin contents in litter (Yang et al., 2007; Rey et al., 2008).

Majority of the studies describing the dynamics of SOC storage and cycling with respect to land use change have focused mainly on the upper 30 cm soil layers where significant land use effects have been reported to exist (Wilson et al., 2011). However, this is less than the rooting depth in many soils, and part of the labile C which has been shown to be well correlated with mineralization in soils may be missed (Trumbore, 2009). For example, as a result of leaching, soluble forms of carbon (e.g. dissolved organic carbon, DOC) are translocated beyond the routinely assessed upper soil layers while partially decomposed root litter inputs (e.g. particulate organic carbon, POC) may be deposited in deeper soil layers. Supply of fresh labile forms of carbon may serve as readily available substrates for microbes and could stimulate microbial mineralization in deeper soil layers (Fontaine et al., 2007). The potential for subsoils to act as net C sinks still remain poorly understood as the decomposability of SOC in subsoils remain less explored, despite the increasing evidence that over 50 % of SOC resides in subsoils (Batjes, 1996; Jobbagy and

Jackson, 2000). The frequent assumption by most simulation models describing SOC is that the same decomposition rates can be applied to SOC pools at all soil depths assuming that the mechanisms controlling SOC are consistent down the entire profile (Lomander et al., 1998; Jenkinson and Coleman, 2008; Salome et al., 2010). However, recent studies have shown that controls on C dynamics differ within the soil profile (Fierer et al., 2003; Fontaine et al., 2007; Salome et al., 2010; Sanaullah et al., 2011).

The amounts and susceptibility of SOC pools within soil profiles is expected to be largely influenced by land use as a result of varying quantity and quality of organic matter inputs entering into these pools. It is further expected that the extent to which organic C is bound to soil mineral particles will exert influences on C mineralization within soil profiles. Our study therefore aimed to:

1. To quantify the amount of CO_2-C mineralized in soil profiles during an extended laboratory incubation under native woodland, native pastures and cultivation;
2. Estimate SOC pool sizes and turnover rates by fitting first order kinetic SOC decomposition models to CO_2-C evolution measurements;
3. Assess the relationship between mineralized C and physically measured SOC fractions namely; DOC, POC and mineral associated organic carbon (MOC) to better understand organic C dynamics in soil profiles.

6.3 Material and methods

6.3.1 Site characteristics

This study was conducted near the township of Uralla in the Northern Tablelands of New South Wales, Australia. Uralla lies at 30.64°S, 151.49°E at an altitude of 1,012 m and receives a mean annual rainfall of 807 mm and has a mean maximum and minimum temperature of 26.4°C and 12.5°C respectively (1901-2012; Bureau of Meteorology 2012). The soils of the area are largely derived from Permian granite and are classified as yellow Chromosols (Isbell 2002) equivalent to Alfisols (Soil Survey Staff 1999).

6.3.2 Land use sampling approach and management history of sample sites

The study was carried out following a sampling approach similar to that used by Wilson et al. (2008, 2010, 2011). Site clusters containing three land uses namely, native pastures, native woodland and cultivation, were randomly selected at three separate locations, giving 3 replicates of each land use, nine sites in total. Different land uses were separated by <500 m from each other at each sample site. Land use type and management history is as described in Table 6.1.

Table 6.1. Land use type and management history

Land use	History
Native woodland	Had never been cleared (undisturbed) and consisted of a mixture of *Eucalyptus* species including Yellow box (*E. melliodora*) and Blakely's Red Gum (*E. blakelyi*) with a range of native grasses and shrubs growing beneath the tree
Native pasture	Had been under current land use for >20 years; Minimally fertilized (superphosphate) with limited oversown legumes (e.g. sub-clover); Dominated by native grasses such as *Microlaena stipoedes;* Paddocks had been historically grazed under set-stocking mainly with sheep although cattle had become more common in recent years.
Cultivation	Forage oats was the dominant crop that had been historically cultivated in alternate years with pasture for > 30 years.

6.3.3 Field soil sampling

Soil sampling was conducted following sampling protocols described by Wilson et al. (2011). A stratified random sampling design was used for each land use and site. A 25 x 25 m plot was randomly established at each site. Three sampling points were randomly selected within each plot. At each sampling point, litter and plant materials were cleared from the soil surface and a truck-mounted hydraulically driven soil corer of 50 mm internal diameter was used to sample to 0.80 m (to weathering rock). The soil core was then placed on a graduated Polyvinyl Chloride (PVC) pipe that had been halved lengthwise and labelled in 10 cm depth increments. Using a sharp cutting knife, each core was accurately divided into 4 depth intervals of 0-10, 10-30, 30-50 and 50-80 cm. Samples were placed in sealed plastic bags, stored in cooler boxes and transported to the laboratory and stored under cool conditions (<4^{o}C) until they could be processed (typically <48 h).

6.3.4 Soil DOC extraction

Extraction of soil DOC followed procedures developed by (Zsolnay, 2003). Briefly, extraction procedures were performed on field-moist soils which had been stored overnight at temperatures ranging from 0 to 4°C after field sampling. Hence, soils were sieved through a 6 mm sieve and subsamples of 15 g were weighed into 50 ml centrifuge tubes. 30 ml of 5 mM $CaCl_2$ solution was added to the mineral soil and gently stirred for 1 minute with a glass rod to make homogeneous slurry. Stirring was done as gently as possible to avoid significant desorption of soluble materials. The slurry was centrifuged at 12,000 *g* for 10 minutes. The supernatant was then filtered through a vacuum or pressure filter unit equipped with <0.45 μm nylon membrane filters. The filtrate was transferred into glass vials and DOC concentration was analyzed using a Shimadzu TOC-5000 A analyzer.

6.3.5 Determination of POC and MOC

POC and MOC were determined following methods described by Cambardella and Elliot (1992). Briefly, soil organic matter (SOM) was first separated into particulate organic matter (POM) and mineral associated organic matter (MOM) fractions using physical fractionation techniques on the basis of size into 53-2000 μm and <53 μm soil fractions respectively. Thirty grams of air-dried, 2 mm sieved soil samples were shaken overnight (16 h) on an end-over-end shaker in 135 ml of 5g l^{-1} sodium hexametaphosphate solution to disperse the soil. The dispersed slurry was passed through a 53 μm sieve on a Fritsch vibratory sieve shaker Analysette 3 Pro. The material that was retained on the sieve included POM (53 to 2000 μm) and sand while the material passing through the sieve was MOM (< 53 μm). The POM material was rinsed repeatedly to separate the organic matter from the sand. The material that passed through <53 μm was similarly collected into weighed beakers and together with the POM, oven dried (70°C) and reweighed. Both fractions were ground using a mortar and pestle to ensure all dried material was homogenous. Soil carbon (C) and nitrogen (N) concentrations associated with the two fractions were determined as POC, particulate organic nitrogen (PON), MOC and mineral-associated organic nitrogen (MON) using LECO CN-1000 autoanalyser (LECO Corporation, St. Joseph, Mich).

6.3.6 SOC mineralisation: extended laboratory incubation study

The incubation was conducted in the laboratory at 25°C under dark conditions following methods described by Paul et al. (2001). Briefly, stones and recognizable plant fragments were removed by hand from fresh moist soil samples. Soils were passed through a 4 mm sieve and sub-samples of 50 g were taken from each of the four depth intervals (0-10, 10-30, 30-50 and 50-80 cm). The soils were adjusted to 60 % of their water holding capacity (WHC), and placed in tared sealed wide-mouth 2 litre glass canning jars. The weight of each glass jar was recorded to ensure water loss could be subsequently monitored and corrected during the incubation period. A vial containing 30 ml of 1M NaOH was placed at the bottom of each jar and used to trap CO_2-C evolved from the soil. A vial containing 20 ml deionised water was also placed beside the alkali trap to maintain humid conditions in the jar during incubation. The jars were tightly sealed and pre-incubated for a week to allow the soil to equilibrate after handling by capturing the initial flush of CO_2-C which is associated with initial recycling of dead biomass and recovery of soil biota.

The amount of CO_2-C evolved during the pre-incubation period was not determined and alkali traps were removed from the jars and disposed. Jars were left open for 1 hour and allowed to equilibrate with the incubation room air in order to maintain aerobic conditions. Soil water loss in the jars was also monitored by weighing each sample jar and adjusting soil moisture to 60 % of WHC. Fresh alkali traps were then placed at the bottom of each jar to begin the main incubation study. Incubations were conducted in triplicates for each of the nine sites and four depth increments. Three blank jars (controls) that contained vials of 1M NaOH but no soils were included during each incubation time interval to provide background CO_2-C concentration. Overall, a total of 117 jars were incubated. Evolved CO_2-C was measured at sampling days 3, 9, 22, 35, 48, 78, 108, 109, 149 and 419 days after start of incubation by titrating each alkali trap with 0.5 M HCl using an automatic titrator (TIM 840 Titration Manager). Aerobic conditions and soil moisture conditions were maintained during each alkali sampling event. The mass of evolved CO_2-C at each sampling event was corrected using blanks and expressed as μg C per g of initial dry mass of soil.

6.3.7 Fitting kinetic models to CO_2-C mineralization data and statistical analyses

First-order kinetic models namely, single (Stanford and Smith, 1972) and double (Rey et al., 2008) exponential models were fitted to CO_2-C evolution data to estimate the SOM mineralization parameters namely, SOC pool sizes (active and slow) and their respective decomposition rate constants. The two models were fitted in order to compare goodness of fit in describing the SOM mineralization kinetics. The single exponential model was described as:

$C_{min} = C_a(1 - \exp(-k_a * t))$ Eq (1),

Where: C_{min} is cumulative mineralized carbon, C_a is the asymptote (size of the active pool), k is the active pool decomposition rate constant and t is the time in days. Hess and Schmidt (1995) have suggested a method to reliably estimate the parameters C_a and k_a, since cumulative CO_2-C data are not independent resulting to misleading estimates of the errors. They suggest analysing discrete/individual, non accumulated data which we obtained by dividing the change in mineralized carbon by the time between successive measurements to get a rate of carbon evolved per day, with time now being at the midpoints of the sampling days. This was modelled by the differential form of Eq (1).

$dC_{min}/dt = C_a k_a \exp(-k_a t)$ Eq (2)

The double exponential model was similarly described in differential form as;

$dC_{min}/dt = C_a k_a \exp(-k_a t) + (C_{soc} - C_a) k_s \exp(-k_s t)$ Eq (3)

where C_{soc} is total soil organic carbon, C_a and $(C_{soc}-C_a)$ are the respective asymptotes (pool sizes) for the active and slow pools, and k_a and k_s are the respective active and slow pool decomposition rate constants. Laboratory mean residence time (MRT) was calculated as the reciprocal of the decomposition rate constant (MRT = 1/k).

The relationship between cumulative CO_2-C and measured SOC fractions i.e. POC, MOC and DOC were examined using linear correlation techniques.

6.4 Results

6.4.1 Modelling SOC mineralization in soil profiles

Results from fitting the two kinetic models showed that our SOC mineralization data was well described by a model with an active C pool which decomposed exponentially according to a first order kinetics. Equation 2 gave similar values of C_a and k_a as in fitting Eq 1 and these are the parameters used to plot the curves in Fig 6.1. Of the 36 site by land use by depth combinations, 30 of the k_s parameters obtained by fitting Eq (3) to SOC mineralization data suggest a rate of carbon evolved per day by the slow pool that far exceeds the rate observed between the last two sampling days. Given these unrealistic estimates of k_s it was decided to use Eq (2) and assume all but a negligible amount of the mineralized carbon was contributed by the slow pool. The difference between the two models (Eq 1 and 2) was that Eq (2) assumes that the slow pool was inert (i.e. $k_s = 0$) and so the second term in Eq (3) was dropped out.

Equation 2 was then applied to all of the SOC mineralization data using the non linear mixed effects (nlme) function in the R software program which fits a non linear mixed effects model. Our initial model estimated a different value of C and k for each of the 12 land use by depth combinations. The model was simplified in stepwise fashion with the last model having only one value for both C and k. Models were then compared by testing twice the change in Log-likelihood (d) as a χ^2 distribution with degree of freedom equal to the difference in degrees of freedom between the two models. If d was small (e.g. <3.84 for df=1, 5.99 for df=2) then the simpler model fitted the data just as well and was therefore preferred.

The simplest model found that there was a strong and significant ($P<0.0001$) land use:depth interaction effect on C (i.e. the size of the active C pool) and that k (i.e. the active pool decomposition rate constant) was strongly influenced by soil depth. Upon testing the depths, at which k values significantly differed, we found k values for depths 10-30, 30-50, 50-80 to be statistically similar under all land uses. Hence a model was fitted with depth as a two level factor (0-10, 10-80) to test whether a common k could be used for the 3 lower depths. This was the simplest model which fitted our data well. For this reason, our subsequent emphasis will be mainly focused on the surface 0-10 cm and combined subsoil layers of 10-80 cm as they represent depths at which significant effects were observed.

6.4.2 SOC mineralization rates and cumulative CO_2-C evolution down the profiles under contrasting land uses

The curves of cumulative CO_2-C evolution and SOC mineralization rates in 0-10, 10-30, 30-50 and 50-80 cm depths under native woodland, native pastures and cultivation are presented in Fig 6.1 upper and lower respectively. SOC mineralization in all soil depths and land uses was characterized by a high initial rate of mineralization ranging between 20 and 30 μg CO_2-C g^{-1} soil day^{-1}, followed by a gradual decline with little or no change in mineralization rates (<5 μg CO_2-C g^{-1} soil day^{-1}) over time (from 150 days to end of incubation) (Fig 6.1, lower). The largest differences in mineralization rates between surface and subsoils were observed at the start of the incubation, and, these differences narrowed and then disappeared as the incubation progressed (Fig 6.1, lower).

The average SOC mineralization rates over the 419 d incubation decreased with increasing soil depth with higher mineralization rates observed in surface (0-10 cm) compared with each of the subsequent soil depth increments which were largely similar between each other across all land uses (Table 6.2). Consequently, the amount of cumulative CO_2-C evolved followed a similar trend, decreasing with increasing soil depth with surface soils consistently found to have significantly larger amounts of CO_2-C than in each of the subsequent soil depth increments across all land uses (Fig 6.1, upper). Differences in the average SOC mineralization rates between land uses were limited to surface (0-10 cm) soil layers with higher rates found for native woodland (5.69 µg CO_2-C g^{-1} soil day^{-1}) compared to cultivation (4.38 µg CO_2-C g^{-1} soil day^{-1}) and native pastures (4.02 µg CO_2-C g^{-1} soil day^{-1}) which were largely similar between each other *(P* < 0.0001).

6.4.3 Land use and soil depth effects on modelled SOC pools and turnover rates

Strong and significant ($P < 0.0001$) land use and depth interaction effects were found for the size of the active C pool suggesting that the size of the active pool changed with soil depth and that the nature of this change differed between the three land uses. The change in the size of the active C pool with depth under the three land uses was shown as follows: under native woodland 0-10 cm > 10-30 cm > (30-50 cm = 50-80 cm), under native pastures 0-10 cm > (10-30 cm = 30-50 = 50-80 cm) and under cultivation 0-10 cm > 10-30 cm > (30-50 cm = 50 -80 cm) ($P < 0.0001$). Significant ($P < 0.0001$) land use effects in the size of the active pool were limited to the surface (0-10 cm) depths with native woodland > cultivation = native pastures. The active C pool accounted for between 80 and 100 % of the cumulatively evolved CO_2-C. However, the fraction of the initial C mineralized from the active C pool was < 10 % in soil profiles under all land uses. Strong and significant ($P < 0.0001$) depth effects were found for the active C pool decomposition rate constant between the surface (0-10 cm) depths and combined subsoil (10-80 cm) depth increments which were shown to be statistically similar between each other. The active C pool decomposition rate constants were 0.015 d^{-1} for the 0-10 cm depth and 0.021d^{-1} for the combined 10-80 cm depth layers and represented laboratory MRTs of 63 and 47 days respectively (Table 6.2).

6.4.4 Relationship between cumulatively mineralized CO_2-C and physically measured SOC fractions (DOC, POC and MOC) down the profiles

Weak relationships were found between mineralized CO_2-C and the physically measured SOC fractions (POC, MOC and DOC) in the combined 0-80 cm soil depths (Fig 6.2 a, b & c). As a result, relationships between mineralized CO_2-C and the above fractions were further explored in surface 0-10 cm and combined subsoils 10-80 cm depths where SOC mineralization had been shown to differ strongly. The mineralized CO_2-C was strongly correlated with DOC and POC in surface soils (Fig 6.3 a, b & c) and less so in the subsoils (Fig 6.4 a, b & c). An unexpected strong relationship was observed between mineralized CO_2-C and MOC in the surface 0-10 cm (Fig 6.3, c) while a weak relationship was observed in the subsoils (Fig 6.4, c).

