Impact of liming on soil organic carbon dynamics in acid soils

Submitted by

Nang Seng Aye

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Department of Animal, Plant and Soil Sciences

School of Life Sciences

College of Science, Health and Engineering

La Trobe University

Bundoora, Victoria 3086, Australia

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Summary

Application of lime to an acid soil with the aim of ameliorating soil acidity that limit plant growth is a common agricultural practice. However, its impact on soil organic carbon (SOC) sequestration is a growing concern. As areas of acidification increase, so do the amount of lime application around the globe. Therefore, it is very important to deeper understand the impact of liming on SOC dynamics for sustainable and environmentally friendly food production.

The first experiment of this thesis explored the effect of long-term liming on changes in SOC content and soil aggregate stability in low-input acid soils. Lime application followed by annual cropping with above-ground residue removal for 34 years decreased SOC content and aggregate stability. However, maintaining native volunteer grass without cultivation for 5 years after initial lime application did not change SOC content, while increased soil aggregate stability. The results suggest that the effect of liming on SOC dynamics and soil aggregate stability largely depends on the accompanying agricultural practices such as tillage, residue management and nutrient management.

The second experiment investigated the impact of lime-induced increases in soil pH on native SOC mineralization in response to C substrate supply, i.e. the priming effect, of two residues with differing in C:N ratio by applying stable isotope technique. Irrespective of residue type, overall decomposition of added residues and the priming effect increased with increasing initial soil pH. The study suggests that the rate of lime application to acid soils should be targeted to increase soil pH value which is high enough for optimal crop productivity but low enough to minimise SOC losses from the soil. The magnitude of native SOC losses was greater with lower C:N field-pea residue compared to the higher C:N wheat residue during a 90-day incubation period. However, the proportion of remaining added residues after 90 days was greater with field-pea residue, indicating that application of lower C:N residue has greater potential to contribute to long-term SOC storage relative to higher C:N residue. This also highlighted the important role of N availability to decomposer organisms in SOC mineralization.

A further experiment scrutinized the interactive effect of soil pH and mineral nitrogen (N) on SOC mineralization in two soils with contrasting indigenous SOC content. Regardless of initial soil pH and SOC content, application of mineral N exhibited non-linear effects on SOC mineralization which greatly depends on the rate of N applied. Low rate of N

application increased, the intermediate rate did not change and the high rate decreased SOC mineralization compared to the control. These findings pointed out the importance of balanced N fertilization for specific soils to improve crop productivity and soil health while minimizing extra SOC loss. The study also highlighted detrimental effects of excessive N fertilization on soil microorganisms, soil health and environment.

The last experiment was conducted by applying ^{13}C-labelled pure extract C sources, glucose and lignocellulose with or without N to address coupling effect of pH and N on C priming. Acidifying (pH 4.1) or liming (pH 6.6) increased priming effect compared to the control soils (pH 4.7). Labile C substrate, glucose yielded a greater priming effect compared to the more recalcitrant C compound, lignocellulose. The addition of mineral N decreased priming effect of both C substrates in all pH values, with this effect more pronounced in lignocellulose-amended soils. The study highlighted the importance of sufficient N application in agricultural field where there is concomitant supply of C substrates with various degree of biodegradability such as root exudates, decaying plant biomass and microbial metabolites to minimize extra SOC loss via the priming effect.

In conclusion, native SOC losses and degrading soil structural stability due to liming acid soils can be minimized by adopting better agricultural practices such as minimum/no tillage, residue retention and balanced fertilization. In order to increase crop production while maintaining SOC in acid soils, net increase in primary production and biomass inputs due to liming should be able to compensate net increase in C respired by liming. Optimal amounts of N fertilization for specific soils would reduce extra SOC loss by priming effect in limed soils. Over-liming should be avoided to minimize extra SOC loss and micronutrient deficiency while maximizing the net profit in agricultural crop production.

Statement of authorship

This thesis includes work by the author that has been published or accepted for publication as described in the text. Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis or any other degree or diploma.

No other person's work has been used without due acknowledgement in the main text of the thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

Melbourne, March 2017

Author

Nang Seng Aye

List of Publications

Refereed papers

Aye NS, Sale PWG, Tang C (2016) The impact of long-term liming on soil organic carbon and aggregate stability in low-input acid soils. *Biology and Fertility of Soils* 52:697-709 (Chapter 3)

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Aye NS, Butterly CR, Sale PWG, Tang C (2017) Non-linear response of soil organic carbon mineralization to nitrogen addition under different pH conditions *Biology and Fertility of Soils* (revision after review) (Chapter 5)

Aye NS, Butterly CR, Sale PWG, Tang C (2016) Influence of initial pH and nitrogen status on soil organic carbon priming by ^{13}C-labelled glucose and lignocellulose *Soil Biology & Biochemistry* (submitted) (Chapter 6)

Presentation in conferences

Aye NS, Sale PWG, Tang C (2014) Impact of liming history on physicochemically protected soil organic carbon and microbial biomass in acid soils. In: *2014 National Soil Science Conference*. Melbourne, Victoria, Australia.

Aye NS, Butterly CR, Sale PWG, Tang C (2015) Residue addition and liming history interactively enhance native C mineralization in acid soils. In: *2015 Agricultural Biosciences International Conference.* Melbourne, Victoria, Australia.

Aye NS, Butterly CR, Sale PWG, Tang C (2016). Interactive effect of pH and mineral N on soil organic carbon mineralization. In: *2016 AgriBio Science Conference*. AgriBio, Centre for AgriBioscience, Bundoora, Victoria, Australia.

List of Abbreviations

Al……...Aluminum

Al^{3+}……Aluminum ion

C……Carbon

^{13}C……..A stable isotope of carbon

$\delta^{13}C$……An isotopic signature, ^{13}C:^{12}C

Ca……..Calcium

Ca^{2+}……...Calcium ion

$CaCl_2$……Calcium chloride

$CaCO_3$.....Calcium carbonate

$CaMg(CO_3)_2$……..Dolomite

$Ca(^{15}NO_3)_2$……^{15}N-labeled calcium nitrate

$CaSO_4.2H_2O$…….....Gypsum

C_{mic}……Microbial biomass carbon

C_{mic}/ C_{org}……The percentage of soil organic carbon present as microbial biomass carbon