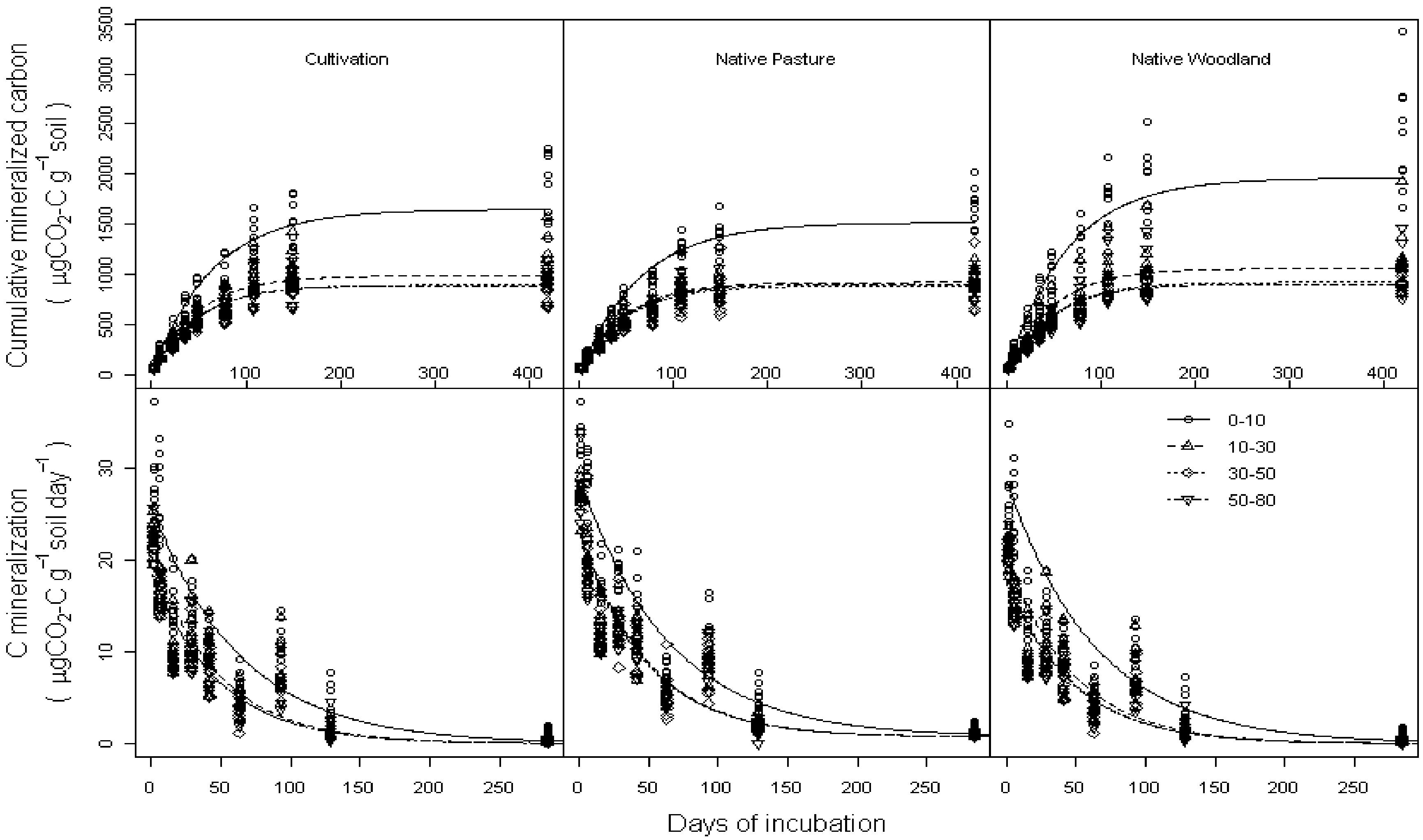

Fig 6.1. Measured (dots) and modelled (line) cumulative CO_2-C evolution (upper, μg CO_2-C g^{-1} soil) and carbon mineralization rates (lower, μg CO_2-C g^{-1} soil day^{-1}) in four soil depths (0-10, 10-30, 30-50 and 50-80 cm) during an extended laboratory incubation under native woodland, native pastures and cultivated soils.

Table 6.2. Parameter values for total soil organic carbon (SOC), cumulative CO_2-C mineralized, average rate of C mineralized over 419 d, and SOC mineralization kinetics in four soil depths (0-10, 10-30, 30-50 and 50-80 cm) under native woodland, native pastures and cultivated soils

Land use	Soil depth (cm)	Total SOC (µg/g soil)	Cumulative CO_2-C mineralized over 419 d (µg/g soil)	Average rate of C mineralized over 419 d (µg/g soil)	Active C pool (µg/g soil)	Mean Residence time of active C pool (days)	Slow C pool (µg/g soil)
Native woodland	0-10	27215	2385	5.69	1964	66	25251
	10-30	14240	1216	2.9	1059	52	13181
	30-50	12250	987	2.35	923	45	11328
	50-80	12043	1000	2.38	906	47	11137
Native pastures	0-10	19901	1684	4.02	1516	66	18385
	10-30	12826	976	2.32	921	52	11905
	30-50	11972	885	2.11	885	45	11087
	50-80	11548	907	2.16	902	47	10646
Cultivation	0-10	20077	1836	4.38	1649	66	18428
	10-30	13818	1137	2.71	987	52	12831
	30-50	12563	901	2.15	894	45	11669
	50-80	11199	922	2.22	890	47	10309

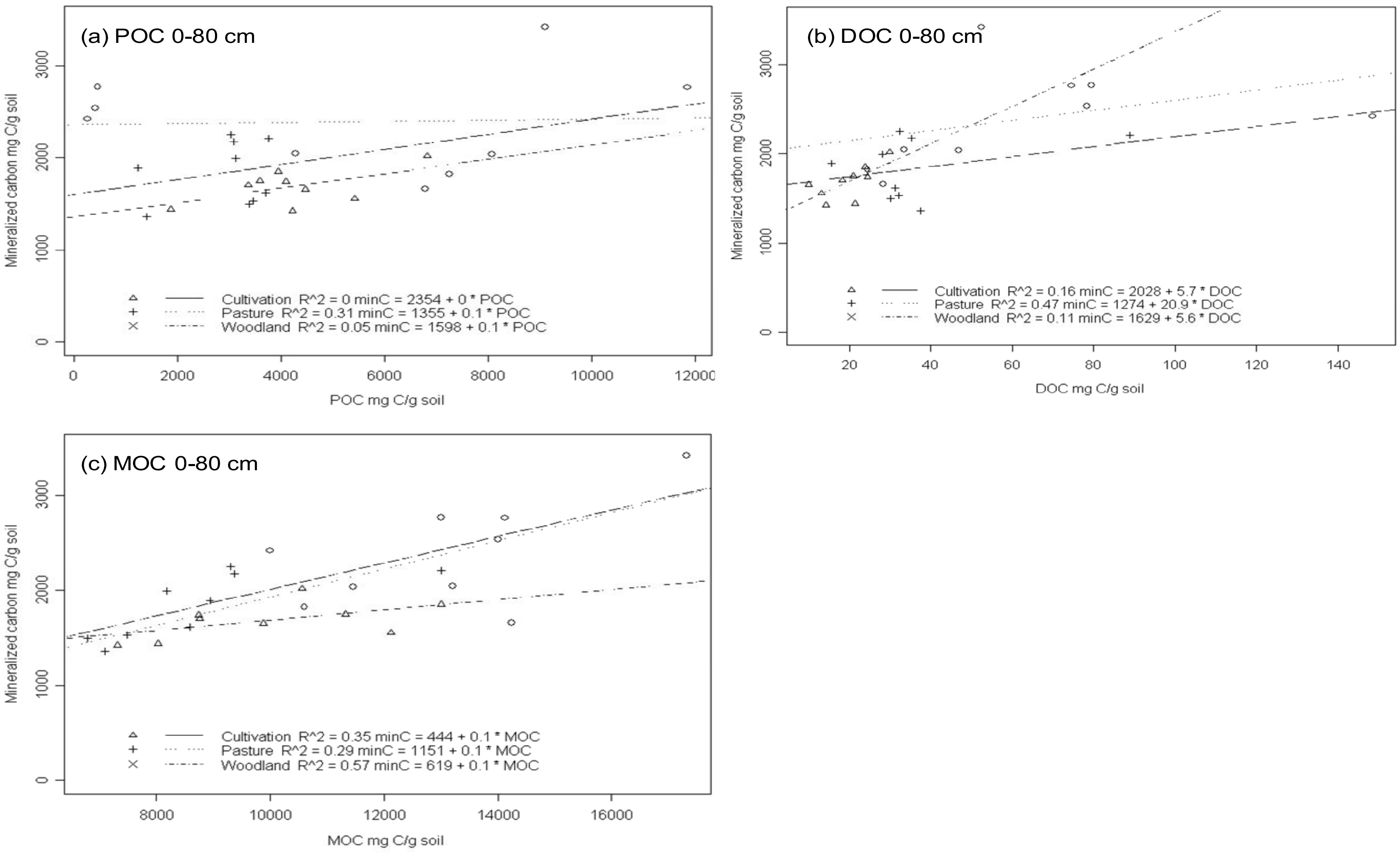

Fig 6. 2 Relationship between mineralized CO_2-C and a) POC (particulate organic carbon), b) DOC (dissolved organic carbon) and c) MOC (mineral-associated organic carbon) in combined 0-80 cm depths under native woodland, native pastures and cultivation

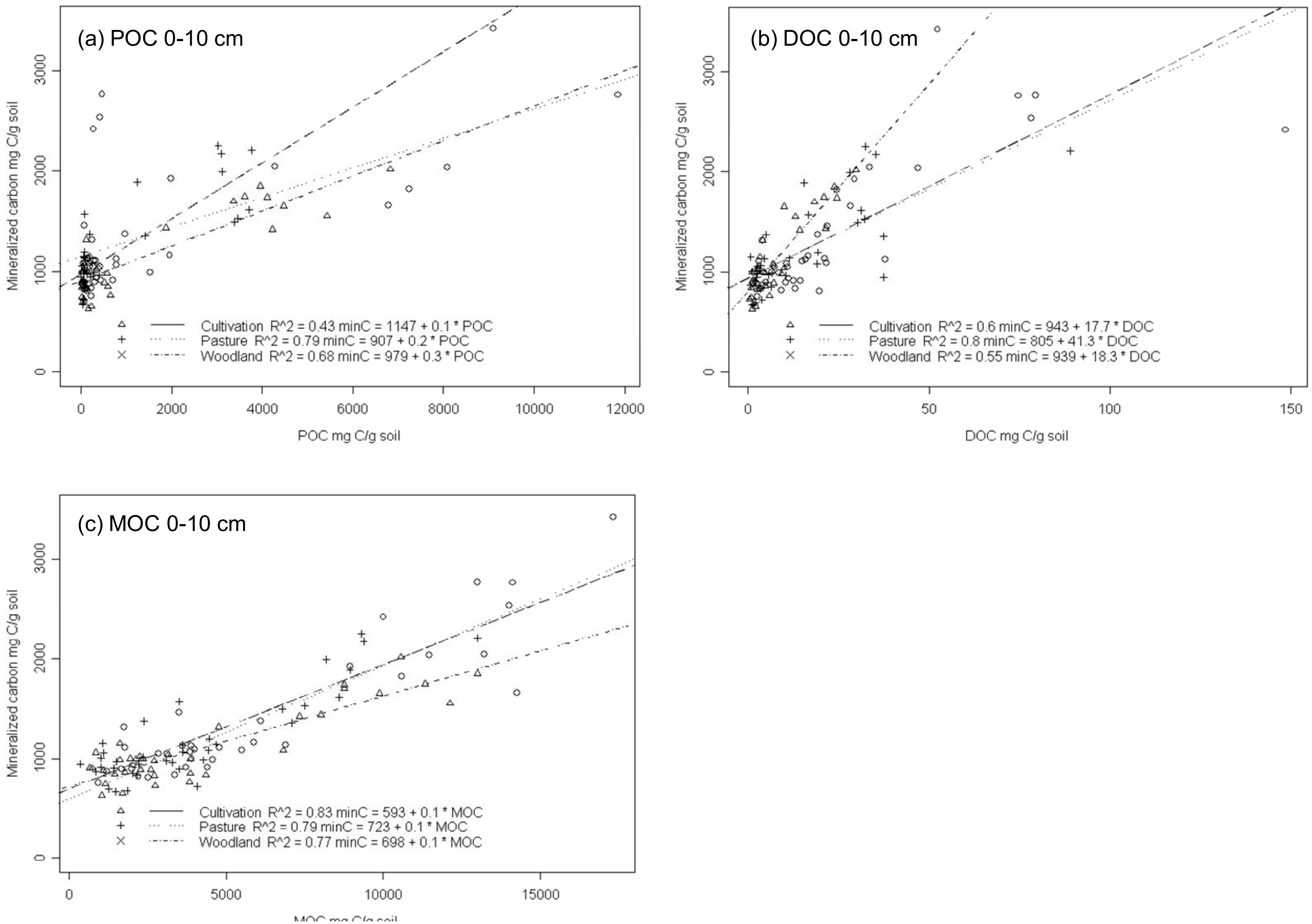

Fig 6. 3 Relationship between mineralized CO_2-C and a) POC (particulate organic carbon), b) DOC (dissolved organic carbon) and c) MOC (mineral-associated organic carbon) in 0-10 cm depths under native woodland, native pastures and cultivation

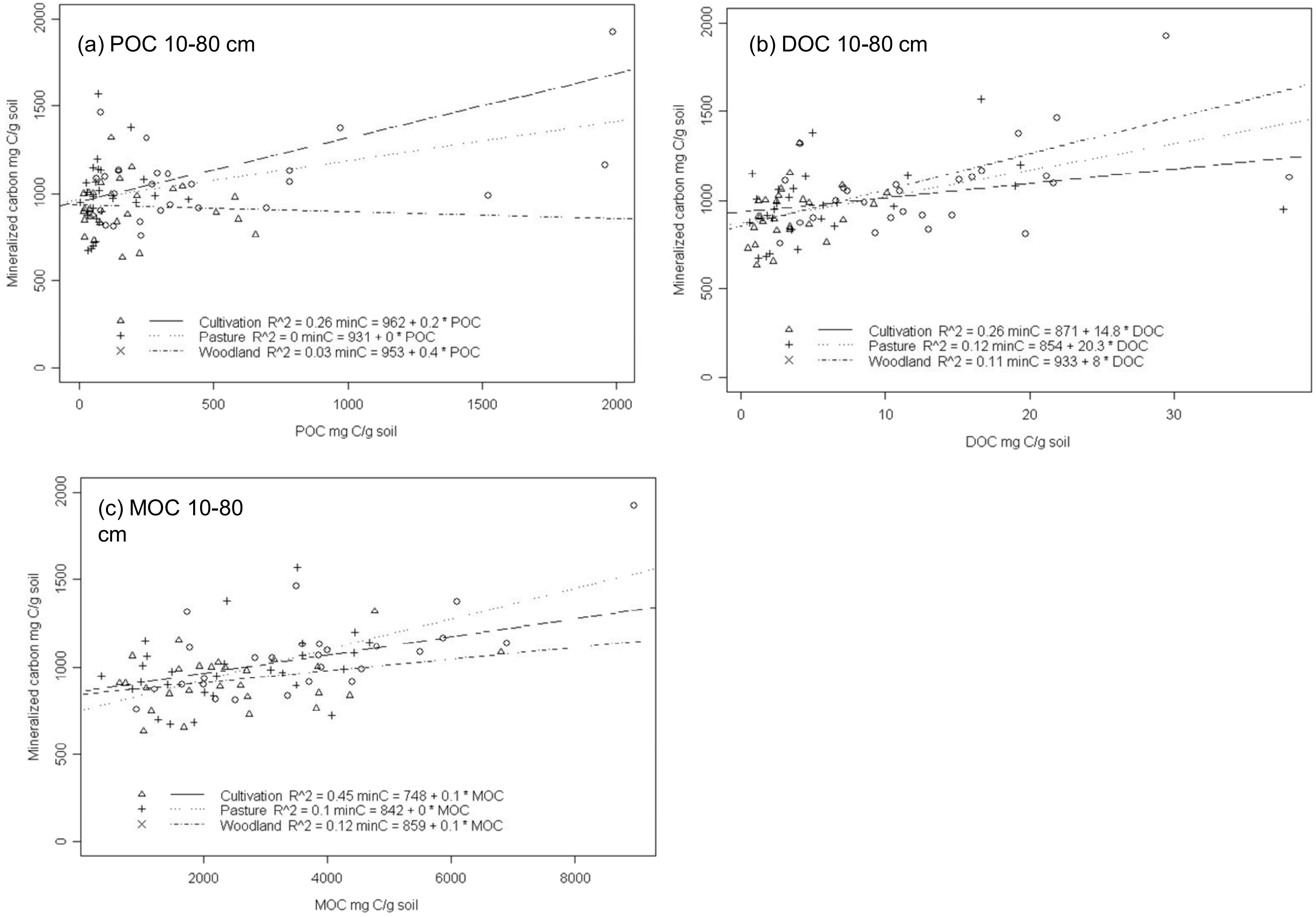

Fig 6. 4 Relationships between mineralized CO_2-C and a) POC (particulate organic carbon), b) DOC (dissolved organic carbon) and c) MOC (mineral-associated organic carbon) in 10-80 cm depths under native woodland, native pastures and cultivation

6.5 Discussion

6.5.1 Modelling SOC mineralization down the profiles

The SOC mineralization was well described by a first-order single exponential kinetic model which described decomposition of a single pool, the active pool as similarly reported by Riffaldi et al. (1996) and Alvarez and Alvarez (2000). The active C pool was shown to account for between 80 and 100 % of the cumulatively evolved CO_2-C. We could not determine the decomposition rate of the slow C pool and therefore assumed that this pool evolved a negligible amount of CO_2-C during the 60 week laboratory incubation suggesting that this pool may be relatively stable in the soils studied. A possible explanation for the relatively stable C pool in these soils might be related to char C which has been found to be present in many Australian soils attributed to past management practices such as biomass burning (Skjemstad et al., 1996, 2002; Clough and Skjemstad, 2000; Dalal and Chan, 2001; Lehman et al., 2008). For example, the amounts of char C in relatively undisturbed soils in the Australian cereal belt have been reported to be approximately 5.1 ± 0.5 t/ha which is 22.4 ± 6.9 % of the total organic C (Skjemstad et al., 1996, 2001). Mechanisms by which C might be stabilized in the soils studied including the contribution of char C will however require further studies.

6.5.2 Land use and depth effects on SOC pools and turnover rate

The size of the modelled active C pools changed with land use and the nature of this change differed with depth reflecting differences in the amounts of labile C substrates among land uses with soil depth. This is well related to the amounts and distribution of DOC and POC (physically measured labile substrates) which differed between land uses and changed differently with soil depth (chapter 4 and 5 respectively). Similar to other studies, our results show that land use plays an important role in influencing the substrates supply entering the SOC active pool as a result of change in the quantity and redistribution of litter input in soil profiles and may in turn influence CO_2 production differently within the different profile layers (Collins et al., 2000; Davidson et al., 2006). Differences in the size of the active C pool among land uses were limited to the surface (0-10 cm) layers with large amounts contained under native woodland compared to other land uses which were similar to each other and are associated with large amounts of organic matter inputs in surface layers therefore contributing more to SOM in the upper soil horizons than in deeper soil layers (Lorenz and Lal, 2005). Both POC and DOC are closely related with recently deposited plant litter input in upper soil horizons (Golchin et al., 1994) and native woodland is generally associated with supplying large amounts of inputs in these upper layers (Wilson et al., 2011). The size of a SOC pool depends on the balance between the rate of C supply into the pool and how fast (decomposable/labile) SOC is decomposing from the pool. In our study, the decomposability of the active C pool was not significantly influenced by land use in any of the soil depths that were studied, probably because the active pool is generally considered to be composed of easily decomposable labile C compounds (Collins et al., 2000). Our results therefore seem to suggest that the size of the active C pool was largely influenced by differences in the supply of labile substrates in soil depths between land uses. However, it is to be noted that these observations are based on results from a controlled laboratory study and there could be still differences in active C pool decomposition rates in the field at similar soil depths due to differences in microclimate among land uses.

Soil depth was found to have a strong influence on the decomposition rates of the modelled active C pools, with subsoils associated with higher decomposition rates and turnover than surface soils. These results suggest that a) an active C pool exists in deeper soil layers, and b) its fast cycling may be attributed to destabilization of the active C stores due to exposure to favourable environmental conditions such as moisture conditions, soil aeration and soil temperature which might have stimulated the activity of microbes already present in subsoils therefore resulting in high decomposition of the active C pool during laboratory incubations. Environmental constraints prevailing in deeper soil layers in undisturbed soil profile such as; oxygen availability, aeration and moisture conditions have been suggested detrimental to SOC mineralization (Gill and Burke, 2002) but are removed when the incubated subsoil and surface soils are under the same standard conditions in the laboratory as in our case. Furthermore, factors such as oxygen availability, soil moisture and temperature have often been considered as decomposition rate-modifying factors (Paustian et al., 1997). Comparable increases in decomposition rates of active C pool in subsoils has been previously observed by Collins et al. (2000) and related to exposure of C associated with aggregates when soil is mixed and brought to the laboratory. Our results demonstrate the existence of active C forms in deeper soil layers with potential for fast C cycling in response to destabilization processes which in our case was partly explained by changes in environmental conditions (Trumbore et al., 1995; Richter et al., 1999; Baisden et al., 2002).

The active C pool represented < 10 % of the initial SOC demonstrating that this pool constitutes a small fraction of the total SOC and that the bulk of the SOC consists of a slow C pool (Gregorich et al., 1994; Paul et al., 1999; Collins et al., 2000). However, though small in size, CO_2-C fluxes from the active C pools may become significant when integrated over the large volume of soil below the surface soils (Trumbore, 2009). Native woodland soils generally had the larger amounts of slow C pools in soil depths compared with the other two land uses which were similar to one another indicating that SOC is more stable under native woodland. Compared with native pastures and cultivation, a more stable SOC pool down the soil profile under native woodland may be linked to biochemically recalcitrant litter (above and below ground litter) inputs with more stable slow C pools which were observed in this study (chapter 7).

6.5.3 Relationship between cumulatively mineralized CO_2-C and physically measured SOC fractions (DOC, POC and MOC) down the soil profiles

Strong correlations between cumulatively mineralized CO_2-C and physically measured SOC fractions (DOC, POC and MOC) occurred mainly in the surface (0-10 cm) soils and less in the combined subsoil (10-80 cm) depth increments and the entire soil profile (0-80 cm) as similarly reported by Alvarez and Alvarez (2000). A possible explanation for the observed depth relationships between cumulatively mineralized CO_2-C and physically measured labile substrates (POC and DOC) include;

- Differences in the amounts of labile C substrates with soil depth among land uses. Availability of larger amounts of easily degradable C substrates in surface soils; DOC and POC (as reported in Chapter 4 and 5) may have led to an increase in microbial biomass and activity resulting in increased mineralization at this depth (Alvarez and Alvarez, 2000; Garcia-Pausas et al., 2008). Consequently, native woodland which had larger amounts of labile substrates in surface soils was found to have larger amounts of CO_2-C evolved. Conversely, microbial activity may have been limited in subsoils due to lower amount of labile substrates e.g. DOC and POC therefore resulting in lower SOC mineralization (Jinbo et al., 2006; Alvarez and Alvarez, 2000).

On the other hand, a possible explanation for the observed depth relationship between cumulatively mineralized CO_2-C and organic C associated with soil mineral fraction include:

- Varying degree of association of organic C with soil mineral particles (clay and silt). MOC was more decomposable in surface soils probably due to a lack of protection from microbial decomposition due to poor aggregation caused by low clay and silt contents in surface soils (chapter 3 of this thesis) (Six et al., 2002). In a study carried out in NSW Australia, Chan et al. (2001) reported that soils taken from the surface 10 cm layer from an Alfisols had a great tendency to lose mineral-associated organic C and related this with the smaller capacity of the clay and silt fraction to protect C. Hassink (1997) similarly reported that contrary to soils from other temperate and tropical regions, Australian soils had a much lower capacity to retain C in their clay and silt fractions and attributed this to soil mineralogy; mainly lower cation exchange capacity and surface area. Similar studies conducted elsewhere, e.g. Ahn Mi-Youn et al. (2009) have also reported a lack of protection against SOM mineralization in low clay surface soils. The adsorption of SOM to soil mineral particles is controlled by the availability of reactive surfaces (Stewart et al., 2008), therefore this process may have been ineffective in our study as the reactive surfaces (clay and silt) were insufficient in surface soils.

  Conversely, the weak relationships found between cumulatively mineralized CO_2-C and MOC in subsoils showed that MOC became less decomposable as organic matter became increasingly associated with clay minerals and therefore becoming protected from microbial mineralization in subsoil layers (Hassink,1997; Ajwa et al., 1998; Kaiser and Guggenberger, 2000; Eusterhues et al., 2003).

6.6 Conclusion

Strong land use and depth effects on the size of the active C pool were found and indicated differences in the supply and deposition in soil profiles of labile C substrates such as POC and DOC between land uses. Shorter turnover of active C pool in subsoils compared with surface soils may be linked to destabilization of active C stores when environmental constraints on decomposition which might inhibit decomposition in the undisturbed profile are removed following incubation under similar conditions in the laboratory. Although the amounts of CO_2-C evolved from the active C pool over 419 days accounted for < 10 % of the initial SOC, the results imply that the active C pools in subsoils may be vulnerable to destabilization and could become a significant source of CO_2 into the atmosphere if disturbed. The priority therefore is to ensure this pool is not destabilized by factors related to changing environmental conditions especially in subsoils. The indication from the negligible amounts of CO_2-C evolved from the slow C pool during the 419 days laboratory incubation is that this pool may be relatively stable in the soils studied and may be important for long term C sequestration. Mechanisms by which C might be stabilized in the soils studied including the contribution of char C will however require further studies so as to establish the long term dynamics of this pool in soil profiles under the land uses studied in northern NSW Australia.

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6.7 References

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	Author's Name (please print clearly)	**% of contribution**
Candidate	Christine Walela	80
Other Authors	Heiko Daniel	3
	Brian Wilson	3
	Peter Lockwood	6
	Annette Cowie	3
	Steven Harden	5

Name of Candidate: Christine Kainyu Walela

Name/title of Principal Supervisor: Prof. Heiko Daniel

[redacted]

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Name/title of Principal Supervisor: Prof. Heiko Daniel

[redacted]

______________ 13/05/2013

Candidate Date

[redacted]

_______________ 13/05/2013

Principal Supervisor Date

Chapter 7. Biochemical composition of above and below ground plant litter from native woodland, native pastures and cultivation and its influence on C mineralization

Christine Walela[1], Heiko Daniel[1], Brian Wilson[1, 2], Peter Lockwood[1], Annette Cowie[1, 3], Steven Harden[4]

[1]School of Environmental and Rural Sciences, Agronomy and Soil Science, University of New England, NSW 2351, Australia

[2]NSW Office of Environment and Heritage, PO Box 0221, Armidale, NSW 2351, Australia.

[3]Rural Climate Solutions, University of New England, NSW 2351, Australia/ Department of Primary Industries, NSW Australia.

[4]NSW Department of Primary Industries, Tamworth Agricultural Institute, NSW, Australia

7.1 Abstract

Understanding the interactions between the initial biochemical composition and subsequent decomposition of plant litter inputs will assist us in identifying sources with more stable plant-derived carbon (C) compounds which might be important for long term soil organic carbon (SOC) sequestration. We determined the effects of land use (cultivation/native woodland/native pasture), litter type (above and below ground) and their interaction on the initial biochemical composition (carbon, nitrogen, water soluble carbon, lignin, tannin and cellulose) and decomposition of litter. Decomposition of litter was studied as the mineralization of C from litter by microbial respiration and was measured as CO_2-C production during 105 d of laboratory incubation with soil. A two-pool model was used to quantify C mineralization kinetics. For all litter types, the active C pool whose decay rate constants ranged from 0.072 d^{-1} to 0.805 d^{-1}, initially constituted < 20 % of the litter mass, while the slow C pool with decay rates of between 0.002 d^{-1} and0.019 d^{-1} comprised > 80 % of the litter mass. The decomposition rate of the slow C pool in litter was strongly and negatively correlated with the initial lignin:N ratio of plant litter suggesting that the interaction between these two litter quality variables had important controls over litter decomposition. The mechanism by which lignin and N interact to influence decomposition of litter were not investigated however, the differences among litter types in the amounts of initial lignin contents may have influenced the mechanism by which lignin and N interact to control decomposition of the litter slow C pool. Compared with other litter types, above and below ground litter from native woodland had higher initial lignin:N ratio and were associated with more stable slow C pools with longer half lives of 109 and 446 days respectively. The more slowly decomposing litter from native woodland is more likely to persist in soils for longer and may enter the soil organic matter (SOM) fraction and contribute to increased C storage down the soil profiles compared with the other two land uses.

Keywords: litter quality, carbon mineralization, land uses, soil organic carbon sequestration, labile and slow pools, and decomposition rate.

7.2 Introduction

Plant litter plays an important role in the global carbon (C) cycle as it is the primary source of soil organic carbon (SOC) (Kögel-Knabner, 2002), the largest terrestrial pool for organic C in the biosphere (Schlesinger, 1997; Lorenz and Lal, 2005). As a source, plant litter is comprised of a complex and variable mixture of C-rich compounds which include labile (e.g. water soluble C, cellulose, and hemicellulose) and biochemically recalcitrant compounds (e.g. lignin, tannin, waxes, suberin, and cutin) (Kögel-Knabner, 2002; Neher et al., 2003; Crow et al., 2009). The labile compounds are lost during the initial stages of decomposition and are thus less likely to enter the long term soil organic matter (SOM) pool (Crow et al., 2009). Conversely, biochemically recalcitrant compounds, which have slower decay kinetics (Berg and Meentemeyer, 2002), accumulate in soils and have the potential to form stable SOM pools through their interaction with soil mineral surfaces (Kleber et al., 2007; Crow et al., 2009). Understanding the influence of biochemical composition of plant litter and its subsequent decomposition will assist us in identifying litter inputs that are sources of more stable plant-derived C compounds which may contribute to long term SOC sequestration.

Various litter quality indices that can be used to determine litter decay have been proposed over the last three decades, however to date there is no consensus on what particular index should be used (Mafongoya et al., 1998). Chemical traits such as C:N ratio have been widely proposed across a broad range of litter types (Pérez Harguindeguy et al., 2000; Prescott, 2010; Zeng et al., 2010). The initial concentration of lignin, lignin:N ratio (Loranger et al., 2002; Berg and McClaugherty, 2003), holocellulose:lignin ratio (McClaugherty and Berg, 1987), (polyphenol + lignin):N ratio (Mafongoya et al., 1998) and hot water extractable organic matter (Ghani et al., 2003) have been proposed within specific categories such as leaf litters. Hot water extractable organic matter has been reported as being linearly related to decomposition while the C:N and lignin:N ratios have been reported as being negatively related to decomposition rate (Melillo et al., 1982; Taylor et al., 1989).

Land use change which is commonly followed by change in both, the quantity and quality of litter inputs, have been identified as processes responsible for affecting changes in net C sequestration (Crow et al., 2009; Wang et al., 2010; Montaine et al., 2010; Potthast et al., 2010). In cases where quantity of litter inputs is similar among land uses, differences in C accretion among land uses may depend on differences in litter quality (Montaine et al., 2010). The initial biochemical composition of above and below ground plant litter have also been shown to differ substantially within and among species therefore influencing their relative decomposition rates (Kögel-Knabner, 2002; Wang et al., 2004; Johnson et al., 2007). In this regard, Johnson et al. (2007) reported slower decomposition of roots compared with the above ground litter components and attributed their results to the chemical recalcitrance of the former compared with the latter.

The majority of research on the biochemical composition of litter and its effect on decay has concentrated on above ground inputs as this litter is deposited on the soil surface thereby strongly contributing to SOC in the upper horizons (Lorenz and Lal, 2005). However, below ground C input also contributes to SOC with some reports showing that roots contribute more C to SOC than above ground inputs (Mafongoya et al., 1998; Wilhelm et al., 2004). Comparing the composition of both above and below ground litter with their respective decay kinetics may improve our understanding of the differential contribution of plant litter materials to SOC (Mafongoya et al., 1998; Johnson et al., 2007). Further, the litter decay kinetics may be used in carbon cycle models estimating the movement of plant derived C to SOC pools (Johnson et al., 2007). Our knowledge of the specific decay rates for above and below ground litter from common land uses found in NSW Australia is still limited and providing such information is valuable. One of the methods used to study litter decomposition is through laboratory incubations which assesses the mineralization of C from litter residue due to microbial activity and is measured as CO_2-C production (Zeng et al., 2010; Cotrufo et al., 2010). This allows the relationship between plant quality and decomposition kinetics to be investigated under controlled conditions with variables such as temperature and moisture content (Johnson et al., 2007).

In this work, we studied the decomposition of above and below ground litter from three contrasting land uses. Our aims were to a) determine the initial biochemical composition of above and below ground litter from the three land uses namely: native woodland, native pastures and cultivation; b) determine the C mineralization dynamics of decomposing plant litter over time and c) examine the interactions between litter chemistry and C mineralization kinetics.

7.3 Materials and methods

7.3.1 Site characteristics

This study was conducted near the township of Uralla in the Northern Tablelands of New South Wales, Australia. Uralla lies at 30.64°S, 151.49°E at an altitude of 1,012 m and receives a mean annual rainfall of 807 mm and has a mean maximum and minimum temperature of 26.4°C and 12.5°C respectively (1901-2012; Bureau of Meteorology 2012). The soils of the area are largely derived from Permian granite and are classified as yellow Chromosols (Isbell 2002) equivalent to Alfisols (Soil Survey Staff 1999).

7.3.2 Field sampling of above and below ground litter

Site clusters containing the three land uses namely, native woodland, native pastures and cultivation were randomly selected at three separate locations. Above and below ground samples were collected from three randomly selected plots within each land use, giving a total number of 54 (3 sites x 3 land uses x 2 litter types x 3 reps) samples. The native pastures were dominated by native grasses such as *Microlaena stipoides.* Above ground biomass from this land use consisted of standing dead biomass and was sampled from quadrats measuring 50 x 50 cm randomly placed in the paddocks (after Sanaullah et al., 2010). Above ground biomass within each quadrat was cut just above the soil surface and collected into plastic bags. Thereafter, roots were collected by excavating soil from within quadrats from the upper 15 cm soil layer. Native woodland consisted of a mixture of *Eucalyptus* species including Yellow box (*E. melliodora*) and Blakely's Red Gum (*E. blakelyi*). Above ground litter from this land use consisted of senescent leaves which were sampled from the ground within a randomly selected 25 x 25 m plot. Sampling for below ground root litter was done within the same plot by randomly selecting three trees, establishing a distance of 3 m from each tree base and excavating roots from 1.0 m soil depth.

The paddocks under cultivation had been predominantly cropped with forage oats (*Avena sativa*) for >20 years. At the time of litter sampling these paddocks were fallow and therefore the oats were grown in glasshouse pots until physiological maturity from which standing dead biomass and roots were harvested. The soil used for sowing forage oats was sampled from the field within the plough layer (0-15 cm). At physiological maturity, above ground litter was sampled by first discarding the stems bearing grains and harvesting only standing dead biomass. Roots were separated from the soil. In the laboratory, all above ground litter from all land uses was sorted to remove any green foliage and retain only brown litter. Roots were separated from soil by sieving through < 4mm and picked out with forceps. All plant above and below ground litter were oven-dried at 60^{o}C to constant weight (~ 48 h). Subsamples were taken from each above ground litter type and ground through < 4 mm.

7.3.3 Litter chemistry laboratory analysis

The initial biochemical composition of all plant litter was determined on subsamples. Total C and N were determined by dry combustion using a CN LECO-1000 autoanalyser (LECO Corporation, St. Joseph, Mich).Water soluble carbon (WSC) was extracted following methods described by Don and Kalbitz (2005). Briefly, 0.2 g, of < 4 mm above and below ground litter was weighed into 130 ml specimen jars. Litter samples were soaked in 100 ml of ultra pure water for 24 h at 25^{o}C. The mixture was then filtered using a < 45 µm nylon filter into 70 ml specimen jars. The samples were analyzed for WSC using a Shimadzu TOC-5000 A analyzer. Acid detergent fibre (ADF) and lignin were determined by the sulphuric acid procedure using Foss FibreCap (tm) (AOAC, method 973.18). Tannin was determined by extraction with an aqueous methanol solution and measured by colorimetry in the presence of Folin-Denis reagent (AOAC, method 952.03). Cellulose was calculated from Acid detergent fibre-lignin.

7.4.4 Carbon mineralization

The kinetics of C mineralization were studied through a laboratory incubation which was performed under controlled conditions of 25°C for 105 d. A sandy soil (yellow Chromosol) taken from our study site from a paddock under forage oats cultivation history (> 20 years) was used as a medium for the incubation of plant materials. Briefly, approximately 5 kg soils were sampled from the top layer (0-5 cm) and gently sieved through a 4 mm mesh on the same day and stored at 4°C. An earlier assessment of the soil taken from this top layer showed that the soil contained 5.6 % clay, 10.4 % silt 84 % sand, 1.2 % C, soil pH (w) of 5.3 and an initial mineral nitrogen of 2.84 mg N kg^{-1} soil. Prior to the main incubation experiment, soils (only) were pre-incubated in 2 l glass jars for one month to avoid capturing the initial flush of CO_2-C which is associated with initial disturbance of soil.

In order to allow decomposition under non-limiting conditions of N availability (Recous et al., 1995; Trinsoutrot et al., 2000) the mineral N content of the soil in each jar was adjusted to 80 mg N kg^{-1} soil. This was done by adding 77.16 mg N kg^{-1} soil as KNO_3. Soils were then moistened to 60 % of their water holding capacity (WHC) and plant litter (1.0 g) was incorporated homogeneously and mixed thoroughly with the moist soil. The soil without addition of any plant material was used as control. Triplicate blanks (empty jars) were incubated in the same way, but without soil-plant mixture. The CO_2 produced by the plant litter amended soil and control treatments was trapped in 20 ml 1M NaOH in 70-ml specimen jars which were placed at the bottom of the jars. A small vial containing 10 ml of deionised water was also placed at the bottom of the jar to maintain humid conditions and prevent soil desiccation. To ensure that the jars remained air-tight, they were reinforced with cling wrap before closing them tight.

The glass jars were placed in the dark at a constant temperature (25°C) room for 105 days. In order to determine the CO_2 evolved from different treatments during the incubation period, the alkali traps were changed periodically on days 2, 5, 8, 14, 21, 28, 35, 49, 63, 77 and 105. Prior to replacing fresh alkali traps and at each sampling time, jars were left open for 1 hour and allowed to equilibrate with the incubation room air in order to maintain aerobic conditions. The moisture of the soil-plant litter mixture and the soil only treatment was checked by periodic weighing of jars during each alkali-changing event and replenished by adding deionised water and maintaining the moisture at field capacity 60 % of their WHC. The alkali traps were then titrated with 0.5 M HCL using an automatic double-point titration (pH 8.3 and 3.7) (TIM 840 Titration Manager). The C mineralization of the added plant litter materials was calculated as the difference in the amount of CO_2-C evolved from the soil-litter and the basal CO_2-C evolved from unamended soil (control) (Wang et al., 2004; Johnson et al., 2007). This calculation assumes any potential priming effects from the addition of plant materials are negligible (Kuzyakov et al., 2000; Wang et al., 2004).