C:N…….....Carbon to nitrogen ratio

CO_2…….....Carbon dioxide

$^{13}CO_2$…….^{13}C-labelled carbon dioxide

C_{org}……Soil organic carbon

EC…….Electrical conductivity

ECEC……Effective cation exchange capacity

Fe……..Iron

FIA ……...Flow injection analyser

g……Acceleration caused by gravity

Gt……..…Giga-tonne

H^+……..Hydrogen ion

HCl…….....Hydrochloric acid

HCO_3^-…….....Bicarbonate

H_2CO_3……...Carbonic acid

H_2O……...Water

H_2SO_3……...Sulphurous acid

H_2SO_4……...Sulphuric Acid

ID……...…..Inner diameter

K……...Potassium

K^+…….....Potassium ion

KCl……...Potassium chloride

K_{EC}……Conversion factor for microbial biomass carbon

K_{EN}……Conversion factor for microbial biomass nitrogen

K_2SO_4……Potassium sulphate

MBC…….Microbial biomass carbon

MBN…….Microbial biomass nitrogen

Mg…….....Magnesium

Mg^{2+}…….....Magnesium ion

Mn…….....Manganese

Mn^{2+}……..Manganese ion

Mo…….Molybdenum

MWD……Mean weight diameter

N……...Nitrogen

^{15}N…….A stable isotope of nitrogen

Na^+……Sodium ion

$Na_2{}^{13}CO_3$……^{13}C-labeled sodium carbonate

NaOH……....Sodium hydroxide

NH_4^+-N …….Nitrogen source as NH_4^+

NH_4NO_3…….Ammonium nitrate

N_2O…….....Nitrous oxide

NO_3^--N……Nitrogen source as NO_3^-

P…….Phosphorus

PDB ……Pee Dee Belemnite

ppm……....Parts per million

qCO_2…….....Microbial specific respiration

rpm…….....Revolutions per minute

S…….....Sulphur

SHMP……....Sodium hexametaphosphate

SOC……...Soil organic carbon

$SrCO_3$…….....Strontium carbonate

$SrCl_2$……..Strontium chloride

TN…….....Total nitrogen

WRB…….....World Reference Base international soil classification

WFPS……...Water-filled pore space

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CHAPTER 1

General Introduction

Agricultural management practices can affect the carbon (C) cycle through controlling soil physical and biochemical properties (Amundson 2001; Janzen 2006; Lal 2003; Lal 2004b; Paustian et al. 1998). It is therefore crucial to comprehensively understand the impact of these management practices on soil C dynamics. Liming has been well known as one of the agricultural practices being used for soil acidity remediation and has a significant influence on the global C budget via physico-chemical (Hamilton et al. 2007; Oh and Raymond 2006; Sumner 1992; West and McBride 2005) and biological pathways (Baker et al. 1999; Curtin et al. 1998; Fuentes et al. 2006; Hamilton et al. 2007). Thus, it is of prime important to investigate implications of liming on soil C balance when considering the impact of agriculture on soil C sequestration (West and McBride 2005).

Changes in soil pH and microbial properties of acid soils following liming have a significant influence on SOC mineralization (Lorenz et al. 2001). Lime-induced increase in soil pH and microbial activity accelerate mineralization of SOC which lead to net losses of SOC (Wang et al. 2015c). On the other hand, increase in crop yield and biomass C inputs in response to liming can increase SOC status (Inagaki et al. 2016). Lime-induced increase in biomass-C inputs might compensate for those accelerations of SOC mineralization rate (Haynes and Naidu 1998). Bridging effect of Ca^{2+} ions (dissolved from lime) between clay particles and organic materials can also create microbially non-preferred spaces within aggregates (Bennett et al. 2014; Ekschmitt et al. 2008). Moreover, other farm management practices such as tillage, residue management and fertilization may also play a crucial role in SOC mineralization (Liu et al. 2016). However, interactive effects of liming and other farm management practices on SOC dynamics in arable land are not well understood.

Application of lime to acid soil also govern soil physical properties, particularly soil aggregation and aggregate stability (Keiblinger et al. 2016). Liming has been reported to improve soil physical properties such as soil infiltration, workability and water-holding capacity (da Costa and Crusciol 2016). In addition, the cementing effect of $CaCO_3$ and flocculation effect of Ca^{2+} due to liming can also improve soil aggregate stability (Bourokba Mrabent et al. 2017; Hati et al. 2008). Nevertheless, the deterioration effect of liming on soil aggregate stability has also been observed due to increased biological degradation and

resultant SOC losses (Chan and Heenan 1998), especially during the first six months of application. Decreases in labile C due to faster mineralization in limed soils can be ascribed to such a negative effect on soil aggregation as labile C is a primary agent for soil aggregation (Haynes and Swift 1990; Tisdall and Oades 1982). Nevertheless, the influence of liming on soil aggregate stability is also related to other farming practices such as intensity of land cultivation and organic material inputs. Also, time span after liming may affect soil aggregate stability as a series of chemical reactions take place after liming which may have a great influence on clay flocculation and dispersion (Haynes and Naidu 1998). However, a thorough investigation of how liming controls soil aggregation by linking with time span after lime application and other management practices is lacking so far.

In addition, the common agricultural practice of crop residue retention in the field can also generate extra native SOC mineralization by means of priming effect which is merely mediated by soil microorganisms (Rousk et al. 2015). Given that liming has a prime influence on soil microbial biomass and activity, it can also have a profound impact on the priming effect. Increases in soil pH have been demonstrated to enhance the priming effect (Bertrand et al. 2007). However, the magnitude and direction of priming effect depend on quantity and quality of added organic materials (Six et al. 2002). For example, the application of easily biodegradable C substrates induced greater size of priming than less biodegradable C substrates (Luo et al. 2016). However, the mechanisms responsible for these impact are not well documented. As C source of various degree of decomposability is supplying in the field, it is important to elucidate the interactive effect of C source and liming practice on SOC dynamics.

Moreover, nitrogen (N) availability to soil decomposer organisms also plays a key role in SOC mineralization (Khan et al. 2007). Nitrogen availability can enhance SOC mineralization by stimulating microbial activity (Kalbitz 2000). However, there are controversial results displayed on this. Nitrogen supply has been shown to increase (Cleveland and Townsend 2006), decrease (Wang et al. 2014), or not affect (Thirukkumaran and Parkinson 2000) on SOC mineralization. Variations in indigenous soil characteristics and the amount of N added among different studies might have resulted these inconsistency outcomes (Ramirez et al. 2012). In spite of recognizing the importance of liming and N fertilization in acid soils, there is lack of comprehensive understanding the coupling effect of these two factors on SOC mineralization.

Furthermore, N also has a great influence on the priming effect (Craine et al. 2007), which varies among the type of crop residues application. For instances, application of N with easily degradable glucose increased priming effect, while that with less degradable crop residues did not significantly affect the priming (Chen et al. 2014). Nevertheless, there are evident reports that N addition with less degradable crop residues decreased soil pH and microbial abundance leading to decreased priming effects (Frey et al. 2014; He et al. 2016). Even though liming and N fertilization are common practices in crop production, so far, knowledge of interactive effects of soil pH and N availability on priming effect is scarce.

Therefore, this PhD project aimed to narrow the aforementioned knowledge gap. Specifically, this project elucidated how liming alter SOC content as well as composition, interactive effect with C sources, soil types and N availability and underlying mechanisms. A series of experiments were implemented to investigate: a) the impact of liming on SOC and aggregate stability in 5- and 34-year-old lime trials (Chapter 3), b) liming effect on native SOC mineralization in response to ^{13}C-labelled field-pea and wheat residues amendments (Chapter 4), c) the interactive effect of liming and mineral N application on SOC mineralization in soils differing in initial soil pH and SOC content (Chapter 5) and d) coupling effect of liming by mineral N on native SOC mineralization following amendments of ^{13}C-labelled glucose and lignocellulose substrates (Chapter 6). On the whole, the results were anticipated to contribute to identifying soil acidity management strategies that would improve crop production and sustaining SOC level in the future.

CHAPTER 2

Review of Literature

2.1 Introduction

Soils have been considered as the largest terrestrial pool for organic carbon (C) and they store about 2000 Gt C, which is approximately three times larger than atmospheric C pool and four times larger than terrestrial vegetation pool (Houghton 1995; Schimel 1995). The dynamics of soil organic carbon (SOC) pool has a profound effect on the global C cycle as it can be a C sink or a C source for atmospheric CO_2 (Lal 2004a). The atmospheric CO_2 concentration has increased from 280 ppm in the pre-industrial era to over 400 ppm today due to anthropogenic activities (Showstack 2013). It has been estimated that agriculture, forestry and other forms of land use contribute over 8 billion metric tons of CO_2 equivalent (Tubiello et al. 2014) or 24% of total global greenhouse gas emission (Edenhofer et al. 2014). Thus, the impact of soil management on soil C stocks needs to be understood to reduce atmospheric CO_2 concentrations and to sustain soil C stocks.

Soil acidification is a soil degradation process that is responsible for limiting plant growth around the world, as well as in Australia. Acidic soils comprise approximately 50% of the world's potentially arable soils (Kochian et al. 2015). Soil acidity has commonly been managed by incorporating finely-ground mineral limestone (Conyers et al. 2011; Toma and Saigusa 1997; Weligama et al. 2008; Williams 1980). However, there are concerns in regard of its impact on soil C sequestration and on CO_2 emissions due to higher soil pHs and increasing microbial activity (Paradelo et al. 2015). As the area of acid soils and liming are increasing worldwide, then a small proportion of the SOC being released as CO_2 can have a substantial impact on atmospheric CO_2 concentrations. Therefore, it is very important to better understand how changes in SOC content and its composition result from liming acid soils. This requires an examination of the physico-chemical and biological responses when acid soils are limed.

Although liming practice has widely been adopted to manage soil acidity, we do not fully understand how liming impacts on soil microbial activity, soil aggregate stability and SOC mineralization. This review will cover changes in SOC status and its composition upon liming. Interactions between liming and crop residue and nitrogen addition, on SOC

dynamics will also be addressed. At the end of the review, knowledge gaps for further research will be highlighted.

2.2 Soil acidification

Soil acidification is a natural process in soil genesis. However, the changes from natural ecosystems to intensive cropping can accelerate soil acidification (Helyar 1976; Xu et al. 2002; Balon and Hedley 2003). Soils forming from acidic parent material which is naturally low in basic cations such as Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} can become acidic. Similarly, the leaching of these cations down through the soil profile by excess rainfall can also result in development of acid soils. This situation is common in high rainfall areas, where precipitation exceeds evaporation, leading to leaching of basic cations (Sparks 2003). Production of H^{+} ions during C, N and S cycling can also contribute to soil acidification. In the C cycle, forming H_2CO_3 due to dissolution of CO_2 and dissociation of carboxylic acids produced by plants and microorganisms are the two main sources of H^{+}. Mineralization and oxidation of organic N and S can also release H^{+} ions (Kunhikrishnan et al. 2016). In addition, soil acidification can result from the use of commercial fertilizers, particularly ammonium-based fertilizers. Furthermore, the removal of cations in harvested parts of crop and the leaching and/or release of organic acids in the decomposition of organic materials, can also lead to soil acidification (Stammer 1992; Tang et al. 2013).

Increasing soil acidification is one of the major challenging issues for Australian agriculture (Scott et al. 2000). Hajkowicz and Young (2005) estimated that Australian agricultural profits would rise A$2.5 billion per annum (around 35%) if acid soils did not occur. About 77-90 million hectares of agriculturally productive land, which represents half of Australia's total agricultural land, is estimated to be affected by soil acidity. There are around 60 million hectares of acid soils have surface soil pH_{CaCl2} <4.8-5.0 and around 23 million hectares have subsoil pH_{CaCl2} <5.5 (Page et al. 2010). About half of these areas occur in Victoria and New South Wales (Scott et al. 2000). Long-term legume-based pasture in Australia also lead to a gradual decline in soil pH (Donald and Williams 1954; Lee 1980).

2.2.1 Effect of soil acidity on plant growth

Acid soils can restrict crop production. The restriction in growth in acidic soils can result from deficient concentrations of available phosphorus (P), molybdenum (Mo), calcium (Ca)

and magnesium (Mg) and from the presence of phytotoxic concentrations of aluminum (Al), manganese (Mn), and hydrogen (H) ions (Brennan et al. 2004; Fageria and Baligar 2008; Xu et al. 2002). In general, nutrient availability for plants is pH-dependent and the optimum pH_{H2O} range for most crops is 6-6.5. This pH range increases the availability of most essential nutrients in the soil solution. An example is with P where inorganic P is mostly adsorbed to metal oxide surfaces or present as Fe- and Al phosphates in acid soils (Sims and Pierzynski 2005) which are unavailable to plants. The most prominent detrimental effects for plants in a strongly acid soil (pH_{H2O} <5) is the occurrence of phytotoxic concentrations of Al ions in the soil solution which retard root growth and consequently reduce crop yield (Foy 1988; Noble et al. 1988; Parker et al. 1988; Kochian et al. 2015).

2.2.2 Effect of soil acidity on soil biology

Soil acidity also has negative effects on soil biology. For example, the activity and biodiversity of soil fauna are reduced by soil acidity. Elevated level of Al in acid soils can exert direct toxic effect on soil biota and hence soil biodiversity (Slattery et al. 2001). Besides, reduction in plant biomass production and substrate availability to soil fauna will also retard cell growth and activity (Kemmitt et al. 2006). Similarly, the activity of soil microorganisms is generally depressed in acid soils (Aciego Pietri and Brookes 2008; Fierer and Jackson 2006). Harrison (1971) reported how the microbial community in the soil was reduced in acid soils by tannin, humic acids, and pH-dependent Al complexes in soil solution. Pal et al. (2007) also found that increased KCl-extractable Al caused reduction of microbial activity in a tea-garden acid soil. The biologically-mediated nitrification process is also markedly decreased below pH_{H2O} 6 and undetectable below pH_{H2O} 4.5 (Adams and Martin 1984; Alexander 1980). Given that soil acidity leads to unfavourable conditions for most of the soil biota (Springett and Syers 1984), then it also reduces biologically-driven decomposition and mineralization processes in soil, which are important for agricultural crop production (Alexander 1980; Bååth and Anderson 2003; Blagodatskaya and Anderson 1998).

2.3 Amelioration of soil acidity

The effective management of soil acidity is challenging and needs to consider not only agronomic and economic factors, but also environmental and soil sustainability issues. Nevertheless, soil acidity has been managed in different ways for a number of centuries

(Gardner and Garner 1953). In the past, soil acidity was managed by means of short- or long-term fallow system (Cramb 2005). Choosing adapted crop species to acid soils has also long been the farmers' practice (Myers and De Pauw 1995). At present, there are a variety of management practices used in different parts of the world depending on soil type, climatic condition, the availability of materials and the farmers' financial status. Soil acidity ameliorating measures include the incorporation of chemical ameliorants (Bauhus et al. 2004; Conyers et al. 2011; Toma and Saigusa 1997; Weligama et al. 2008) and/or organic materials (Muhrizal et al. 2003; Tang et al. 2013) which have liming effects. In addition, adopting acid tolerant cultivars (Tang et al. 2003) and improved farming practices (Coventry et al. 1992) can also overcome soil acidity constraints to some extent.

2.3.1 Methods of soil acidity management

The most commonly used ameliorants for acid soils are finely-ground calcitic lime ($CaCO_3$) and dolomite ($CaMg(CO_3)_2$), while gypsum ($CaSO_4.2H_2O$) has also been used. However, finely-ground limestone is most widely used, and is known as the foundation for crop production, or "workhorse", in acid soils (Fageria and Baligar 2008). Lime neutralize soil acidity in two steps. In the first step, Ca and Mg from lime react with H^+ on the exchange complex and H^+ ions are replaced by Ca^{2+} and Mg^{2+} on the exchange sites (negatively charged particles of clay or organic matter), forming HCO_3^-. Then HCO_3^- react with H^+ to form CO_2 and H_2O thereby increase soil pH. Reaction rate of lime mainly depends on moisture, temperature and quantity and quality of lime (Kunhikrishnan et al. 2016). Lime application also increase soil-exchangeable Ca and Mg (Fageria and Baligar 2008). In the case of gypsum, pH does not increase appreciably as it does with $CaCO_3$, but it can increase plant growth in acid soils by reducing Al toxicity (McLay et al. 1994; Toma and Saigusa 1997). Jayasinghe et al. (2009) also reported that using fly ash can obtain beneficial effect in acid soils in Okinawa, Japan by improving soil physical and chemical properties.