7.4.5 Fitting kinetic models to determine C mineralization kinetics

To quantify the kinetics of C mineralization in above and below ground litter, curves were fitted with a double exponential model using R software. Prior to fitting the model, the amount of % C remaining in the soil from the plant litter relative to the initial C contents was first calculated following equation 1 (Johnson et al., 2007; Sanaullah et al., 2010).

$$\% \ C_{remaining} = [(C_{initial} - CO_2\text{-}C_{evolved})/C_{initial}]\ 100 \qquad \text{Equation 1}$$

Hence % C remaining was calculated as the fraction of the initial C ($C_{initial}$) added in plant litter after subtracting the C evolved as CO_2 (CO_2-$C_{evolved}$).

The double exponential model was fitted on the % C remaining data using equation 2 (Wieder and Lang, 1982; Johnson et al., 2007).

$$C_t = C_a \exp(-k_a t) + (100-C_a)\exp(-k_s t) \qquad \text{Equation 2}$$

Where C_t is the percentage of C remaining at time t, C_a is the initial percentage of C in the active pool, ($100-C_a$) is the initial percentage of C in the slow C pool, k_a (% C d^{-1}) is the active pool decomposition rate constant and k_s (% C d^{-1}) is the slow pool decomposition rate constant. Half–lives were calculated as: HL= 0.693/k where HL is time in days and k corresponds to either k_a or k_s. In order to validate the best curve fit, the residual standard error (RSE) of the curve fitting was calculated.

7.4.6 Statistical analysis

The effects of land use (i.e. cultivation/native woodland/native pasture), litter type (i.e. above and below ground) and their interaction on the initial biochemical composition (i.e. carbon, nitrogen, water soluble carbon, lignin, tannin and cellulose) and percentage of C remaining after 105 days were analysed by ANOVA (Genstat, version 13.3). With three samples per land use at a site the block terms were site and land use within site, except in those initial biochemical composition variables with only one composite sample per land use at a site where the only block term was site. Data were square root transformed, where required, to normalize the residuals. Land use by litter type means are shown in Tables 7.2 and 7.3 with differences determined by LSD at P=0.05 indicated by letters, with means within a column having a letter in common not being significantly different. Decomposition kinetics were estimated by fitting Equation 2 to C mineralization data using the non linear mixed effects (nlme) function in the R software program. Pearson correlation coefficients were used to investigate the relationship between initial biochemical composition of plant litter and C mineralization parameters including, C_a, K_a and K_s.

7.5 Results

7.5.1 Biochemical composition of above and below ground plant litter

Highly significant ($P < 0.001$) land use and litter type interactions were found for the biochemical composition of litter which included; total carbon, total nitrogen, C/N ratio, cellulose, water soluble carbon (Table 7.1). Similarly, significant land use and litter type interactions were found for tannin ($P = 0.034$), tannin/N ratio ($P = 0.005$), lignin/N ratio ($P = 0.007$), (lignin and tannin)/ N ratio ($P = 0.006$) and Cellulose/N ratio ($P = 0.002$) (Table 7.1). These results indicate that the differences in biochemical composition between and within land uses were dependent on litter type. Significant land use main effects were found for lignin and lignin + tannin ($P < 0.001$). The litter type main effect was significant for lignin ($P = 0.023$) and nearly significant for lignin + tannin ($P = 0.054$), with no significant land use and litter type interaction observed (Table 7.1). The results for lignin and lignin + tannin indicate that the three land uses had consistent differences in these properties across litter types.

The biochemical composition of the litter residues obtained from the three land uses is shown in Table 7.2. Total C concentration ranged from 29.5 % in oat roots to 48.8 % in native woodland leaves. The magnitude of total C concentration in above ground litter was in the order of native woodland > native pastures = cultivation ($P < 0.05$) while that of below ground was in the order of native woodland > native pastures > cultivation ($P <0.05$). Above ground litter residues from the three land uses, consistently had larger concentrations of total C than their below ground components. Total N concentration ranged from 0.46 % in oat straw to 1.32 % in native woodland leaves. Strong differences were similarly observed for total N concentration in litter residues within similar land uses with native woodland leaves > native woodland roots, native pastures roots > native pastures standing dead biomass and oat roots > oat straw. The C/N ratio in all the 6 litter residues was > 30, being largest in oat straw (94.4) and smallest in oat roots (34.0) from the same land use. The water soluble carbon concentration ranged from 20 mg/kg in standing dead biomass from native pastures to 99 mg/kg in oat straw.

Native woodland roots had significantly larger tannin concentrations compared with leaves components from the same land use. Cellulose concentration was largest in standing dead biomass (44.3 %) from native pastures and oat roots (42.6 %) and smallest in native woodland leaves (19.1 %). The mean lignin concentration across land uses was in the order of native woodland (11.60 %) > native pastures (5.50 %) > cultivation (2.58 %). Lignin was high in roots than the corresponding above ground litter components in all land uses. The combined concentrations of lignin and tannin were found to be largest in native woodland (11.55 %) followed by native pastures (5.52 %) and cultivation (2.72 %). The lignin/N and (lignin + tannin)/N ratio was found to be significantly higher in roots from native woodland and lowest in roots from cultivation. On the other hand, cellulose/N ratio was highest in above ground litter from native pastures (83) and lowest in above ground litter from native woodland (15).

Table 7.1. F statistics and P values for the analysis of variance for initial biochemical composition of plant litter from native woodland, native pasture and cultivation

	Total C (%)			Total N (%)			C/N ratio			Tannin (%)			Lignin (%)			Cellulose (%)		
	F	d.d.f	*P*	*F*	d.d.f	*P*	*F*	d.d.f	*P*	*F*	d.d.f	*P*	*F*	d.d.f	*P*	*F*	d.d.f	*P*
Land use	245.43	48	<0.001	35.12	46	<0.001	12.23	46	<0.001	0.97	12	0.406	64.34	12	<0.001	65.54	12	<0.001
Litter type	383.64	48	<0.001	4.14	46	0.048	59.88	46	<0.001	2.12	12	0.171	6.74	12	0.023	19.55	12	<0.001
Land use x litter type	30.34	48	<0.001	14.23	46	<0.001	37.56	46	<0.001	4.52	12	0.034	2.71	12	0.107	21.00	12	<0.001

	Water soluble Carbon (mg/kg)			Tannin/N ratio			Lignin/N ratio			Lignin and Tannin (%)			(Lignin + Tannin)/ N ratio			Cellulose/ N ratio		
	F	d.d.f	*P*	*F*	d.d.f	*P*	*F*	d.d.f	*P*	*F*	d.d.f	*P*	*F*	d.d.f	*P*	*F*	d.d.f	*P*
Land use	19.81	48	<0.001	3.98	12	0.047	15.70	10	<0.001	67.99	12	<0.001	14.23	10	0.001	29.72	10	<0.001
Litter type	0.26	48	0.612	0.64	12	0.440	0.01	10	0.942	4.55	12	0.054	0.01	10	0.935	0.05	10	0.831
Land use x litter type	13.73	48	<0.001	8.39	12	0.005	8.49	10	0.007	3.05	12	0.085	8.94	10	0.006	11.78	10	0.002

d.d.f is the estimated denominator degrees of freedom used in the derivation of the F and P statistics

Table 7.2 Mean biochemical composition of litter residues obtained from above and below ground litter types (% dry wt).

Initial Biochemical composition of litter	Litter type	Total C (%)	Total N (%)	Tannin (%)	Cellulose (%)	Water soluble carbon (mg/kg)	Lignin (%)
Native woodland	Senescent leaves	48.8^{a}	1.32^{a}	0.05^{b}	19.1^{d}	38^{cd}	9.66
Native pastures	Standing dead biomass	42.6^{c}	0.54^{cd}	0.10^{ab}	44.3^{a}	20^{d}	4.34
Cultivation	Oat straw	41.7^{c}	0.46^{d}	0.20^{a}	34.6^{bc}	99^{a}	2.72
Native woodland	Roots	44.7^{b}	1.02^{ab}	0.22^{a}	32.4^{c}	76^{b}	13.74
Native pastures	Roots	33.8^{d}	0.77^{bcd}	0.13^{ab}	39.0^{ab}	35^{cd}	6.75
Cultivation	Roots	29.5^{e}	0.87^{bc}	0.14^{ab}	42.6^{a}	56^{bc}	2.44

		C/N Ratio	Tannin/ N ratio	Lignin/ N ratio	(Lignin + tannin)/N ratio	Cellulose/ N ratio	Lignin + Tannin
Native woodland	Senescent leaves	37.9^{c}	0.04^{b}	7.7^{b}	7.8^{b}	15^{c}	9.64
Native pastures	Standing dead biomass	80.3^{a}	0.19^{ab}	8.2^{b}	8.4^{b}	83^{a}	4.45
Cultivation	Oat straw	94.4^{a}	0.44^{a}	6.0^{b}	6.4^{b}	76^{a}	2.91
Native woodland	Roots	49.9^{b}	0.26^{ab}	15.7^{a}	15.9^{b}	38^{b}	13.86
Native pastures	Roots	44.7^{bc}	0.17^{ab}	8.8^{b}	9.0^{b}	51^{b}	6.82
Cultivation	Roots	34.0^{c}	0.16^{b}	2.9^{b}	3.0^{b}	49^{b}	2.54

Values in a column sharing the same letter are not significantly different (P=0.05)

7.5.2 C mineralization and decomposition kinetics in above and below ground plant litter

The amounts of cumulative CO_2-C evolved (µg C g^{-1} soil) from litter residues by the end of the105 d incubation period showed a strong land use and litter type interaction ($P < 0.001$) indicating that evolution of CO_2-C from soil amended with litter residues changed with litter type but that the nature of this change differed between the land uses studied. After 105 days, the cumulative CO_2-C evolution ranged from 2182 µg CO_2-C g^{-1} soil in native woodland roots to 6732 µg CO_2-C g^{-1} soil in oat straw (Table 7.3). Generally, above ground litter residues evolved higher cumulative CO_2-C than their corresponding below ground components from all land uses by the end of the incubation period.

The initial amount of C (from litter residues) added to the soils less the net amount of CO_2-C evolved provided an estimate of C remaining in the soil from the litter residues as a function of time. A first-order double exponential kinetic model (Equation 2) was then fitted on this estimate and used to describe mineralization kinetics. The C mineralization kinetics was well described by the double exponential model which assumes that litter was partitioned into two pools; a rapidly decomposing active pool and a slowly decomposing pool. The measure of fit, the residual standard error (RSE), resulting from fitting the nonlinear least square (NLS) model is presented in Fig 7.1. The percentage of C remaining in litter residues after 105 d incubation showed strong and significant ($P < 0.001$) land use and litter type interactions indicating that the differences between land uses were largely dependent on litter type.

The highest percentage of C remaining after 105 d incubation was found in native woodland roots (75.5 %) and lowest in oat roots (17.7 %) (Table 7.3). Plant litter (both above and below ground) from cultivation and native pastures (above ground litter only) had at least 50 % less C remaining than native woodland roots (Table 7.3). Under cultivation, over 80 % of C was lost from both above and below ground litter within the 105 d incubation compared to litter residues from other land uses. Roots from native woodland and native pastures had 32.2 % and 40.9 % more C remaining than their corresponding above ground components (Table 7.3). Compared to above ground litter components, the loss of C in roots from native woodland and native pasture within 105 d incubation was 24.5 % and 35.6 % respectively while, from the above ground litter residues was 54.7 % and 76.5 %.

The estimated decomposition kinetics of the incubated litter, which include, the active and slowly decomposing litter pools, their respective decomposition rate constants and half lives, are presented in (Table 7.3). The initial fraction of the total C that was in the active C pool in the litter residues ranged from 11.6 % in native woodland roots to 21.1 % in leaves from the same land use. Conversely, the initial fraction of the total C that was in the slow C pool in the litter residues ranged from 78.9 % to 88.4 % in leaves and roots from native woodland respectively. By the end of the 105 d incubation, the active pool in all litter residues from the three land uses had been completely exhausted leaving only the slow pool of the litter residues. The fraction of the initial C that remained in the slow pool at the end of the incubation was found to be in the order: native woodland roots (75.5 %) > native pastures roots (64.4 %) > native woodland leaves (43.3 %) > native pastures standing dead biomass (23.5 %) > oat straw (19.3 %) > oat roots (17.7 %) ($P < 0.001$).

The active pool had k (decomposition rate constant) ranging from 0.072 d^{-1} to 0.805 d^{-1} compared with 0.002 d^{-1} to 0.019 d^{-1} for the slow pool. The above decomposition rates of the active pool represented relatively short half-lives for all litter types from all land uses and ranged from 1 to 10 days. Generally, the active pool of oat roots and straw had the fastest decomposition rates with half-lives of 1 and 2 days respectively (Table 7.3). Half-lives for the slow pool varied widely between litter types within and among land uses and ranged from 37 to 446 days (Table 7.3). Half-lives for the slow pool were highest in native woodland roots (446 days) followed by native pastures roots (268 days). These half-lives were higher than those of their corresponding above ground components which were 109 and 57 days respectively. Shorter half-lives for the slow pool were found for oat roots (37 days) than oat straw (48 days).

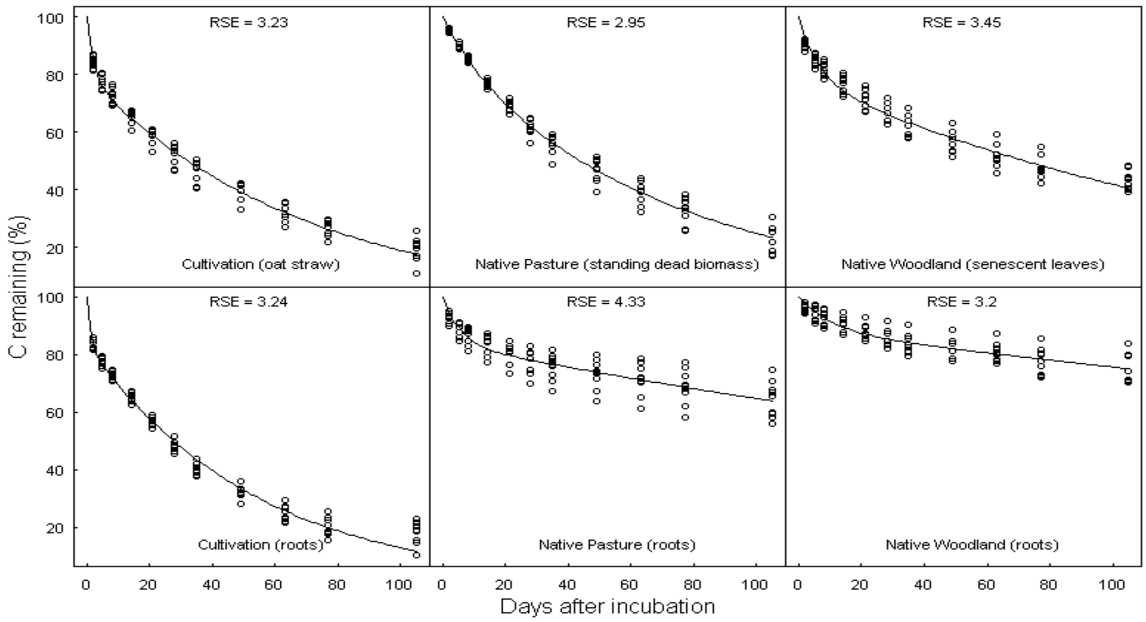

Fig 7.1 Percentage of C remaining as measured (open circles) in above and below ground plant litter obtained from cultivation, native pastures and native woodland. Plant litter was incubated for 105 d at 25°C and 60 % WHC (*n =3*). Data were fitted (solid lines) with Equation 2.

Table 7.3 Percentage of C remaining in above and below ground litter residues after 105 days and decomposition kinetics.

Land use	Litter type	Cumulative CO_2-C evolved (μg C g^{-1} soil) (after 105 days)	% C Remaining after 105 days	C_a	C_s	K_a	K_s	HL_a	HL_s
Native woodland	Senescent leaves	5532^{c}	43.3^{b}	21.1	78.9	0.158	0.006	4	109
Native pasture	Standing dead biomass	6530^{b}	23.5^{c}	15.8	84.2	0.072	0.012	10	57
Cultivation	Oats straw	6732^{ab}	19.3^{d}	20.9	79.1	0.443	0.014	2	48
Native woodland	Roots	2182^{e}	75.5^{a}	11.6	88.4	0.094	0.002	7	446
Native pasture	Roots	2396^{e}	64.4^{a}	16.1	83.9	0.183	0.003	4	268
Cultivation	Roots	4840^{d}	17.7^{d}	16.4	83.6	0.805	0.019	1	37
Lsd $_{(<0\ 05)}$		362	3.8						

C_a is the initial % C in the active C pool

C_s is the initial % C in the slow C (100-Ca) pool

K_a is the active pool decomposition rate constant (d^{-1})

K_s is the slow pool decomposition rate constant (d^{-1})

HL_a and HL_s are half- lives for active and slow pools respectively (d)

7.5.3 Relationship between biochemical composition of above and below ground litter and C mineralization kinetics

The results on the relationship between biochemical composition of litter and C mineralization kinetic parameters are presented in Table 7.4. Across all litter types from all land uses, C_a was not significantly correlated to any of the plant litter biochemical compositional constituents that were measured. Highly significant negative correlations were observed between K_a and lignin (r = -0.58, $P < 0.01$), lignin/N ratio (r = -0.51, $P < 0.01$), lignin + tannin (r = -0.57, $P < 0.01$), (lignin + tannin)/N ratio (r = -0.58, $P < 0.01$) and total carbon (r = -0.59, $P < 0.01$). The K_s was similarly significantly and negatively correlated to total carbon (r = -0.41, $P < 0.01$), total nitrogen (r = -0.33, $P < 0.05$), lignin (r = -0.59, $P < 0.01$), lignin/N ratio (r = -0.65, $P < 0.01$), lignin + tannin (r = -0.59, $P < 0.01$) and (lignin+ tannin)/N ratio (r = -0.64, $P < 0.01$) (Table 7.4).