Crop residues and organic manure application practices have also been used as affordable means for managing soil acidity in many developing countries (Hue 1992; Muhrizal et al. 2003; Wang et al. 2011; Wong et al. 2000; Xu and Conventry 2003). In tropical countries where lime or gypsum is too expensive or not readily available for farmers, organic materials are widely used to manage soil acidity. Many workers (Bessho and Bell 1992; Muhrizal et al. 2003; Murakami et al. 2005; Teutscherova et al. 2017; Wong and Swift 1995) demonstrated that suitable organic materials are effective substitutes for liming. They can

result in improved plant growth at low pH values by the detoxification of Al ions and by the formation of Al-organic complexes (Brady and Weil 1996). It has been reported that organic residue amendments in acid soils not only improve soil physical properties but also enhance essential nutrient availability and crop yields (Haynes and Mokolobate 2001; McLay et al. 1999; Wang et al. 2011). More recent research (Chan et al. 2008; ; Dai et al. 2017; Mehmood et al. 2017; Steiner et al. 2007) suggests that both pyrolytic biochar and low-energy consuming residue biochars can also be used as ameliorants for soil acidity.

The use of improved agricultural practices to minimize soil acidity and prevent soil acidification has been increasing (Fageria and Nascente 2014; Seguel et al. 2016; Tang et al. 2011). Improved practices can also be expected to sustain crop production and minimize environmental risk. For example, reduced or minimum tillage practices enhance the positive effects of liming, resulting in increased microbial biomass N, and lead to rapid N turnover that promotes plant growth in acid soil (Soon and Arshad 2005). Optimizing nitrogen application can also reduce soil acidity and improve productivity (Conyers et al. 1996). Avoiding the use of acidifying fertilizers such as ammonium sulphate and urea can also be decreased soil acidification (Wortmann et al. 2014). Minh et al. (1998) also demonstrated that mulching and surface tillage along with maintaining a shallow water table was effective in preventing dry season Al^{3+} toxicity in acid sulphate soils in Vietnam.

In recent years, soil acidity management by growing Al-tolerant crop cultivars has increased in areas where surface liming is not so effective. This occurs in areas with subsoil acidity (Fageria and Nascente 2014; Opala et al. 2014; Seguel et al. 2016). Aluminum-tolerant plant genotypes can reduce Al phytotoxicity through two mechanisms. The first is by Al exclusion from the roots (Delhaize et al. 1993; Kochian et al. 2005; Tice et al. 1992) while the second is by the exudation of Al-chelating organic acids (Delhaize et al. 1993; Ma et al. 2001; Ryan et al. 1995a; Ryan et al. 1995b). Using acid-tolerant varieties can also reduce the lime requirement for acid soils (Kochian et al. 2015).

2.4 Soil organic carbon

Soil organic matter is derived from the microbial decomposition of plant or animal residues or remains. It makes up just 2-10% of the soils mass but has a critical role in the physical, chemical and biological functioning of agricultural soils (Kononova 2013). Carbon is a key component in soil organic matter such that SOC is seen as a direct measure of soil organic

matter. The non-living organic matter in soil comprises mixtures of plant and animal residues/remains at various decomposition stages. The components in this soil fraction vary from particulate organic matter which has undergone minimal decomposition, substances synthesized by microorganisms, through to inert organic matter which represents completely decomposed residues from the mineralisation process (Fig. 2.1).

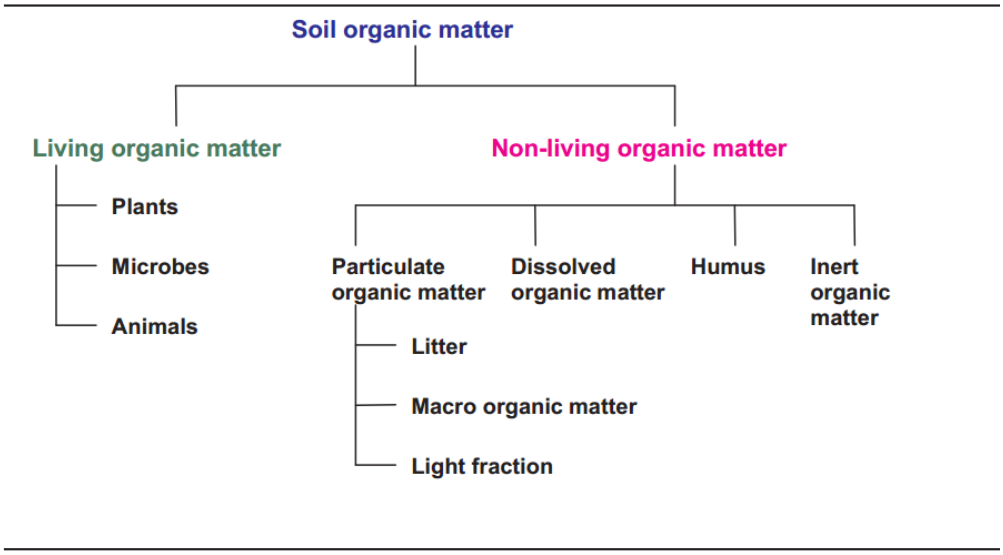

Figure 2.1: Components of soil organic matter (Walcott et al. 2009).

2.4.1 The carbon cycle

The terrestrial C cycle begins with the assimilation of atmospheric CO_2 by plant photosynthesis to form organic compounds in plant tissues. These compounds in turn serve as sources of chemical energy when plant tissues are decomposed in the soil, which releases most C back to the atmosphere during microbial respiration (Fig. 2.2). About half of the organic C added to the soil mainly as shoot litter and roots. Only a very small portion of this C (about 0.7%) is transformed to stable humus substances (Schoenholtz et al. 2000), which are estimated to have turnover time of 27 years (Jenkins 1988). Decomposition processes involve initial breakdown of residues into small particles and repeating the cycle through the soil microorganisms and gradual transformation to recalcitrant C (Stockmann et al. 2013).

An equilibrium in soil C levels is established in mature undisturbed soil, between inputs (above- and below-ground residues) and outputs by mineralization and leaching. However, this long-term balance becomes disrupted with the advent of large-scale soil cultivation.

Given that crop-growing areas occupy about 20% of global land areas (Allmaras et al. 1999), it follows that the C stored in soil and released to atmosphere is greatly influenced by farm management practices. Litter inputs to soil provide C source for heterotrophic microorganisms, and readily decomposable C induce rapid growth of microbial communities (Ågren and Bosatta 1996). Hence, C return back to atmosphere by decomposition of litter, which occurs during the C cycle, is largely controlled by quality of the litter itself.

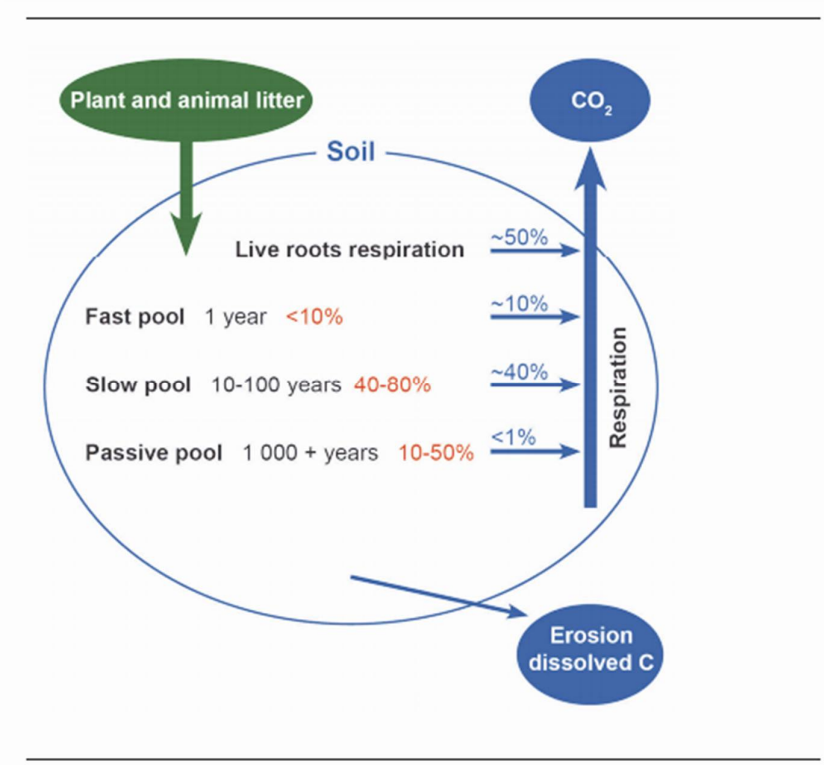

Figure 2.2: A simple diagram of the soil C cycle. The proportion of soil C in each pool is shown in red, and the contribution to respiration from each of the pools is shown in blue (Walcott et al. 2009)

2.4.2 Soil organic carbon pool

The magnitude of the terrestrial C pool within ecosystems varies widely with plant growth rates, respiration rates and C turnover times. Total SOC is generally separated into labile (active) and stable (passive) C pools (Parton et al. 1987). The labile C pool has been the focus in short-term C and N cycling studies due to its rapid turnover and its sensitivity to management practices (Fisher and Montagna 1990; Parton et al. 1987). Soil microbial

biomass, the light fraction and the particulate organic matter fractions are considered as major components of the functional C pool. They are being studied to investigate the nutrient supplying fractions of soil organic matter (Palm et al. 2001).

The light fraction and the particulate organic matter fraction mainly consist of crop residues and residues of some animals and microorganisms. Their turnover rates can vary from a few days to a few years (Stevenson and Cole 1999; Woomer et al. 1994). These fractions may contain as much as 30-40% of the total SOC that is associated with microbial biomass and enzyme activity (Gregorich and Ellert 1993). Thus, an understanding of the mechanisms that give rise to changes in labile-C fractions is becoming increasingly important in understanding the potential for SOC storage in soils.

2.4.3 Importance of soil organic carbon

The SOC pool is a very important soil component. It is considered to be a major potential C sink for atmospheric CO_2 mitigation, and it also plays a key role in sustainable crop production. Increasing SOC content benefits agricultural production and ecosystem services through increasing soil aeration, permeability, moisture retention, nutrient cycling and storing C which otherwise could be warming atmosphere (Keesstra et al. 2016). It also provides substrates for microorganisms and regulates the soil microbial community structures and activities. Upon decomposition, it also releases essential nutrients for plant growth (Saul-Tcherkas and Steinberger 2009).

Preservation of SOC is undoubtedly important for the potential of minimising global warming and for soil quality. For example, a 10% increase in the size of the SOC pool is estimated to be equivalent to 30 years of anthropogenic CO_2 emissions and so changes in this pool could drastically affect atmospheric CO_2 concentrations (Amundson 2001b). On the other hand, small increases in the oxidation rate of SOC as a result of increasing temperatures and/or soil management changes could lead to substantial increases in atmospheric CO_2 levels (Davidson and Janssens 2006). Denman et al. (2007) estimated that escalating global CO_2 emissions from anthropogenic activities reached approximately 8.7 Gt C each year while global atmospheric CO_2 increases occurred at the rate of 3.8 Gt C (Chidthaisong 2005). This highlights the importance of the regulatory capacity of biospheric C pools (Le Quéré et al. 2009). Despite these important roles, quantifying SOC formation and decomposition in natural and agricultural systems presents great challenges because of

its complexity. Understanding changes in SOC levels in soil as a result of land management practices is urgently needed to minimize CO_2 emissions to atmosphere, and to promote crop productivity.

2.4.4 Factors influencing SOC dynamics

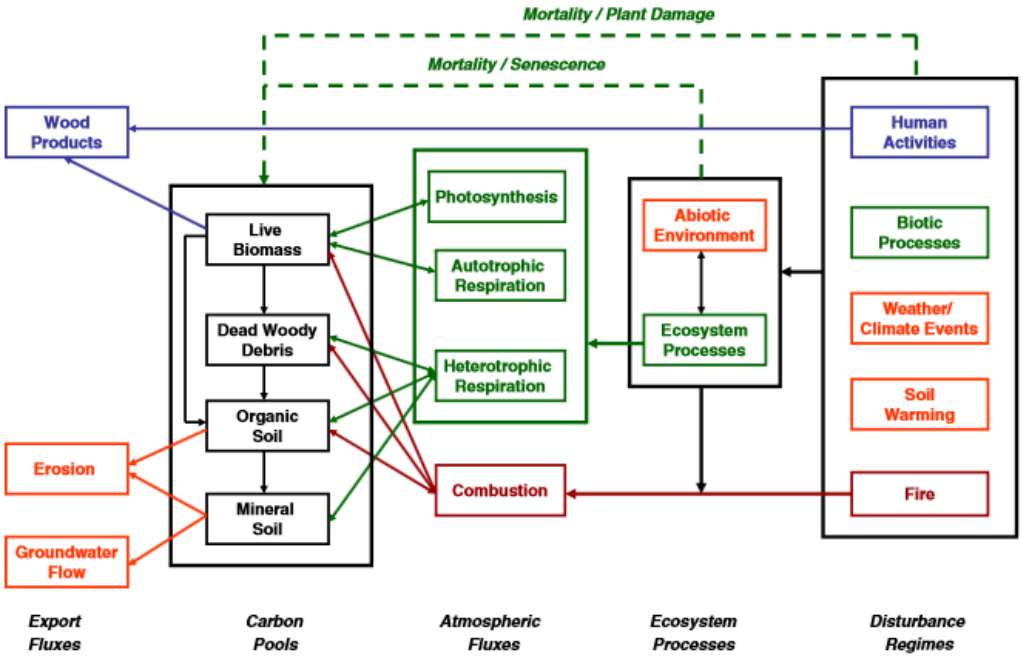

Figure 2.3: Schematic diagram of the relationship of disturbances to terrestrial C cycle, including key components (disturbance regimes, ecosystem processes, atmospheric fluxes, export fluxes) and the processes controlling C cycling (Kasischke et al. 2013)

The size of SOC pools is determined by a range of climatic, land use and soil-related factors (Houghton and Woodwell 1990; Schimel et al. 1994). Disturbances of natural terrestrial C cycle by human activities altering ecosystem processes and climate events greatly control net global SOC stock (Fig. 2.3). The decomposition rate of SOC is largely controlled by the rate of plant residue inputs, soil moisture content and temperature (Raich and Schlesinger 1992). However, CO_2 release due to temperature rises will also depend on composition of SOC pools which have different sensitivities to temperature change (Craine et al. 2010; Fang et al. 2006; Giardina and Ryan 2000).