Table 7. 4 Correlation coefficients (r) between biochemical composition of plant litter and decomposition kinetics

	Mineralization curve parameters		
Biochemical composition of litter residues (54 combinations of 2 litter types x 3 land uses x 3 sites x 3 reps)	Ca	Ka	Ks
Total Carbon	0.08	-0.59^{**}	-0.41^{**}
Total Nitrogen	-0.01	-0.16	-0.33^{*}
C/N ratio	0.10	-0.09	0.18
Water soluble carbon	-0.05	0.22	0.13
Biochemical composition of litter residues (18 combinations of 2 litter types x 3 land uses x 3 sites)			
Tannin	0.07	0.25	-0.10
Tannin/N ratio	0.29	0.29	0.05
Lignin	-0.26	-0.58^{*}	-0.59^{**}
lignin/N ratio	-0.04	-0.51^{*}	-0.65^{**}
Lignin and Tannin	-0.26	-0.57^{*}	-0.59^{**}
(Lignin and Tannin)/N ratio	-0.03	-0.50^{*}	-0.64^{**}
Cellulose	0.07	0.41	-0.05
Cellulose/N ratio	0.39	0.23	-0.01
ADF	-0.07	0.11	-0.44

To be significant |r| has to be greater than:

* 0.2686 (5%), ** 0.3482 (1%) for 54 combinations; and

* 0.4683 (5%), ** 0.5897 (1%) for 18 combinations.

7.6 Discussion

7.6.1 Biochemical composition of above and below ground plant litter

The differences in biochemical composition of plant litters indicate significant quality differences between litter types within and among the land uses studied. Plant litter (leaves and roots) from native woodland was found to contain significantly larger concentrations of lignin and tannin compounds compared with litter types from other land uses which were also significantly different from each other, a result which is consistent with other studies (Rowell et al., 2001; Wang et al., 2004). Generally, forest litter has been reported as having higher lignin and tannin content compared with crop residues or pastures/grass an observation mainly attributed to the presence of woody tissues in forest litter as opposed to large amounts of polysaccharides in crop and grass litter (Lorenz and Lal, 2005). Both lignin and tannin compounds are known to contribute to the recalcitrance of plant litter by physically and chemically protecting the more easily degradable structural polysaccharides (cellulose) from microbial degradation (Mafongoya et al., 1998; Lorenz and Lal, 2005). The straw from oats was found to have significantly larger amounts of WSC, compounds that are of high quality and are primarily responsible for promotion of microbial growth and activity (Smith, 1994).

Our results indicate that litter from cultivation and native pastures may be considered to be relatively high in quality, with the above ground litter being of higher quality than the below ground components in the respective land uses. Although they had high C:N ratios, plant litters from the two land uses were largely characterized by large amounts of easily decomposable carbon fractions such as WSC, cellulose compounds, low lignin:N and (lignin + tannin):N ratio. Conversely, plant litter from native woodland may be considered to be relatively low in quality as it contained large amounts of biochemically recalcitrant compounds such as lignin and tannins, with below ground litter containing larger amounts of these compounds compared with above ground litter. These litter quality differences between plant litter types within and among land uses would further be expected to impact on their subsequent C mineralization.

7.6.2 C mineralization and decomposition kinetics in above and below ground plant litter

The addition of litter to the soil had strong and significant effects on C mineralization during the 105 d incubation. The differences in the amount of residue C remaining in soils amended with different litter types implied that the litter materials used in this study decomposed differently (Kuzyakov et al., 2000; Johnson et al., 2007; Trinsoutrot et al., 2000). The C mineralization kinetics of the litter used in this study was well described by a double exponential model which partitioned litter mineralization into two organic C pools; the active and slow pools, which differed both in size and decomposition rates. The differences observed between the size of the active and slow C pools reflect differences in the amounts of C inputs entering into the respective pools. Over 80 % of the C was in the slow pool, while < 20 % of the C was in the active pool in the above and below ground litter in all land uses. Johnson et al. (2007) found pool sizes of similar magnitude, 25 to 35 % active and 65 to 75 % slow pool from an incubation study carried out for 498 d under controlled conditions similar to ours.

In the short term, there was a 10 fold difference in the decomposition rate of the active C pool among litter types which was reflected in the early differences in decomposability between litter types. Differences in the decomposability of the active C pool during the initial stages of decomposition may be partly explained by the results from chapter 4 of this thesis that showed that the dissolved organic matter (DOM) (which forms part of the active C pool) extracted from these litters differed qualitatively. In the above mentioned study (chapter 4), the relative proportion of aromatic C in DOM extracted from litter was highest in native woodland, followed by native pastures then cultivation. These results should not however be overemphasized as the differences in the half lives among litter types were between 1 and 10 days which implied that the compounds in the active C pool were highly degradable and easily available for rapid microbial decomposition during the initial stages of incubation. In the long term, the major differences in degradability among litter types was determined by the degradability of the slow C pool whose half lives ranged from 37 to 446 days. The decomposition rates of C associated with active and slow pools reported in our study are within the range reported by Wang et al. (2004) for similar litter types and land uses in Australia, and elsewhere by Trinsoutrot et al. (2000) and Sanaullah et al. (2010). Wang et al. (2004) reported decomposition rate constants ranging from 0.042 d^{-1} to 0.24 d^{-1} for the active pool, and 0.00026 d^{-1} to 0.012 d^{-1} for the slow pool, in a laboratory incubation study conducted at 25^{o}C for 356 days. This study was conducted under similar laboratory conditions for a shorter period of 105 d and found ranges of 0.072 d^{-1} to 0.805 d^{-1} and 0.002 d^{-1} to 0.019 d^{-1} for the fast and slow pools respectively.

Our study therefore demonstrated that the major difference in degradability among litter types in the long term was in the degradability of the slow C pool rather than that the active C pool. The litter (above and below ground) obtained from native woodland was associated with slow C pool with greater relative stability compared with litter from native pastures and cultivation. This result is attributed to significantly higher amounts of lignin and tannin compounds (biochemically recalcitrant C compounds) in litter obtained from native woodland than in the other two land uses. The decomposition rate of the slow C pool in litter was strongly and negatively correlated with the initial lignin:N ratio of plant litter suggesting that the interaction between these two litter quality variables had important controls over litter decomposition (Melillo et al. 1982; Taylor et al. 1989; Talbot et al. 2012).

The mechanisms by which lignin and N interact to influence decomposition of litter were not investigated in this study however several explanations have been reported including; a) lignin may form cross-linkages to the more labile N thereby chemically protecting N from hydrolysis during litter decay (Talbot et al. 2012), and b) lignin may physically protect the more labile cell wall components (e.g. structural polysaccharides and N) from microbial attack during litter decomposition when lignin is deposited in cell walls within the hemicellulose-protein matrices (Boerjan et al. 2003). In this study, it is possible that differences among litter types in the amounts of initial lignin contents may have influenced the mechanism by which lignin:N interact to control decomposition of the slow C pool in litter. In this respect, native woodland litter (leaf and root) which had larger amounts of initial lignin contents was associated with larger slow C pools in litter which were relatively more stable compared with the other litter types. More slowly decomposing plant residues (in this case native woodland litter) are more likely to enter the SOM fraction and become stabilized as humified SOM which may persist in soils for significant periods of time therefore contributing significantly to SOC stocks (Schlesinger 1990). The mechanism by which lignin and N interact to influence decomposability of litter will however require further testing.

7.7 Conclusions

Our laboratory incubation study showed that in the long term, the differences in decomposability between litter types was determined by differences in the decomposition rate of the slow C pool rather than that of the active C pool. The decomposition rate of the slow C pool in litter was strongly and negatively correlated with the initial lignin:N ratio of plant litter suggesting that the interaction between these two litter quality variables had important controls over litter decomposition. Compared with other litter types, above and below ground litter from native woodland had higher initial lignin:N ratio and were associated with more stable slow C pools with longer half lives of 109 and 446 days respectively. The more slowly decomposing litter from native woodland is more likely to persist in soils for longer and may enter the soil organic matter (SOM) fraction and contribute to increased C storage down the soil profiles compared with the other two land uses. The mechanism by which lignin and N interact to influence decomposability of litter will however require further testing

Results from this study may be used to partly explain results on larger SOC stocks found under native woodland especially in surface (0-20 cm) soil layers where land use differences in SOC stocks were found (chapter 3 of this thesis). The surface soils under native woodland soils were found to contain 11 and 9 t/ha more SOC stocks than native pastures and cultivation respectively. A supply of low quality litter with slowly decomposing slow C pools definitely explains accumulation of large quantities of SOC in these surface soils. The low quality litter (above and below ground) under native woodland may also explain the large C:N ratios associated with the particulate organic matter (POM) size fraction in the entire soil profile (0-80 cm) (chapter 5 of this thesis). POM is closely related to plant litter inputs and the nature of low quality litter both from above and below ground sources explains the wider C:N ratios throughout the soil profiles under native woodland. As a result, our study showed that the C associated with POM under native woodland soils decreased with depth at a significantly slower rate than under cultivation and native pasture which were similar to each other. Results from this study may also be linked to those of SOC mineralization in chapter 6 which showed that the slow C pool did not evolve much during the 60 week laboratory incubation suggesting that this pool was relatively stable in these soils. While the mechanisms of stabilization of this pool was not understood, the intrinsic recalcitrance (larger initial lignin contents) of litter input under native woodland may partly explain the significantly larger slow SOC pools found in the surface (0-10 cm) soil layers compared with other two land uses. At this depth (0-10 cm) the slow SOC pool under native woodland were 25,251 µg/g soil compared with 18,428 µg/g soil under cultivation and 18,385 µg/g soil under native pastures (Chapter 6).

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	Author's Name (please print clearly)	**% of contribution**
Candidate	Christine Walela	75
Other Authors	Heiko Daniel	3
	Brian Wilson	3
	Peter Lockwood	6
	Annette Cowie	3
	Steven Harden	5

Name of Candidate: Christine Kainyu Walela

Name/title of Principal Supervisor: Prof. Heiko Daniel

[redacted]

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Name/title of Principal Supervisor: Prof. Heiko Daniel

[redacted]

______________ 13/05/2013

Candidate Date

[redacted]

_______________ 13/05/2013

Principal Supervisor Date

Chapter 8. Litter quality effects on nitrogen mineralization and immobilization: A laboratory study

Christine Walela[1], Heiko Daniel[1], Brian Wilson[1, 2], Peter Lockwood[1], Annette Cowie[1, 3], Steven Harden[4]
[1]School of Environmental and Rural Sciences, Agronomy and Soil Science, University of New England, NSW 2351, Australia
[2]NSW Office of Environment and Heritage, PO Box 0221, Armidale, NSW 2351, Australia.
[3]Rural Climate Solutions, University of New England, NSW 2351, Australia/ Department of Primary Industries, NSW Australia.
[4]NSW Department of Primary Industries, Tamworth Agricultural Institute, NSW, Australia

8.1 Abstract

The availability of inorganic nitrogen (N) is known to affect the stability of soil organic carbon (SOC) pools and therefore carbon sequestration in the long term. A laboratory incubation experiment was conducted to study the N mineralization (NO_3-N, NH_4-N and total inorganic N) dynamics of above and below ground litter obtained from three land uses namely, native woodland (*Eucalyptus species*), native pastures (*native grasses*) and cultivation (*forage oats*). Above ground litter consisted of senescent leaves, standing dead biomass and oat straw from the above respective land uses, while the below ground litter were the respective root components. The relationship between the initial biochemical composition of the plant litter and N mineralization over the incubation period was assessed. N mineralization was strongly influenced by litter type ($P < 0.05$) and depended on the initial biochemical composition of litter. Significant relationships were observed between the initial substrate composition namely; C:N ratio, % lignin and % water soluble carbon (WSC) and inorganic N release from litter in the early stages of incubation (day 4 to 35). During the early stages of litter decomposition, the biochemically recalcitrant lignin, influenced the susceptibility of substrates to microbial attack and thereafter a demand for N by microbial decomposers. However, the subsequent release of N from substrates however, depended on the C:N ratio. Differences in the magnitude of initial N immobilization among substrates of similar lability were related to differences in the initial decomposability of substrates and were explained by differences in the relative amounts of easily degradable WSC. Compared with above ground litter, roots which had larger amounts of biochemically recalcitrant lignin, mineralized slowly resulting in a low N demand by microbial decomposers. Consequently, roots were associated with the larger amounts of potentially mineralizable N at the end of the 105 d incubation than their above ground components. This availability of N has important implications for C sequestration in that, N has the ability to interact with lignin thereby forming stabilized C compounds that are less easily degradable by microbial decomposers leading to more stable C pools especially in subsoils.

Key words: N mineralization, N immobilization, soil organic carbon, decomposition, plant litter quality.

8.2 Introduction

The availability of adequate amounts of inorganic nitrogen (N) from decomposing plant litter is important for the formation of stabilized soil organic carbon (SOC) compounds that are less easily degraded by microbial decomposers and may in turn foster an increase in carbon (C) sequestration (Hagedorn et al. 2003; Gill et al. 2006; Christopher and Lal 2007; Berg and McClaugherty 2008). As plant residues decompose, organic N is released in mineral forms (ammonium and nitrate) into the soil, at the same time; the more labile C substrates are consumed by microbes, leaving the more recalcitrant C compounds (e.g. lignin) (Christopher and Lal 2007; Berg and McClaugherty 2008). A portion of the released inorganic N reacts with lignin creating more recalcitrant aromatic C compounds (Berg and Matzner 1997; Berg 2000; Berg and McClaugherty 2008, Knicker 2011). Whereas the main paradigm of increasing N availability in the soil has been to increase nutrient cycling therefore enhancing biomass production, there is now increasing awareness of the importance of supplying N to enable sequestration of SOC (Christopher and Lal 2007; Knicker 2011). With the growing interest to identify land uses with potential to sequester significant amounts of C in the soil, it is necessary to also understand the factors affecting availability of N due to its considerable influence on the recalcitrant soil organic matter (SOM) pool (Knicker 2011). The release of residue N has been shown to be mainly influenced by the initial biochemical composition (quality) of litter (Jensen et al. 2005), however, its availability in the soil thereafter is also determined by the competition between plants, bacteria and fungi (microbial immobilization) (Knicker 2011). Understanding the N mineralization and immobilization patterns of decomposing plant litter as influenced by its biochemical composition will enhance our understanding of the factors controlling the availability of N which will have important implications for C sequestration in the long-term.

The C:N ratio of residues is generally suggested as an important factor controlling the occurrence of net N mineralization or net N immobilization (Trinsoutrot et al. 2000; Cabrera et al. 2005). Some studies however, have found variation in extent of mineralization of organic residues with similar C:N ratios (Palm and Sanchez 1991). In most of these studies N mineralization has been shown to be poorly correlated with C:N ratio showing that C:N ratio alone cannot explain all the differences in N mineralization (Palm and Sanchez 1991; Constantinides and Fownes 1994; Neve and Hoffman 1996; Nakhone and Tabatabai 2008). Consequently, it is now believed that compositional variables play an important role in N mineralization and it is necessary therefore to identify specific biochemical constituents that may influence this process when considering different litter types.

Land use change alters the amount and quality (biochemical composition) of plant litter inputs and therefore N supply and availability (Knicker 2011). Moreover, the initial biochemical composition of different components of the same plant has been shown to differ thus influencing the N mineralization and availability (Abiven et al. 2005). In this respect, Abiven et al. (2005) suggested the importance of separately considering different plant parts when assessing the dynamics of plant residue decomposition and N mineralization.

This study is a component of a research project that seeks to understand the C sequestration potential in soils under major land uses, namely native woodland, native pastures and cultivation in northern NSW Australia. Given the close interrelationship between the C and N cycles, Knicker (2011) commented that a realistic evaluation of the potential of soils as a C sink requires better understanding of the impact of soil organic nitrogen (SON) on SOM formation. This study aims to contribute to knowledge of the N release pattern and availability from above and below ground litter inputs that are important sources of SOC in these land uses. Such information is lacking for the land uses studied.

Our aim was to a) study the N mineralization (NO_3-N, NH_4-N and total inorganic N) dynamics of above and below ground litter obtained from native woodland, native pastures and cultivation; and b) assess the relationship between the initial biochemical composition of the plant litter and N mineralization over the incubation period.

8.3 Materials and methods

8.3.1 Soil characteristics and plant litter

This study was conducted near the township of Uralla in the Northern Tablelands of New South Wales, Australia. Uralla lies at 30.64°S, 151.49°E at an altitude of 1,012 m and receives a mean annual rainfall of 807 mm and has a mean maximum and minimum temperature of 26.4°C and 12.5°C respectively (1901-2012; Bureau of Meteorology 2012). The soils of the area are largely derived from Permian granite and are classified as yellow Chromosols (Isbell 2002) equivalent to Alfisols (Soil Survey Staff 1999). The soil used for the incubations was topsoil (0-15 cm) collected from a site which had been predominantly cultivated with forage oats for > 20 years. The chemical and physical properties of the soil had been determined from an earlier study (chapter 3) as containing: 1.2 % organic C, 2.84 mg N kg^{-1} soil mineral N, pH (water) 5.3, 84 % sand, 10.4 % silt, 5.6 % clay and a bulk density of 1.17 g/cm^3.

Site clusters containing three land uses namely: native pastures, native woodland and cultivation were randomly selected at three separate locations. Above and below ground samples were collected from three randomly selected plots within each land use, giving a total number of 54 (3 sites x 3 land uses x 2 litter types x 3 reps) samples. Native woodland consisted of a mixture of *Eucalyptus* species including Yellow box (*E. melliodora*) and Blakely's Red Gum (*E. blakelyi*) while native pastures were dominated by native grasses such as *Microlaena stipoides.* The paddocks under cultivation had been predominantly cropped with forage oats (*Avena sativa*) for > 20 years. Above ground litter components comprised senescent leaves, standing dead biomass and oats straw sampled from native woodland, native pastures and cultivation respectively. Roots were sampled as the below ground litter components from the above respective land uses. At the time of collecting litter from the field, the paddocks under cultivation were fallow and hence, oats were grown in pots containing the study site soil in a glasshouse until physiological maturity, when above and below ground litter was harvested. The plant litters were dried at 60°C and then ground to pass a 4 mm sieve. The initial biochemical composition of each litter type is given in Table

8.1. Methods for the determination of the biochemical composition of litter are reported in chapter 7 of this thesis.

8.3.2 N mineralization – laboratory study

N mineralization was studied through laboratory incubation at constant temperature (25°C) and moisture (60 % of water holding capacity, WHC) for 105 d. Briefly, 50 g of field moist-sieved (< 4 mm) soil were adjusted to 60 % of their WHC and pre-incubated in 2 l glass jars for seven days. Mineral N content in the soil was adjusted to 80 mg N kg^{-1} dry soil with KNO_3 to allow decomposition without N limitation (Abiven and Recous 2007). Soils were again moistened to 60 % of WHC and plant litter (1.0 g, 4 mm size) was incorporated and mixed thoroughly. Three replicates were used for each litter type. Soil without addition of plant material was used as control and was incubated in triplicate. A set of three empty jars (blanks) were also included. The CO_2 produced by the plant litter-amended soils and control soils was trapped in 20 ml 1M NaOH in 70-ml specimen jars which were placed at the bottom of the 2 l jars. A small vial containing 10 ml of deionised water was also placed at the bottom of each jar to maintain humid conditions. The soil mineral N (NH_4 - N and NO_3 – N) was quantified on six occasions during the incubation (after 4, 10, 21, 35, 63 and 105 days). Briefly, 2 g of soil were randomly sampled from each litter-amended soil and control into centrifuge tubes and the soil mineral N was extracted with 20 ml of 2M KCL solution, by tumbling end over end (15 rpm) for one hour. The samples were immediately filtered into vials using a Whatman no. 42 paper. Samples were immediately analysed for soil mineral N on the Skalar auto analyser. Alkali traps were removed from jars and disposed at each sampling occasion. Prior to replacing fresh alkali traps the jars were left open for 1 hour in order to maintain aerobic conditions. The moisture content in amended and control soil was checked by periodic weighing of jars during each sampling event and adjusted to 60 % of WHC using deionised water.