Changes in land use have significant impacts on soil C storage. Land use change from cropping to pastures or to permanent forest has been shown to substantially increase the amount of C in soil (Guo and Gifford 2002). For instance, net annual rate of C storage after

pasture and forest establishment is about 0.34 t C ha^{-1} (Post and Kwon 2000). Land clearing for the purpose of cropping and changing to monocultures leads to SOC losses. Majority of these losses can be attributed to lower amounts of organic matter input, rapid decomposition rates of crop residues, and decreased physical protection of organic matter in soil aggregates, due to aggregate breakdown by tillage (Dalal and Mayer 1986; Haas et al. 1957; Post and Kwon 2000). When soil is cultivated, it stimulates soil C loss by accelerating the oxidation of SOC by microbial activity and by breaking down soil aggregates to expose SOC to microbial attack (Peterson et al. 1998).

Decomposition of SOC by microbial activity is considered to be a very important process in C and nutrient cycling (Attiwill and Adams 1993). The decomposition is affected more by biological and environmental factors, than by the molecular structures of carbon compounds (Schmidt et al. 2011). In spite of representing less than 5% of soil organic matter, microorganisms play an essential role in SOC decomposition and nutrient cycling (Stevenson 1994). Microbial breakdown of soil organic matter influences the potential C sequestration by the soil in terrestrial ecosystems and the amount of CO_2 released to the atmosphere (Denman et al. 2007; Friedlingstein et al. 2006; Schuur et al. 2009; Trumbore and Czimczik 2008).

Agricultural management practices affect the C cycle by influencing the physical and biochemical properties of the soil (Amundson 2001a; Janzen 2006; Lal 2003; Lal 2004b; Paustian et al. 1998). Among the different farming practices, liming is a practice used for managing soil acidity. However, liming has significant effects on the soil C cycle via physico-chemical (Page et al. 2010; Hamilton et al. 2007; Kunhikrishnan et al. 2016; Oh and Raymond 2006; West and McBride 2005) and biological processes (Baker et al. 1999; Curtin et al. 1998; Fuentes et al. 2006; Hamilton et al. 2007). Tilman et al. (2001) projected that lime use is likely to increase around three-fold in the next 50 years as worldwide expansion of intensive agriculture increases. Furthermore, Robertson et al. (2000) pointed out in an analysis of the global warming potential of agricultural systems that agricultural liming is the second most important cause of global warming via increased CO_2 emissions, after N_2O emissions. Therefore, it is vitally important to understand the implications of liming on the soil C balance, when considering the impact of agriculture on global climate change (West and McBride 2005).

2.5 Effect of liming on soil organic carbon dynamics

2.5.1 Effect of liming on CO_2 emissions from soil

The application of lime to ameliorate acid soils and to prevent acidification, can have a marked effect on SOC dynamics. A key measurement in the research on SOC dynamics is to measure CO_2 emissions from soil, as an indicator of the C loss from the SOC. In this respect, liming has been shown to increase CO_2 emissions due to the increased decomposition of soil organic matter (Bertrand et al. 2007; Fuentes et al. 2006; Kirkham et al. 2007; Murakami et al. 2005; Neale et al. 1997; Shah et al. 1990;). This has been attributed to the improved microbial growth and activity when soil acidity is ameliorated (Priha and Smolander 1994; Staddon et al. 2003). In contrast, other research has found that liming reduced CO_2 emissions while at the same time enhancing soil fertility and crop production (Briedis et al. 2012a; Šimek et al. 1999). Greater net increase in plant production and C input by liming compared to that increase in C respired was ascribed to this positive effect of liming. A complication here is that increased CO_2 emissions are also likely to result from the chemical dissolution of lime, forming HCO_3^- ions, which in turn react with H^+ ions to form CO_2 and water, as discussed above (Fageria and Baligar 2008). Thus, the effect of liming on SOC decomposition is still not clearly understood as several complex biochemical and biological mechanisms are involved in this process (Paradelo et al. 2015). The inconsistent results suggest that there might be many other specific factors such as climate, indigenous C content and crop management practices impacting on the effect that liming has on CO_2 emissions from soil (Nair 2012).

2.5.2 Effect of liming on SOC dynamics in different cropping systems

There are several possible mechanisms whereby liming might impact on SOC levels in acid soils. Firstly, the increase in microbial activity with higher soil pHs, along with increases in the availability of labile C will increase SOC decomposition and hence SOC loss (Ahmad et al. 2014; Chatzistathis et al. 2015). Secondly, liming can have an opposite effect by improving SOC stabilization through complexing the SOC with Ca^{2+} ions (Bennett et al. 2014; Manna et al. 2007). This could result in SOC becoming occluded and inaccessible to decomposer organisms (Kaiser et al. 2012). Finally, the increase in plant growth and biomass inputs in response to liming can also lead to increase in SOC storage (Briedis et al. 2012b; Fornara et al. 2011; Haynes and Beare 1996). Thus increases in SOC content in

limed soils can result from increased biomass production in a given area as a result of soil fertility improvements (Caires et al. 2006). It seems that losses of SOC by accelerating microbial decomposition in response to liming can be compensated by increasing return of plant biomass to the soil.

Management therefore will impact on the change in net SOC content following liming, and will largely depend on the balance between C inputs and respiratory C losses from the soil. A number of published examples illustrate this point. For example, Hati et al. (2008) revealed that the SOC in a cropped soil was maintained over 30 years of liming in conjunction with balanced fertilization in acidic Alfisols in India. Maintaining SOC and improved physical environment of soil was attributed to increase in above- and below-ground biomass C inputs through doubling crop yields in response to integrated use of mineral nutrients and lime. Another example is provided by Fornara et al. (2011) who reported that the SOC of a permanent pasture soil was increased 2-20 times following more than 100 years of liming with balanced mineral nutrient application. The authors attributed this result partly to the protection of the SOC from oxidation by the increased soil aggregation, and to large biomass inputs from pasture roots and shoots.

Tillage as a crop management practice also plays an important role in SOC dynamics. Tillage frequently accompanies liming in order to incorporate lime through the topsoil layers. The issue is that breakdown of soil aggregates by tillage can expose SOC in the aggregates to microbial decomposition (Merante et al. 2017). Aggregates are known to protect the SOC within them from biodegradation (Howlett et al. 2011) and soil aggregation is recognized as one of the major factors responsible for soil C sequestration (Cambardella and Elliott 1993; Six et al. 1998). The C can be stored in macro-aggregates (>250 mm) for short-term and micro-aggregates (0.053-0.25 mm) for long-term (Howlett et al. 2011). Ekschmitt et al. (2008) also proposed biologically non-preferred spaces within aggregates as a key mechanism for C stabilization. Thus, the extent of any reduction in SOC will also depend on the intensity of any accompanying tillage (Merante et al. 2017).

All these observations suggest that liming should be accompanied by sound management systems with appropriate tillage, crop residue and fertility management practices to minimize SOC loss and maintain sustainable crop production. In addition, most of the studies on liming effects on SOC changes have been conducted over short time periods and very little is known about the long-term effect of liming. Hence, deeper understanding of

the liming effect on SOC dynamics and aggregate stability would be useful in developing sustainable practices for soil acidity management.

2.5.3 Interactive effects of liming and nutrient availability on SOC dynamics

Fertilizer applications to agricultural fields, with the aim of increasing crop yields, also play an important role in determining the SOC balance (Khan et al. 2007). The issue is that inorganic N fertilizer applications can increase both organic matter inputs, and C mineralization. Thus, the effect of N fertilizer can have varying effects on net SOC change (Powlson et al. 2011; Russell et al. 2009; Waldrop et al. 2004). Nitrogen fertilization has been shown to enhance SOC storage through increasing crop production and plant biomass C inputs (Nadelhoffer et al. 1999). In addition, it has been reported that the breakdown of organic material in the soil can be reduced by the chemical reaction of inorganic N forms (both ammonium and nitrate) on lignin residues and phenolic compounds, resulting in increased SOC storage (Berg and Matzner 1997). The effect is mediated by inorganic N forms reducing the activity of ligninolytic enzymes (Gallo et al. 2004). Nitrogen fertilizer can also reduce the decomposition of SOC by reducing soil pH, when acid-forming N fertilizers are used (Zhang et al. 2008), and this can suppress microbial activity (Bowden et al. 2004). Nitrogen fertilization can also have the opposite effect on SOC storage in the soil by stimulating microbial decomposition of easily degradable SOC forms through enhancing microbial activity, especially when it is applied to N-limited environment, leading to C losses from the soil (Kalbitz 2000).

The effect of N on SOC storage in soil also depends on its effects on the quality of C inputs (Fog 1988). Nitrogen applications generally result in higher residue quality with lower C:N ratios (Russell et al. 2009) and this can lead to the increased decomposition of the SOC and lower SOC content in the soil (Johnson et al. 2005; Parton et al. 2007). Moreover, effect of N also varies on different C pools. The addition of N fertilizer also increases the turnover rate of more labile SOC pools although that of the more slowly cycling pool decreases (Cusack et al. 2011). Thus, a positive C balance in response to N can only be achieved if N fertilization stimulates C biomass inputs more than C decomposition rates and SOC losses. A number of researchers (Cao and Woodward 1998; Körner 2000) report that the predicted increases in SOC storage due to increasing crop production and plant biomass C inputs following N application are not likely to be achieved due to increases in SOC losses that also occur.

As discussed above, the applications of both lime and N can increase SOC content through increasing plant biomass production (Derome 1991), while also enhancing microbial activity and thus SOC mineralization and SOC losses (Moore et al. 1987; Zhu et al. 2016). This raises the issue of how lime and N applications might interact in their effects on SOC content in soil. It is likely that, the interactive effect of lime and N on the SOC content in soil will depend on a number of factors such as initial soil properties and the amount of amendments applied. One could speculate that the application of lime and N fertiliser together would increase the mineralisation of crop residues with low C:N ratios, and therefore limit any increases in SOC content in soil. However, very little is known about the interactive effect of lime and N on SOC mineralization. As liming and N fertilization are routine practices in agricultural crop production, knowledge of how they interactively influence SOC dynamics is required in order to tackle soil acidity issue sustainably.

2.6 Effects of liming on soil properties

2.6.1 Liming effect on soil physical properties

Maintaining optimum soil physical conditions is important for soil fertility and liming has a range of effects on the physical fertility of the soil. Liming practice is believed to improve soil physical properties through improving workability (Gardner and Garner 1953), reducing soil strength and associated improvements in crop establishment (Scott et al. 2003). Liming can exert both positive and negative effects on aggregation and aggregate stability through changes in pH and ionic strength in the soil solution (Haynes and Naidu 1998). Increase in negative charge and thus the ratio of negative to positive charges along with increasing pH in soil solution can increase the percentage of dispersible clays (Castro and Logan 1991). At the same time, the declining activity of Al^{3+} due to the precipitation of hydroxyl-Al polymers would also induce repulsive force between clay particles (Haynes and Swift 1988). However, such detrimental effects of liming on soil structure are only evident during the first year after liming (Roth and Pavan 1991).

A number of mechanisms are proposed to explain the beneficial effect of liming on soil structure and soil aggregation. For example, Chan and Heenan (1998) proposed that the gradual increase in aggregate stability after 3 years of lime application at Wagga Wagga, Australia, was due to the bonding between Ca^{2+} ions, organic matter and soil particles to form micro-aggregates. Improvements in soil structural stability along with increasing Ca^{2+} concentrations after 13 years of annual lime application was also found in Chile (Muñoz et

al. 2012). Edwards and Bremner (1967) also suggested earlier that the chemical-physical interaction between some organic molecules and polyvalent cations and mineral particles govern aggregate stability. In addition, the presence of sufficient Ca^{2+} ions can exert flocculating and cementing effects on soil aggregation (Baldock and Skjemstad 2000; Clough and Skjemstad 2000). Calcium ions can compress the electric double layer and thus diminish the repulsive force between clay particles in dispersive soils. Moreover, the cementing effect of lime and newly precipitated Al and Fe oxides and hydroxides can contribute to soil structural improvements (Haynes and Swift 1988).

Increasing crop yields by liming can contribute to soil aggregation by increasing the return of crop residues. These can in turn provide different organic matter compounds such as humic molecules and polysaccharides which are primary soil aggregate binding agents (Haynes and Beare 1996). Increasing plant growth and microbial activity by liming would also increase root enmeshing activity and microbial mucilage bonding, leading to improvements of soil structure (Oades 1984). Increases in aggregate stability and cation exchange capacity after 12 years of liming sodic soils in New South Wales, Australia (East Cookies Plain) was attributed to increases in biomass inputs and SOC (Bennett et al. 2014). All these results suggest that liming acid soils can have positive effects on soil structure in the long term. However, no detailed research has been conducted to measure these effects directly and so the mechanisms are not fully understood (Davies and Payne 1988).

2.6.2 Liming effect on soil chemical properties

Application of lime to acid soil has a marked effect on soil chemistry. It is well-known that liming increases soil pH, exchangeable Ca, extractable P and effective cation exchange capacity (ECEC) and decreases exchangeable Al and Mn (Olego et al. 2016; Viade et al. 2011). Liming improves soil P availability by solubilizing soil organic matter and mineralizing organic P during lime dissolution (Fageria 2016). The decreasing solubility of Al in soil solution due to its precipitation with hydroxyl anions and to the increased binding of Al to organic matter is mainly responsible for reducing Al^{3+} toxicity with liming (Olego et al. 2016). The replacement of exchangeable Al^{3+} by Ca^{2+} and Mg^{2+} ions on the colloidal surface also contributes to reducing Al^{3+} toxicity.

It is noteworthy that the over-liming of acid soil can decrease the availability of micronutrients such as iron, manganese, zinc, copper and boron, and can lead to severe

micronutrient deficiencies (Fageria and Baligar 2008). Over-liming can also lead to K deficiency in soils initially low in K due to antagonistic effect of Ca and Mg on K uptake (Tsialtas et al. 2017). Furthermore, high rates of lime application can decrease soil P availability due to the formation of poorly soluble Ca-P compounds at high pH (Hopkins and Ellsworth 2005). Elevated pH levels from over-liming may persist for some decades in limed soils and the correction of any micronutrient deficiencies yielded can be quite expensive. Therefore, lime addition should occur at the optimum rate to improve optimum nutrient availability and crop production.

2.6.3 Liming effect on soil biochemical and biological properties

There are several biochemical and biological mechanisms which are related to the beneficial effect of liming on crop production. Liming can create a more favorable environment for microbial activity by increasing soil pH, which in turn enhances mineralization of soil organic matter to release essential plant nutrients such as P and N (Frostegård et al. 1993; Higgins et al. 2012; Saarsalmi et al. 2011). Increased nodulation by legume plants growing in acid soils, and increased rhizobium activity and N fixation in limed soils, can also contribute to increased N availability (Mengel and Kamprath 1978). Both Ca and Mg are important nutrients in the initiation of nodule formation and the growth of nodules so supplying these nutrients through liming can improve the N status of the soil (Lund 1970; Norris 1958). Increases in the soil biota such as mycorrhizal fungi, ammonia oxidizers, gram-negative bacteria and actinomycetes following liming have previously been demonstrated (Bäckman et al. 2003; Frostegård et al. 1993; Staddon et al. 2003). However, many other factors such as quantity and quality of lime materials, soil type and climate, etc. will impact on the effect of liming on soil biochemical processes.