Table 8.1 Initial biochemical composition of above and below ground litter obtained from native woodland, native pastures and cultivation

Land use	Litter type	Total C (%)	Total N (%)	Tannin *(%)*	Cellulose (%)	Water Soluble C (mg/kg)	Lignin (%)
Native woodland	Senescent leaves	48.8^{a}	1.32^{a}	0.05^{b}	19.1^{d}	38^{cd}	9.66
Native pastures	Standing dead biomass	42.6^{c}	0.54^{cd}	0.10^{ab}	44.3^{a}	20^{d}	4.34
Cultivation	Oats straw	41.7^{c}	0.46^{d}	0.20^{a}	34.6^{bc}	99^{a}	2.72
Native woodland	Roots	44.7^{b}	1.02^{ab}	0.22^{a}	32.4^{c}	76^{b}	13.74
Native pastures	Roots	33.8^{d}	0.77^{bcd}	0.13^{ab}	39.0^{ab}	35^{cd}	6.75
Cultivation	Roots	29.5^{e}	0.87^{bc}	0.14^{ab}	42.6^{a}	56^{bc}	2.44
		C/N Ratio	Tannin/ N ratio	Lignin/ N ratio	(Lignin + tannin)/N ratio	Cellulose/ N ratio	Lignin + Tannin
Native woodland	Senescent leaves	37.9^{a}	0.04^{b}	7.7^{b}	7.8^{b}	15^{c}	9.64
Native pastures	Standing dead biomass	80.3^{a}	0.19^{ab}	8.2^{b}	8.4^{b}	83^{a}	4.45
Cultivation	Oats straw	94.4^{a}	0.44^{a}	6.0^{b}	6.4^{b}	76^{a}	2.91
Native woodland	Roots	49.9^{b}	0.26^{ab}	15.7^{a}	15.9^{b}	38^{b}	13.86
Native pastures	Roots	44.7^{bc}	0.17^{ab}	8.8^{b}	9.0^{b}	51^{b}	6.82
Cultivation	Roots	34.0^{c}	0.16^{b}	2.9^{b}	3.0^{b}	49^{b}	2.54

Values in a column sharing the same letter are not significantly different (P=0.05)

8.3.3 Statistical analyses

N mineralization (NH_4-N, NO_3-N and total inorganic N) was determined for each sampling day and expressed as µg N g^{-1} soil. Concentrations of NH_4-N, NO_3-N and total inorganic N were plotted against time to show temporal pattern of N mineralization. Lines through the mean of the data for each sample day were added to aid visualisation of the trends. Potentially mineralizable N on the last day of incubation (day 105) was determined and an analysis of variance (ANOVA) was applied to determine differences between the litter types. The blocking terms for this analyses were site and land use within site, while the treatment terms were land use (native woodland/native pastures/cultivation), litter type (above or below ground) and their interaction (land use x litter type). Correlations between the initial biochemical composition of litter and NH_4-N, NO_3-N and total inorganic N were calculated for each sampling day.

8.4 Results

8.4.1 Changes in the amounts of total inorganic N in amended and unamended soils during 105d incubation

The changes in the amounts of total inorganic N, NO_3-N and NH_4-N in the amended and unamended (control) soils over the 105 d incubation are shown in Fig 8.1, Fig 8.2 and Fig 8.3 respectively. Compared to all the amended soils, the unamended (control) soil showed a steady net mineralization throughout the incubation, increasing gently from the initial 80 µg N g^{-1} soil to134.68 µg N g^{-1} soil at day 105. The soils amended with above ground litter components showed three stages of net N mineralization and immobilization: (a) an initial net N immobilization; (b) an interval when N mineralization approximately balanced immobilization; and (c) net N mineralization during the latter days of incubation. The soils amended with oat straw induced the largest amount of N immobilization at the start of incubation (day 4) (- 60.40 µg N g^{-1} soil) compared with soils amended with leaves from native woodland (-38.44 µg N g^{-1} soil) and biomass from native pastures (- 4.61 µg N g-1 soil) which also differed strongly between each other ($P < 0.05$). The maximum net N immobilization was reached at days 10 (-82.04µg N g^{-1} soil), 35 (-59.53 µg N g^{-1} soil), and 63 (-76.36 µg N g^{-1} soil) in soils amended with oat straw, leaves from native woodland and biomass from native pastures respectively. The interval during which only small changes in the amounts of total inorganic N occurred was from days 10 to 63, 21 to 35 and 35 to 63 in soils amended with oat straw, leaves from native woodland and biomass from native pastures respectively. Net N mineralization did not begin until day 35 in soils amended with leaves from native woodland and until day 63 in soils amended with oat straw and biomass from native pastures. Generally, net N mineralization during these latter days of incubation occurred at rates below that of the control soils.

The incorporation into the soil of oat roots caused a net N immobilization (-27.77 µg N g^{-1} soil) at the beginning of the incubation (day 4) followed by a net N mineralization which occurred less that of the control soil until the end of the incubation. Soils amended with native pasture roots showed net N mineralization right from the beginning of the incubation till the end, with rates almost similar to the unamended soils during the entire incubation period. Conversely, N mineralization in excess of the control was found in soils amended with native woodland roots at days 4 and 10 only with subsequent N mineralization shown to occur below that of the control soil. The potential N mineralization at day 105 was strongly and significantly ($P < 0.001$) influenced by litter type, with soils amended with below ground litter found to have significantly larger amounts of total inorganic N compared with their above ground litter components. The magnitude of this differences as shown by the means of total inorganic N was in the order of 114.6 μg N g^{-1} soil (native pastures roots) = 109.0 μg N g^{-1} soil (native woodland roots) = 94.2 μg N g^{-1} soil (oat roots) > 49.9 μg N g^{-1} soil (native woodland leaves) = 29.9 μg N g^{-1} soil (native pasture biomass) = 27.7 μg N g^{-1} soil (oat straw). Generally, by the end of the 105 d incubation, the amounts of total inorganic N in soils amended with above ground litter remained below the initial 80 μg N g^{-1} soil (negative net N mineralization), while inorganic N increased in soils amended with below ground litter (positive net N mineralization) (Fig 8.1).

8.4.2 Changes in the amounts of NO_3-N and NH_4-N in amended and unamended soils during 105 d incubation

The amounts of NH_4-N measured in the soil during the entire incubation period were low throughout the whole incubation period and the largest proportion of inorganic N was in the form of NO_3-N. Hence, the changes in the amounts of NO_3-N in soils amended with above and below ground litter components followed almost a similar pattern as that of total inorganic N in soils (Fig 8.2). A net N immobilization in NH_4-N was observed in soils amended with above ground litter from day 4 to 35, after which NH_4-N mineralization was observed in trace amounts. Incorporation in soil of below ground litter components caused a net N mineralization as NH_4-N at the beginning of the incubation (day 4) with soils amended with roots from native woodland showing the largest increase (+ 18.20 μg N g^{-1} soil). The amounts of NH_4-N however declined showing only trace amounts over the subsequent days of incubation (Fig 8.3). The unamended (control) soil showed net N mineralization in NO_3-N and NH_4-N throughout the entire incubation period.

8.4.3 Correlations between net N mineralization and initial biochemical composition of above and below ground litter

Relationships between biochemical components of litter residues and net N mineralization ranged from strong negative correlation (r = -0.64) to strong positive correlation (r = 0.64) (Table 8.2). The net effect of litter amendments on total inorganic N and NO_3-N dynamics was poorly to strongly negatively correlated to C:N during the entire incubation period (r values ranging between -0.29 and -0.64) (Table 8.2). The total mineral N and NO_3-N mineralized during early incubation (i.e. day 4) were moderately negatively correlated with water soluble carbon (WSC) (r = -0.41 and -0.49 at $P < 0.001$). The strongest correlation (positive) for total inorganic N, NO_3-N and NH_4-N mineralized was with % lignin for different days (Table 8.2). These correlations were found at days 4, 10, 21 and 35 for total inorganic N, days 10, 21, and 35 for NO_3-N and days 4 and 10 for NH_4-N mineralized (Table 8.2).

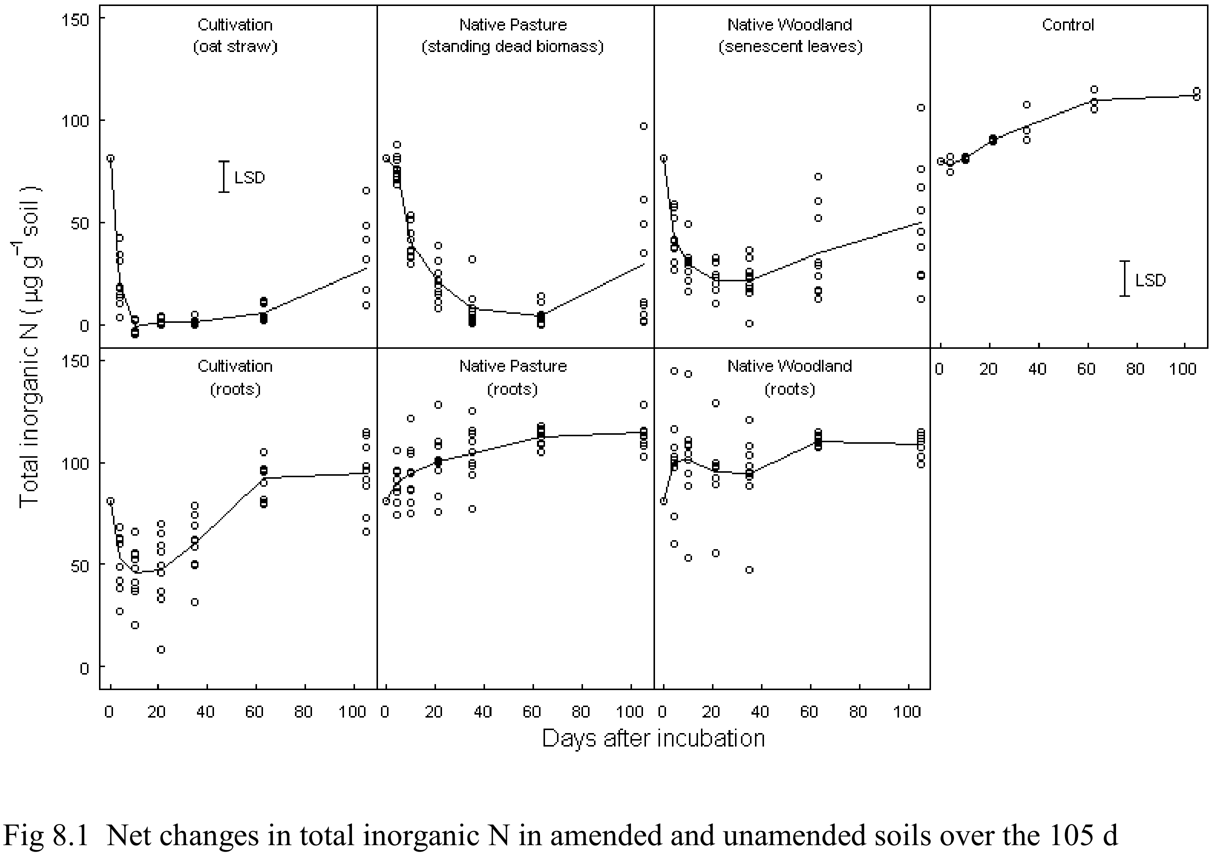

Fig 8.1 Net changes in total inorganic N in amended and unamended soils over the 105 d incubation. Open circles represent total inorganic N concentration for each sampling day, n = 9.

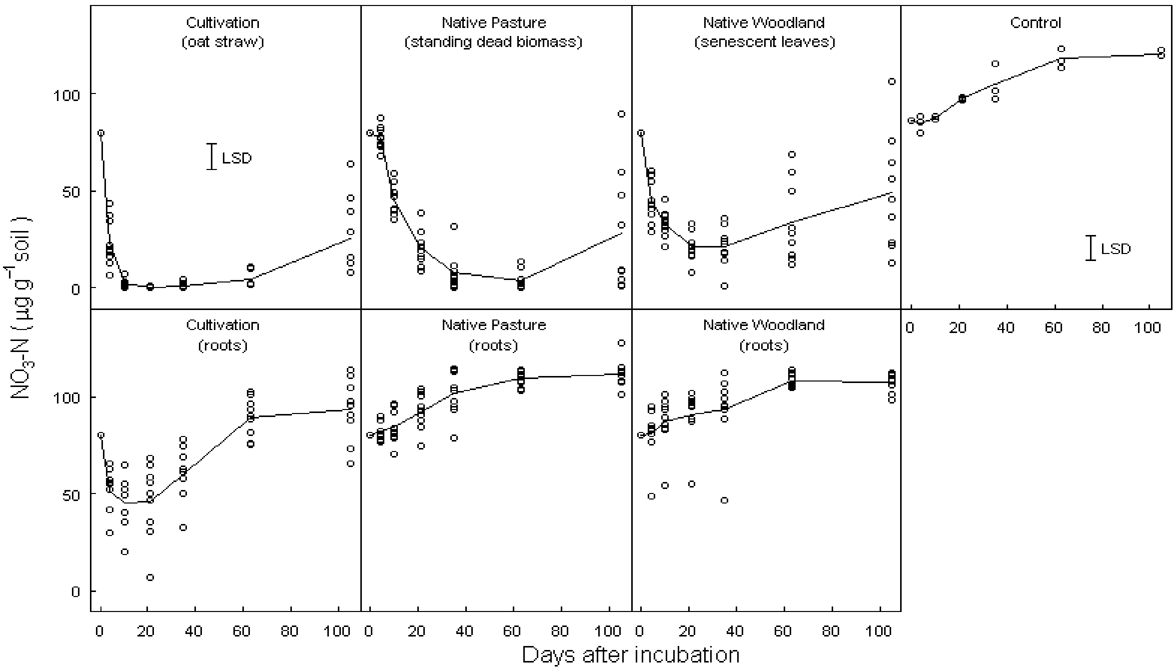

Fig 8. 2 Net changes in NO_3-N in amended and unamended soils over the 105 d incubation. Open circles represent NO_3-N concentration for each sampling day, *n* = 9.

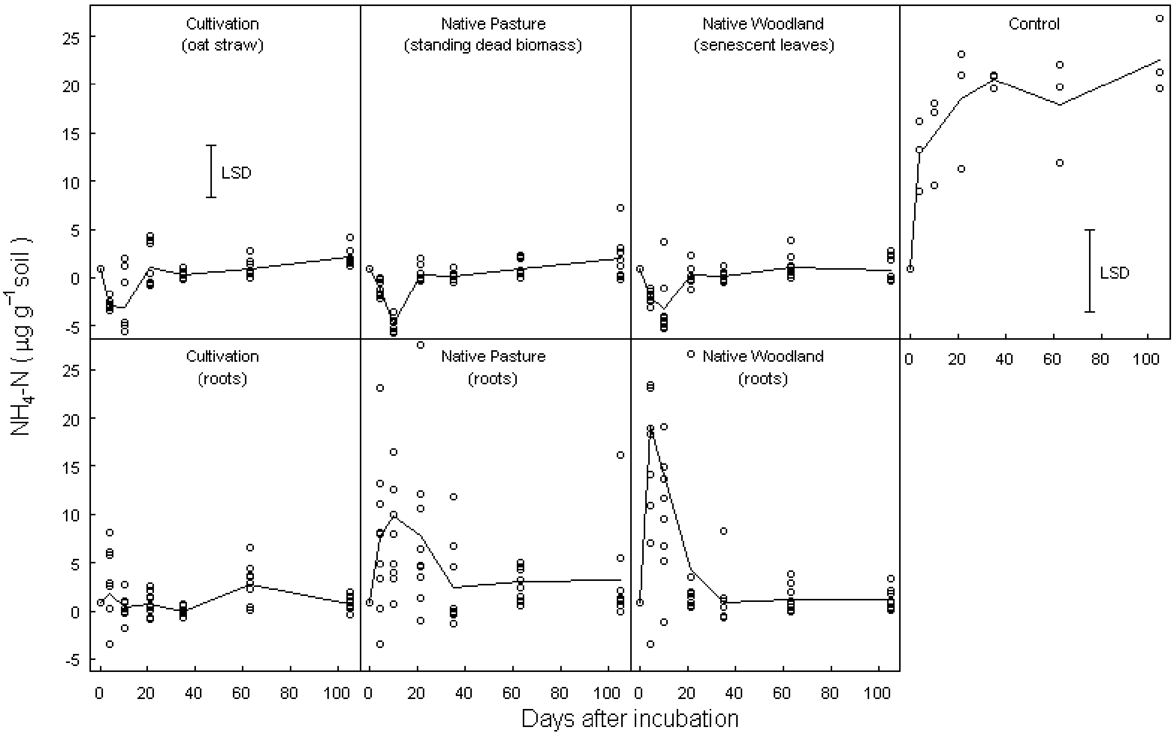

Fig 8. 3 Net changes in NH_4-N in amended and unamended soils over the 105d incubation. Open circles represent NH_4-N concentration for each sampling day, *n* = 9.

8.4.4 *Relationship between N mineralization and intital biochemical composition of litter*

Table 8.2 Correlation coefficients on N mineralization (NO_3-N, NH_4-N and total inorganic N) at each sampling day with the initial biochemical composition of above and below ground litter

Biochemical composition	Total inorganic N (µg g^{-1} soil)						NO_3-N (µg g^{-1} soil)						NH_4-N (µg g^{-1} soil)					
	D4	D10	D21	D35	D63	D105	D4	D10	D21	D35	D63	D105	D4	D10	D21	D35	D63	D105
% C	-0.09	-0.19	-0.30	-0.40	-0.48	-0.45	-0.12	-0 20	-0.31	-0.40	-0.47	-0.45	0.01	-0.09	-0.1	-0.05	-0.51*	-0.06
% N	0.22	0.31	0.28	0.32	0.34	0.33	0.12	0.28	0.27	0.31	0.35	0.34	0 36	0.31	0.18	0.18	0.09	-0.15
C:Nrat	-0.33*	-0.48**	-0.49**	-0.56**	-0.64**	-0.62**	-0.29*	-0.49**	-0.49**	-0 57**	-0.63**	-0.63**	-0.30*	-0.32*	-0.19	-0.18	-0.34*	0.11
WSC (mg/kg)	-0.41**	-0.27*	-0.13	-0.08	0.03	0.03	-0.49**	-0.32*	-0.13	-0.07	0.03	0.03	-0.08	-0.05	-0.12	-0.13	-0.11	-0.1
% Tannin	0.07	0.15	0.23	0.25	0.23	0.22	-0.05	0.07	0.21	0.24	0.23	0.21	0 34	0.42	0.33	0.34	0.08	0.23
T:Nrat	-0.27	-0 22	-0.14	-0.14	-0.17	-0.16	-0.34	-0 29	-0.17	-0.15	-0.17	-0.17	-0.01	0.07	0.11	0.06	-0.1	0.21
% Lignin	0.57*	0.64**	0.55*	0.48*	0.40	0.36	0.44	0.61**	0.55*	0.48*	0.40	0.36	0.67**	0.64**	0.37	0.24	-0.15	0.01
L:Nrat	0.46	0.51	0.44	0.34	0.24	0.22	0.39	0.50	0.45	0.35	0.25	0.22	0.47	0.47	0.22	0.05	-0.26	0.12
% LT	0.57	0.64	0.56	0.48	0.40	0.36	0.44	0.61	0.56	0.48	0.41	0.36	0.68	0.65	0.38	0.25	-0.14	0.02
L:Trat	0.45	0.50	0.44	0.33	0.23	0.21	0.38	0.49	0.44	0.34	0.24	0.21	0.47	0.47	0.22	0.05	-0.26	0.12
% Cellulose	0.23	0.13	0.13	0.13	0.09	0.09	0.31	0.16	0.14	0.13	0.09	0.09	-0.02	0.00	0.04	-0.01	0 31	0.15
CE:Nrat	-0.14	-0 31	-0.32	-0 37	-0.44	-0.41	-0.07	-0.3	-0.32	-0 37	-0.44	-0.42	-0.27	-0.3	-0.16	-0.17	-0.14	0.2

To be significant |r| has to be greater than:

* 0.2686 (5%), ** 0.3482 (1%) for % (Carbon) C %, (Carbon: nitrogen) C: N and Water Soluble Carbon (WSC); and

* 0.4683 (5%), ** 0.5897 (1%) for the rest of litter quality variables (i.e. Tannin (T), Lignin (L), Cellulose (C).

NB: Only relationships that had high correlations have been shown by asterisk

8.5 Discussion

8.5.1 Net N mineralization and relationship with initial biochemical composition of above and below ground litter

The N mineralization patterns exhibited in the amended and unamended soils in this study are consistent with those commonly observed by others (e.g. Palm and Sanchez 1991, Constantinides and Fownes 1994; Recous et al. 1995; Hadas et al. 2004; Jensen et al. 2005; Abiven et al. 2007; Sall et al. 2007;Yamasaki et al. 2011). N mineralization was strongly influenced by litter type ($P < 0.05$) with significant relationships identified between initial % lignin, % WSC and C:N ratio in the early days of incubation (day 4 to 35). The above ground litter residues, which contained less biochemically recalcitrant lignin, decomposed faster and a net N immobilization was observed due to microbial demand for N exceeding that which could be supplied by the decomposing litter, as reflected in the high C:N ratios (Mtambanengwe and Kirchmann 1995; Recous et al. 1995; Trinsoutrot et al. 2000; Abiven et al. 2005; Manzoni et al. 2008; Potthast et al. 2010; Yamasaki et al. 2011).

The differences in the magnitude of initial N immobilization (at day 4) in soils amended with above ground litter may be explained by differences in the amounts of water soluble carbon (a source of readily decomposable C) among litter types. Compared to leaves from native woodland and biomass from native pastures, oat straw had larger quantities of WSC which may have caused a flush of microbial growth during the early stages of incubation (day 4 and 10) leading to rapid decomposition and subsequent N mineralization, however, a high N demand may have led to microbial immobilization of the N released (Recous et al. 1995; Trinsoutrot et al. 2000). Differences in the magnitude of initial N immobilization among above ground litter types suggest differences in the initial decomposability among substrates. This is consistent with our findings from C mineralization studies (chapter 7 of this thesis), which reported similar differences in decomposition rates of the active C pool (which generally comprises easily degradable C compounds such as WSC) of these same litters. Hence, decomposition rates of the active C pool were found to be 0.443 d^{-1} (2 days half life) for oat straw, 0.158 d^{-1} (4 days half life) for leaves from native woodland and 0.072 d^{-1}(10 days half life) for biomass from native pastures.

The subsequent slowing down in N mineralization following initial N immobilization is associated with slow decomposition of substrates which may have resulted from a continued deficiency in substrate N. The net N mineralization observed during the latter stages of incubation may be attributed to an ongoing mineralization of the native SOM as was observed in the control soil.

Below ground litter substrates, which contained larger amounts of biochemically recalcitrant lignin concentrations compared with above ground litter components showed slow decomposition demonstrating that despite having high C:N (similar to the above ground components) the demand for N in the former was lower than in the latter. This was displayed by the low initial N immobilization in soils amended with oat roots. The initial N mineralization observed in soils amended with native pasture roots was attributed to mineralization of native SOM indicating that the supply of N was sufficient to supply the microbial demand. A positive net N mineralization was observed in soils amended with native woodland roots at day 4 and 10 and is linked to slow decomposition demonstrating a low N demand when substrates with large amounts of recalcitrant C compounds such as lignin are decomposed (Recous et al. 1995).

Compared with above ground litter components, roots were associated with the largest amounts of potentially mineralizable N at the end of the 105 d incubation. The availability of N in the soil has important implications for subsequent C sequestration as N influences the formation of stabilized SOC compounds through its interaction with lignin (Berg and McClaugherty 2008; Knicker 2011). Further, N plays an important role in inhibiting formation of lignolytic enzymes thereby suppressing degradation of lignin in soils (Berg and Matzner 1997; Berg and McClaugherty 2008).

A positive net mineralization was observed in the amounts of NH_4-N in the soils amended with root litter of pasture and native woodland; however this was followed by a quick decline. The loss of NH_4-N in our study was linked more to nitrification than microbial immobilization as evidenced by a gentle rise in NO_3-N as NH_4-N declined. The positive net N mineralization observed in the unamended soils indicated that the mineralization of N from the native SOM was sufficient to supply the microbial needs. The slow decomposition of the unamended soils may further be attributed to the nature of SOM which is considered to be highly humified (C:N ratio of 15, Chapter 3) compared to the amended soils.

8.6 Conclusions

N mineralization dynamics were strongly influenced by litter type with significant relationships identified between the initial C:N ratio, % lignin and % WSC. The litter substrates used in this study (both above and below ground litter) were considered as having insufficient N to meet microbial needs as shown by the high C:N ratios (> 30). In the early stages of litter decomposition, the biochemically recalcitrant lignin, influenced the susceptibility substrates to microbial attack and thereafter a demand for N by microbial decomposers. However, the subsequent release of N from substrates however, depended on the C:N ratio. Differences in the magnitude of initial N immobilization among above ground litter types were related to differences in the initial decomposability of substrates which was explained by differences in the relative amounts of easily degradable WSC. This result was well related to the results of C mineralization studies in chapter 6 of this thesis which reported a 5 fold difference in the early decomposability of the active C pool between above ground litter types. Our results showed that compared with their above ground components, root litter which had large amounts of recalcitrant lignin, decomposed slowly as a result of low N demand by microbial decomposers. Compared with above ground litter components, roots were associated with larger amounts of potentially mineralizable N which has important implications for long term C sequestration in that, N has the ability to interact with lignin thereby forming stabilized C compounds that are less easily degradable by microbial decomposers leading to more stable C pools in especially in subsoils.

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	Author's Name (please print clearly)	**% of contribution**
Candidate	Christine Walela	80
Other Authors	Heiko Daniel	3
	Brian Wilson	3
	Peter Lockwood	6
	Annette Cowie	3
	Steven Harden	5

Name of Candidate: Christine Kainyu Walela

Name/title of Principal Supervisor: Prof. Heiko Daniel

[redacted]

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Chapter 8: Nitrogen mineralization and immobilization from above and below ground plant litter from native woodland, native pastures and cultivation.	100 % Walela Christine with contributions from supervisors, statistical advice and technical advice

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Name/title of Principal Supervisor: Prof. Heiko Daniel

[redacted]

______________ 13/05/2013

Candidate Date

[redacted]

_______________ 13/05/2013

Principal Supervisor Date

Chapter 9. General conclusion and future research

9.1 Introduction

In view of a rapidly changing climate system resulting from a global increase in greenhouse gas (GHG) emissions, the global soil pool has received much attention regarding its role in GHG abatement. Historically, soils have lost huge amounts of carbon (C) to the atmosphere making them major sources of C (IPCC 2007). Conversely, soils have been widely recognized as having the potential to sequester significant amounts of C through more judicious land use or management practices, especially where C depletion has occurred (Lal 2004). Many studies on the soil carbon sequestration potentials for most developed nations have been conducted (Houghton *et al*., 1999; Smith *et al*., 2000, 2010; Follett *et al*. 2000; Sleutel *et al*. 2003; Freibauer *et al*. 2004). However, information quantifying the soil C storage potential is lacking in Australia as only limited work has been conducted to quantify C stocks (Sanderman *et al*. 2010; Wilson *et al*. 2011). Moreover, our present knowledge on C stocks in Australia including New South Wales (NSW) relies on studies that have been conducted within a narrow range of land use or management systems, mainly grazing management practices (Dalal and Chan 2001; Chan 2001; Lodge *et al*. 2003) and field agronomic trials that were designed to test best management practices and not C studies (Sanderman *et al*. 2010) with few studies conducted on a broad range of land uses (Young *et al*. 2005; Wilson *et al*. 2008, 2011). Further, almost all of the studies cited above have been conducted in surface soils (30 cm or less).

There is however a growing interest in storage of C in subsoil horizons as a number of studies have showed that despite their low concentrations subsoils may contribute more than half of the total profile C (Batjes 1996). Moreover, there is accumulating evidence that compared with surface soils; C in subsoil layers is more stable as evidenced by increase in radiocarbon age at depth (Trumbore 2009). While the processes that lead to high stability of C in subsoils are not entirely clear, the speculation has been, these layers represent a potential sink for organic C as they may not yet be saturated with C (especially those with low C concentrations) (Rumpel and Knabner 2011). It has been proposed that land use may influence soil organic carbon (SOC) stocks in subsoil layers (Jobbagy and Jackson 2000) however firm conclusions have been hampered given that few studies have been conducted below surface soil layers (Lorenz and Lal 2005).

There are substantial gaps in our understanding of SOC dynamics and its implications especially in northern New South Wales Australia. Specifically, the relative quantities of C that can be stored in soils and the long term dynamics (stability) of C under a broad range of land uses is largely unknown and need to be determined before accurate and reliable predictions of soil C change resulting from land use change can be made. In this chapter, the effects of land use on a) quantity of SOC down the soil profiles, b) dissolved organic carbon (DOC) storage at different soil profiles, c) composition of dissolved organic matter (DOM) extracted from plant litter, d) quantity and quality of organic C among particle size fractions down the soil profiles, e) SOC mineralization at different soil depths, f) initial biochemical composition of plant litter and g) C and N mineralization kinetics is discussed. The results are compared to those of similar studies conducted in Australia and elsewhere, major conclusions drawn from this research are highlighted and areas for future research which flow from this thesis are identified.

9.2 Synthesis of the main findings and implications

A series of questions were asked about: a) the effect of different land use on SOC stocks at different soil depths (chapter 3); b) the role of subsoils SOC in soil C storage and potential to sequester atmospheric CO_2-C in subsoils (chapter 3); c) the influence of different land use on DOC storage down the soil profiles (chapter 4); d) the composition of DOM extracted from plant litter obtained from different land uses (chapter 4); e) the influence of land use on the storage and quality of C among particle size fractions down the soil profiles (chapter 5); f) the influence of land use on SOC mineralization including the size of SOC pools and turnover rates down the soil profiles (chapter 6); g) the influence of soil depth on turnover rate of C pools (chapter 6); h) the initial biochemical composition of above and below ground litter and its influence on litter C mineralization dynamics (chapter 7); and i) N mineralization and immobilization from above and below ground litter (chapter 8).

The main empirical findings are chapter specific and are summarized within the respective empirical chapters. The following sections synthesize the empirical findings to answer the study's research questions.

9.2.1 SOC stocks down the soil profiles under native woodland, native pastures and cultivation

This section synthesizes the empirical findings to answer the study's research questions about: a) the effect of different land use on SOC stocks at different soil depths (chapter 3); and b) the role of subsoil SOC in soil C storage and potential to sequester atmospheric CO_2-C in subsoils (chapter 3).

The effects of land use on SOC stocks were largely restricted to the surface (0 to 20 cm) soil layers with native woodland soils shown to contain 11 and 9 t/ha more SOC than native pastures and cultivation respectively, which were statistically similar to each other. Significantly larger SOC stocks in surface soils under native woodland may be partly attributed to a) above ground organic matter inputs with more slowly decomposing slow C pools of litter (chapter 7), that are more likely to enter the soil organic matter (SOM) fraction and contribute to increased C storage than the other two land uses, and b) larger C inputs in surface soils under native woodland compared with the cultivation and native pastures which are largely associated with reduced inputs in surface soil layers through crop harvest and grazing respectively (Wilson *et al*. 2011). The results showed no significant difference in SOC stocks between cultivation and native pastures in surface soil layers probably because only a small number of sites were sampled (9 sites) and therefore difficult to detect differences. Previous studies conducted in this region have reported similar relative patterns in soil C between land uses (Young *et al*. 2005; Wilson *et al*. 2008, 2010, 2011). The results from this study are important for land holders, natural resource managers and policy makers in NSW region as it highlights the significance of native woodland in storing larger SOC in the surface (20 cm) compared with native pastures and cultivation.

Assuming that the SOC stocks determined in this study represent equilibria (i.e. that steady state has been reached with respect to the initial management) and that the native woodland soil C stocks represent the state of soil C stocks prior to change/conversion in land use i.e. to either native pastures or cultivation, then relative changes (gains or loss) in C resulting from such conversions may be calculated. Using observations from this study, the results indicate that the amounts of C in either native pastures or cultivation soils represent considerable losses in soil C (up to 38 %) as a result of conversion from native woodland suggesting that there is a potential to store C in these C depleted soils (Smith *et al*., 2010). There is an urgent need to stop further losses in soil C stocks in both of these land uses. Notably, whether soils become a source or a sink for C depends on management of the soil. Application of judicious land use or management practices such as a) improved efficiency of crop residue use in cultivated soils and b) improved livestock management to reduce soil disturbance in native pasture land use may partly reduce further C losses and may lead to increases in soil C stocks. Responses of SOC as a result of application of C sequestration activities such as conversion of cultivation to improved pasture has however been reported to be modest for granite soils in this region with increase in soil C estimated to yield between 0.06 and 0.15 t/ha/yr in the surface (30 cm) soil layers over a period of 20 to 50 yrs (Wilson *et al*. 2011). The estimates reported by Wilson *et al*. (2011) are well below those reported elsewhere in other international jurisdictions with cooler or higher rainfall where estimates of soil C sequestration potential have been shown to range from 0.3 to 0.8 t/ha/yr (IPCC 2000; Lal 2004; Smith *et al*. 2000; Follett *et al.* 2000; Nabuurs *et al*. 1999; Smith *et al*. 2007). Generally Australian soils are associated with a less potential to sequester additional SOC as a result of land use change mainly due to climatic extremes in much of the Australia continent which is characterized by year to year variability in temperature and especially soil moisture which have major effects on SOC decomposition (Sanderman *et al*. 2010).

Subsoils (20 to 80 cm) contained 40 % of the total profile SOC stocks across all land uses demonstrating the potential for SOC storage in deeper soil layers (Batjes 1996). There has been much speculation that land use may influence SOC storage at depth (Lorenz and Lal 2005). Our study however suggests that although there were large quantities of SOC at depth, changes in SOC due to land use only occurred in surface soil layers. It will therefore be challenging to manage deep soil carbon to affect significant change in the soils and land uses studied. For land use to effect real changes in subsoil C, large quantities of slowly decomposing organic matter inputs will need to be supplied in these layers. It is therefore concluded that the priority should be to understand SOC at depth, its form and processes of incorporation to ensure it remains undisturbed.

9.2.2 Land use effects of DOC concentrations in soil profiles and composition of DOM extracted from plant litter

This section synthesizes the empirical findings to answer the study's research question about: a) the influence of different land use on DOC concentration at different soil depths (chapter 4); and b) the composition of DOM extracted from plant litter obtained from different land uses (chapter 4).

The relative proportion of aromatic C in DOM extracted from litter was highest in native woodland, followed by native pasture then cultivation indicating qualitative differences in DOM fractions which may in turn influence biodegradability of DOM between litter types. The properties of the various organic compounds contained in DOM have been reported to control its biodegradation (Kalbitz *et al*. 2003). Kalbitz *et al*. (2003) reported that DOM fractions rich in aromatic structures and poor in carbohydrates were likely to be less microbially degraded compared to fractions with more labile (more rich in carbohydrates) compounds and hence more stable.

The influence of land use on DOC concentrations was maintained in the entire soil profile with native woodland soils found to contain significantly larger concentrations compared with native pastures and cultivation soils which also differed significantly between each other in the top 40 cm only. Larger DOC concentrations in the entire soil profiles under native woodland may be attributed to a continued large input of litter mainly in surface soil layers (Wilson *et al*. 2011) and supply of labile compounds released from live growing roots (Paul and Clark 1996). These results may however be partly biased against cultivation and native pastures as oats had been harvested at the time of sampling and native pastures comprised biomass that was senescent. This study acknowledges timing of soil sampling as part of the limitation and recommends future comparison of land use influence on DOC storage to be made during a crop/pasture growing season as the living plant biomass is certain to influence DOC concentrations. The results from this study may be taken as a snap-shot of DOC storage in soil profiles at the time of soil sampling. Though the chemistry of soil derived DOC at different soil depths was not determined, the stability of DOC may still be deduced from the properties of DOM extracted from litter from which it was derived (Kalbitz *et al*. 2003). Consequently, it is possible that soil DOC under native woodland may be less biodegradable compared to the other two land uses as DOM extracted from native woodland plant litter partly constituted aromatic compounds. Studies have reported that DOM fractions containing aromatic compounds may survive biodegradation as DOM percolates in subsoil and may be preferentially sorbed to soil mineral surfaces thereby contributing to accumulation of C in subsoil layers (Maie 1997; Kaiser *et al*. 2003).

DOC concentrations decreased with increasing soil depth with the rate of decline shown to diminish in subsoil (> 20 cm) layers which may be related to the abrupt change in texture in the soils studied. It is possible that an abrupt increase in clay content in subsoil layers (> 20 cm) may have led to sorption of DOC on the soil mineral surfaces and therefore DOC was unavailable in soil solution. This result suggests that sandy surface soils may play an important role in rapidly transporting DOC to subsoil clayey layers where it may be sorbed and contribute to C storage (Stevens *et al*. 1999).

In this study, soil derived DOC represented between 0.01 to 0.1 % of the total SOC showing that DOC constitutes a small fraction of the total SOC as similarly reported by Zsolnay and Alessandro (1996). Other studies have however reported that DOC was responsible for 20 and 9 % of total SOC stocks in a forest and grassland soil showing that DOC transport and adsorption in the mineral soil may contribute significantly to SOC storage in some soils (Sanderman and Amundson 2008). Although this study found the contribution of soil DOC to total SOC to be relatively small, the amounts of DOC adsorbed in clayey subsoil layers may build overtime and may contribute substantially to C stocks in deeper layers. However, more studies are needed to further establish the stability of DOC derived from both soils and litter in order to determine its long term dynamics in soils.

9.2.3 Land use effects on the storage and quality of organic SOC among particle size fractions

This section synthesizes the empirical findings to answer the study's research questions about: the influence of land use on the storage and quality of SOC among particle size fractions down the soil profiles (chapter 5).

Across all land uses, the mineral-associated organic carbon (MOC) accounted for between 73 and 99 % of the total SOC down the soil profiles indicating the strong capacity for soil minerals (clay and silt) to adsorb organic C which may in turn render it unavailable to microbes thereby slowing its decomposition, a finding consistent with other studies (Degryze *et al*. 2004; Yamashita *et al*. 2006; Gregorich *et al*. 2006). The contribution of MOC to total SOC increased with increasing soil depth which is most likely because in the surface layers there was a high proportion of less decomposed SOC, hence a lower proportion of MOC in surface soils than in deeper soil layers. This result indicated that subsurface C was more strongly protected than surface C. Further, the decrease in C:N ratios of MOC with increasing soil depth with values approaching close to those of soil microbes (between 5 and 10) suggest that the MOC in deeper soil layers consists of a highly altered older C fraction and may be relatively stable compared to MOC in surface soils. Association of organic C with soil mineral particles is one of the soil processes reported to slow down C mineralization (Sollins *et al.* 1996; Six *et al.* 2002). Given that in chapter 3 this study reported that at least 40 % of total SOC in the soil profile was found in subsoils (20 to 80 cm) layers, the result from this study (chapter 5) implies that C in subsoils may be more protected from decomposition because of a) adsorption to clays which may limit microbial accessibility to organic C and b) a limitation of less decomposed substrate which may limit microbial activity. This information is important for land holders and natural resource managers to inform the protection of existing C stocks in subsoil layers.

While the results demonstrate the potential for subsoils to store C that may be more protected from decomposition, the challenge however will be how to increase C storage in subsoils through land use and management in order to affect significant change, as observed in chapter 3. If indeed land use or management may be altered in such a way that subsoil C stocks can be increased, the next issue that may arise is if MOC in subsoils exhibit saturation behaviour. There has been recent claim that reactive mineral surface area is a finite resource, especially in topsoils (Six *et al*. 2002; Stewart *et al*. 2007) but it’s not clear if the same concept may apply for subsoils. Carbon saturation has been referred to as the maximum level of C that a particular soil will be able to retain as stabilized C based on the physicochemical properties of the soil with additional inputs remaining as unprotected particulate organic C (POC) which may be readily lost (Stewart *et al*. 2008).

Compared with native pastures and cultivation which were largely similar, native woodland soils contained larger amounts of MOC in the entire soil profile suggesting that C may be more protected from microbial attack and therefore more slowly decomposed. Differences in MOC among land uses are reflective of differences in the amounts of structurally stabilized organic matter compounds among land uses (Golchin *et al.* 1994).

This explanation is further linked to the results presented in chapter 7 which showed that plant litter under native woodland initially contained larger amounts of biochemically recalcitrant compounds mainly lignin and tannin compounds compared to the other two land uses. Further, the results from litter C mineralization (chapter 7) showed that both above and below ground litter from native woodland was characterized by more stable slow C pools with longer half lives compared with litter from the other two land uses. Following results from this thesis, the processes that lead to stabilization of C in the entire soil profile under native woodland may therefore be summarized as: both above and below ground litter inputs are initially comprised of large amounts of biochemically recalcitrant compounds (lignin and tannin) (chapter 7); as a result, these plant litter inputs have slower decay kinetics characterized by more stable slow C pools (chapter 7) which subsequently accumulate in soils and enter into soil mineral fractions therefore forming stable C pools (chapter 5).

Native woodland soils were associated with consistently wider C:N ratios in the particulate organic matter (POM) fraction throughout the soil profile compared with native pastures and cultivation. Native pasture had higher C:N ratio than cultivation in each depth increment to 50 cm. These results indicate differences in the quality of organic matter inputs (litter) entering the POM fraction (Yamashita *et al*. 2006; Golchin *et al*. 1994). As a result, the rate of decline in POC in soil profiles was found to be slower under native woodland compared with the other two land uses. Results from the C mineralization kinetics of the active C pool of plant litter (chapter 7) may be used to further understand the stability of POC in soils as POC is closely related to recently deposited plant litter. In this respect, there was a 10 fold difference in the decomposition rate of the active C pool among litter types which was reflected in the early decomposability of litter residues (chapter 7). Differences in the decomposability of the active C pool in litter may therefore explain the observed differences in the rate of decline of POC in soils among land uses (chapter 5). It is worth noting that, as shown in chapter 7, the active C fraction in litter was characterized by a high turnover (<10 days) among all land uses indicating that POC is easily mineralized and may not contribute to long term C sequestration in soils. As previously reported by others, the results of this study indicate that due to its high turnover rate, POC may be used as an indicator to show differences induced by land use or management changes (Dalal and Mayer 1987; Janzen *et al*. 1992; Cambardella and Elliot 1992; Chan 2001; Chan *et al*. 2002).

9.2.4 SOC mineralization in soil profiles

This section synthesizes the empirical findings to answer the study's research question about: a) the influence of land use on SOC mineralization including the size of SOC pools and turnover rates in soil profiles (chapter 6); and b) the influence of soil depth on turnover rate of C pools (chapter 6).

SOC mineralization kinetics was well described by decomposition of a single pool; the slow pool evolved a negligible amount of C which suggested that this pool may be relatively stable in the soils studied. A possible explanation for the relatively stable C pool in these soils might be related to char C which has been found to be present in many Australian soils attributed to past management practices, such as burning after land clearing, for grassland management and fire control, and wildfire (Skjemstad *et al*. 1996, 2002; Clough and Skjemstad 2000; Dalal and Chan 2001; Lehman *et al*. 2008). Quantifying the contribution of char C to the stable pool will however require further studies.

Native woodland had consistently larger C pools in all soil depths compared with native pastures and cultivation. This result is related to differences in the amounts and distribution of labile C substrates such as DOC and POC (chapter 4 and 5) down the soil profile among land uses. The results highlight the importance of land use in influencing the size and distribution of labile substrate in the soil profile.

The mean residence time of the active C pool at various soil depths was however not significantly influenced by land use probably because the active C pool generally contains easily decomposable labile C compounds (Collins *et al*. 2000). This result may be well related to the mineralization kinetics of the litter active C pool (chapter 7) as plant litter is the main source of C in soils (Kögel-Knabner 2002). In chapter 7, this study reported a 10 fold difference in the active C pool decomposition rate constant between litter types from different land uses; however these differences were in the short term with half lives of < 10 days. This suggests that irrespective of litter type, the litter active C pool comprised of easily degradable C compounds and consequently these results may be related to the soil active C pool decomposition rates which did not significantly differ at various soil depths among land uses.

The mean residence time of the active C pool was strongly dependent on soil depth with results showing the existence of a fast cycling active C pool in subsoils with shorter turnover of 47 days compared with 66 days in surface soils. Such results may have resulted from destabilization of active C stores when environmental constraints on decomposition (poor aeration, oxygen supply, moisture conditions) which might inhibit decomposition in the undisturbed profile are removed when the incubated subsoil and surface soils are under similar conditions in the laboratory. Comparable increases in active C pool decomposition rate in subsoils has been similarly reported by Paul *et al.* (1999) and Collins et al. (2000) who related their results to exposure of C associated with aggregates when soil is mixed and brought into the laboratory. Although the amounts of CO_2-C evolved from the active C pool over 419 days accounted for < 10 % of the initial SOC, the results imply that the active C pools in subsoils may be vulnerable to destabilization and could become a significant source of CO_2 into the atmosphere if disturbed, especially when large amounts of SOC stocks occurring below the surface soils are considered. The priority therefore is to ensure this pool is not destabilized by factors related to changing environmental conditions especially in subsoils.

9.2.5 C mineralization dynamics and interaction with initial biochemical composition of plant litter

This section synthesizes the empirical findings to answer the study's research question about: the initial biochemical composition of above and below ground litter and its influence on litter C mineralization dynamics (chapter 7).

The initial biochemical composition of litter differed between litter types and land uses showing differences in initial quality of litter as similarly reported by Kögel-Knabner (2002). Larger amounts of biochemically recalcitrant lignin and tannin compounds were found in litter from native woodland compared with the other two land uses. Litter quality differences between plant litter types within and among land uses were shown to subsequently influence litter C mineralization rates as discussed below.

For all litter types, the active C pool whose decay rate constants ranged from 0.072 d^{-1} to 0.805 d^{-1}, initially constituted < 20 % of the litter mass, while the slow C pool with decay rates of between 0.002 d^{-1} and0.019 d^{-1} comprised > 80 % of the litter mass. The results indicated that while differences in the decomposability of the active C pool were found in the early stages of decomposition, in the long term, the major differences in degradability among litter types was determined by the slow C pool whose decomposition rate differed substantially. The decomposition rates of C associated with the active and slow pools reported in this study are within the range reported by Wang *et al*. (2004) for similar litter types and land uses in Australia. Differences in decomposition kinetics between above ground litter and their corresponding below ground litter components highlights the differential contribution of plant litter inputs to SOC storage and cycling in soil profiles (Lorenz and Lal 2005; Johnson *et al*., 2007). In this study, below ground litter (roots) obtained from native woodland and native pastures had slower decay kinetics compared to their corresponding above ground litter components which emphasizes the importance of recalcitrant root residues in sustaining organic matter content of soil (Yang *et al*. 2011). Root residues represent a direct input of organic matter into the soil system and therefore it is expected that the accumulation of recalcitrant root residues from native woodland and native pastures will contribute significantly to subsoil C sequestration (Sanderman *et al*. 2010). However, since the decomposition rate of the SOC slow C pool could not be determined during the extended laboratory incubation, it is impossible to determine differences in long term dynamics (stability) between surface and subsoils in this study. Our study however highlights the importance of separately considering different plant parts when studying the plant decomposition dynamics as different components vary in their decomposition dynamics within and between land uses (Abiven *et al*. 2005).

The decomposition rate of the slow C pool in litter was strongly and negatively correlated with the initial lignin:N ratio of plant litter suggesting that the interaction between these two litter quality variables had important controls over litter decomposition (Melillo *et al*. 1982; Taylor *et al*. 1989; Johnson *et al*. 2007; Talbot *et al*. 2012). The mechanisms by which lignin and N interact to influence decomposition of litter were not investigated in this study however several explanations have been reported including; a) lignin may form cross-linkages to the more labile N thereby chemically protecting N from hydrolysis during litter decay (Talbot *et al*. 2012), and b) lignin may physically protect the more labile cell wall components (e.g. structural polysaccharides and N) from microbial attack during litter decomposition when lignin is deposited in cell walls within the hemicellulose-protein matrices (Boerjan *et al*. 2003). In this study, it is possible that differences among litter types in the amounts of initial lignin contents may have influenced the mechanism by which lignin:N interact to control decomposition of the slow C pool in litter. In this respect, native woodland litter (leaf and root) which had larger amounts of initial lignin contents was associated with larger slow C pools in litter which were relatively more stable compared with the other litter types. More slowly decomposing plant residues (in this case native woodland litter) are more likely to enter the SOM fraction and become stabilized as humified SOM which may persist in soils for significant periods of time therefore contributing significantly to SOC stocks (Schlesinger 1990).

9.2.6 N availability and release from above and below ground plant litter

This section synthesizes the empirical findings to answer the study's research question about: N mineralization and immobilization from above and below ground litter (chapter 8).

Significant relationships were observed between the initial biochemical composition of plant litter namely C:N ratio, % lignin and % water soluble carbon (WSC) and inorganic N release from litter in the early stages of incubation (day 4 to 35). The results suggest that during the early stages of litter decomposition, the biochemically recalcitrant lignin influenced the susceptibility of substrates to microbial attack and thereafter the demand for N by microbial decomposers. However, the subsequent release of N from substrates depended on the C:N ratio. Compared with their corresponding above ground litter components, root litter, which had large amounts of recalcitrant lignin, decomposed slowly and induced a lower N demand across all land uses. As a result, roots were associated with the largest amounts of potentially mineralizable N at the end of the 105 d incubation. As reported in chapter 7, the availability of N in the soil has important implications for subsequent C sequestration, as N influences the formation of stable slow C pools through its interaction with lignin (Berg and McClaugherty 2008; Knicker 2011). Further, N plays an important role in inhibiting formation of lignolytic enzymes thereby suppressing degradation of lignin in soils (Berg and Matzner 1997; Berg and McClaugherty 2008). This study shows that the above and below ground litter components have distinct N mineralization patterns as influenced by the initial biochemical composition namely C:N ratio, % lignin and % water soluble carbon.

9.2.7 Overall synthesis and contribution of the study to the understanding of SOC storage and stability at different soil depths under native woodland, native pastures and cultivation

This study has addressed substantial gaps that were identified in literature regarding SOC storage and stability in soil depth profiles under native woodland, native pastures and cultivation. As a result of laboratory analytical work together with long and short term laboratory mineralization experiments this study has advanced the understanding of SOC dynamics and its implications in the land uses studied in northern New South Wales Australia. Specifically, the relative quantities of C that can be stored in soils taking into account subsoils have been determined. I have demonstrated that subsoils have potential to store substantial amounts of SOC stocks however, differences in SOC stocks due to land use only occurred in the surface (20 cm) soil layers where native pastures and cultivation had 9 and 11 t/ha less C than native woodland. Consequently, it will be challenging to manage deep soil C to effect significant change.

The influence of land use on DOC concentrations was maintained in the entire soil profile with native woodland soils found to contain significantly larger concentrations compared with native pastures and cultivation soils which also differed significantly between each other in the top 40 cm only. DOC concentrations constituted between 0.01 and 0.1 % of the total SOC showing that the contribution of DOC to SOC storage was relatively small in the soils and land uses. Although DOC constituted a low flux, the amounts of DOC adsorbed in clayey subsoil layers may build overtime and may contribute substantially to C stocks in deeper layers. A key management strategy would be to increase the production of DOC by increasing surface litter inputs which may favour the production of DOC that may then be transported to deeper soil layers. Furthermore, this study shows that the sandy textured surface soils may play an important role in rapidly transporting DOC to subsoil clayey layers where it may be sorbed. More studies are however, needed to further establish the stability of DOC at different soil depths and DOM from which it is derived in order to determine its long term dynamics.

Across all land uses, between 73 and 99 % of the total SOC was associated with soil mineral particles (clay and silt) with associations shown to increase with soil depth suggesting that duplex soils may play an important role in storing relatively stable forms of C albeit in low concentrations. The active C pool in deeper soil layers is sensitive to destabilization especially exposure to favourable environmental conditions such (aeration, oxygen supply, favourable moisture conditions) and therefore it is important that the current C stores remain undisturbed. The initial lignin:N ratio in litter was the best predictor of decomposition rate of litter slow C pool and may be used to identify litter sources with relatively more stable slow C pools. On the other hand, the initial C:N ratio, % lignin and % water soluble carbon (WSC) significantly influenced inorganic N release from litter in the early stages of incubation.

In conclusion, in surface (20 cm) soil layers where land use differences in SOC stocks were shown to occur, native pastures and cultivation had 9 and 11 t/ha less C than native woodland. Larger amounts of DOC were contained in the entire soil profiles under native woodland compared to the other two land uses suggesting that the production of DOC under native woodland may be an important process through which soluble forms of C may be translocated to deeper soil layers albeit in small concentrations. Further, the fraction of DOC extracted from native woodland litter was shown to partly constitute aromatic compounds suggesting that DOC may be more resistant to microbial degradation and may persist in soil solution for a longer period compared to fractions from native pastures and cultivation litter. SOC stocks in soil profiles under native woodland were shown to be relatively more stable than the other two land uses as a) significantly larger amounts of C were associated with the soil mineral organic matter fraction; b) the particulate organic matter fraction had wider C:N ratios; and c) the above and below ground litter was associated with relatively more stable slow C pools with estimated half lives of 109 and 446 days respectively. The results from this thesis are relevant to landholders, natural resource managers and policy makers as they inform that native woodland soil have an important role in storing a) larger SOC stocks in surface (20 cm) layers compared with native pastures and cultivation which were generally similar, b) relatively less decomposable C down the soil profiles as larger amounts of C are associated with the mineral fraction, and c) more slowly decomposing organic matter inputs which were characterized by relatively stable slow C pools in litter compared with native pastures and cultivation in the northern region of NSW Australia.

9.3 Future research areas

The following were identified as areas for future research effort:

1. Investigate land use effects on total SOC stocks in soil profiles using increased intensity of soil sampling in order to represent spatial variation and increase capacity to detect differences in total SOC especially where differences in C might be small such as in non-wooded land uses (cultivation and pastures) – The cost implication of conducting such an intensive sampling was a limitation in the current study and it would be interesting to investigate if real differences in total SOC occur between the non-wooded land uses particularly in surface soils;
2. Compare land use effect on DOC stocks at various depths during crop/pasture growing season. This is important as the living plant biomass is certain to influence DOC concentrations;
3. Determine the stability of DOC derived from both soils and litter in order to determine its long term dynamics in soils;
4. Determine the long term stability of soil slow C pool and investigate the mechanisms by which C might be stabilized including the contribution of char C at various depths under the three land uses;
5. Determine the mechanism by which lignin and N interact to influence decomposability of litter. This will further enhance our understanding of the stability of litter inputs sources among the land uses studied; and,
6. Understand the mechanism of SOC and N stabilization.

9.4 References

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