2.7 Carbon priming effect

The amount of C stored in soil is the balance between the rate of accumulation and decomposition of soil organic matter. The common agricultural practice of returning crop residues to the soil provides a source of plant nutrients and has a positive effect on soil health. However, the application of these organic materials can affect the turnover of endogenous SOC by the process known as the 'priming effect' (Bingeman et al. 1953; Kuzyakov 2010). Such changes can result in the acceleration (positive priming) or the retardation (negative priming) of SOC mineralization and this depends on the initial soil

properties and on the nature of the added organic substances (Kuzyakov and Domanski 2000a). Changes in the activity of soil microorganisms that result in the mineralization of SOC and plant residues mediate the priming effect. Even though the number of studies on the priming effect of endogenous SOC has increased in the last two decades, the understanding of mechanisms underpinning this complex phenomenon remains elusive (Rousk et al. 2015).

2.7.1 Mechanisms of priming effect

The priming effect is defined as the change in pre-existing SOC content when exogenous C substrate is supplied to the soil (Bingeman et al. 1953). In practical terms, it is measured by changes in CO_2 efflux rates when organic material is added to the soil. However, the source of the extra CO_2-C (primed C) cannot be detected, as changes in CO_2 efflux can also result from increased microbial biomass turnover which is not related to endogenous SOC turnover (De Nobili et al. 2001; Mason-Jones and Kuzyakov 2017). Therefore, the process is called apparent priming effect if extra CO_2 is released from rapid microbial respiration and not from native SOC, while it is called real priming effect if extra CO_2 is released from pre-existing SOC (Blagodatskaya and Kuzyakov 2008).

Two potential mechanisms have been proposed to explain apparent priming effect. The first one is the pool substitution phenomenon where exogenous C substrate becomes additional microbial substrate. The substrate is taken up in the microbial biomass and thereby enhances release of CO_2 from a larger microbial biomass turnover but not from pre-existing SOC (Jenkinson et al. 1985). A second mechanism is called “microbial triggering” and was proposed by De Nobili et al. (2001). Here, microbial activity increases when microbes sense molecular signals from the added substrate without using the substrate C as an energy source, thereby increase extra CO_2 efflux without affecting native SOC. The existence of this triggering effect has recently been validated by employing non-metabolizable, low-molecular-weight organic substance (Mason-Jones and Kuzyakov 2017). They speculated that this triggering effect arises from chemosensory mechanism which enables microbes to detect specific substances in their environment and react accordingly (Mauriello 2013).

There are likely to be a number of microbial-based mechanisms involved in the real priming effect which can either be positive or negative (Blagodatskaya and Kuzyakov 2008; Mason-Jones and Kuzyakov 2017). Four mechanisms are thought to be responsible for the positive

priming effect that increases the decomposition of native SOC. The first is the co-metabolism theory; where the synthesis of enzyme by decomposer organisms to decompose the added substrate also catalyzes the degradation of endogenous SOC (Horvath 1972; Kuzyakov and Domanski 2000a). The second is the shifting microbial community mechanism. Here, the exogenous C substrate supply supports the growth of some specific microbial species that change or 'shift' the microbial community composition in favor of SOC decomposers (Fontaine et al. 2003). The third mechanism is due to extracellular enzyme synthesis. Here, the added substrate serves as available energy sources for microorganisms to produce energetically-expensive extracellular enzymes that degrade native SOC (Hamer and Marschner 2005). The final mechanism is termed microbial N mining. Here, the application of readily available C source shifts the soil from being C-limited to a N-limited environment, thereby leading to an increase in the degradation of native SOC in order to acquire N to balance the microbial stoichiometry ratio (Fontaine et al. 2011).

The negative priming effect (slowing down native SOC mineralization) is usually assumed to be driven by preferential substrate utilization of easily-degradable and readily-accessible added substrates by microbes (Kuzyakov 2002). However, a toxicity effect from added substrates for soil microorganisms, and/or an inhibition of enzyme activities or a structural change or changes to the native SOC by binding organic materials with mineral soils, can also be responsible for this negative priming effect (Fierer et al. 2001). This process is commonly observed shortly after application of large amounts of easily-degradable substrates such as low-molecular weight organic materials (glucose, amino acids or root exudates) and is generally only significant in the short-term (Blagodatskaya et al. 2007).

2.7.2 Quality and quantity of carbon substrates and the priming effect

The addition of fresh organic materials with different degrees of degradability to decomposer organisms generally induce different patterns of C priming (Six et al. 2002). Degradability here refers to susceptibility of substrates to enzymatic degradation followed by the uptake of reaction products by the soil microorganisms (Chen et al. 2014). From meta-analysis of over 171 experimental datasets, Luo et al. (2016) summarized that addition of easily-degradable substrates generally induce larger priming effects than less degradable substrates. Microorganisms can access C from easily-degradable substrates (lower C:N ratio) more readily than from less degradable (higher C:N ratio) complex organic materials

rich in lignin compounds (Fontaine et al. 2003). Therefore, the nature of the priming effect can change over time following substrate application, according to substrate availability from the exogenous organic material to soil microbes. The priming effect can range from -48% to >2000% in the first five days from fresh C inputs (Luo et al. 2016). Such large variations might be induced by different environmental factors such as nutrient supply and/or energy availability, due to the quality and quantity of the fresh C inputs.

The magnitude of priming effect is closely related to the rate of substrate decomposition. For example, Wang et al. (2015b) demonstrated that application of easily degradable leaves (C:N 29) induced a greater priming effect compared to that of less degradable stalks (C:N 44) during the early decomposition stage (<12 days) while that of stalks residue was greater during the later stage (>12 days). These changes in priming effect between the two residues were explained by differences in their degradability, resulting in different C and N availability to the microbes. Both co-metabolism and N mining mechanisms could be involved in this early surge of priming effect in leaf-amended soils. However, the priming effect with stalk residue at the later stage might have been dominated by N mining mechanisms due to limited N supply from higher C:N stalk residues (Wang et al. 2015b). This suggests that, depending on the nutrient content and the degradability of residues, there will be different amounts of priming involved.

Not only the quality of the substrates, but also the amount that is added, governs the size and direction of priming effect. The priming effect generally increases proportionally with the mass of added substrate-C (Mary et al. 1993). This could be explained by changes in the microbial community structure in response to different amount of substrates supply (Griffiths et al. 1998). However, the amount of added substrates relative to indigenous microbial biomass C can also play a crucial role in the priming effect. Blagodatskaya and Kuzyakov (2008) reported that the addition of readily available organic C up to 15% of the microbial biomass, induced a linear increase in the priming effect, whereas the priming effect decreased exponentially and even became negative in certain circumstances if the added amount of C exceeded 50% of the microbial biomass C. This suggests that the priming effect involves a succession of processes partly connected with changes in the microbial community and their functions (Hamer and Marschner 2002). This complexity in the priming process makes it difficult to quantify and successfully relate with C inputs (Hamer and Marschner 2005). Wang et al. (2015b) also suggested that the priming effect is mainly determined by microbial C:N ratios and microbial demands for C and other nutrients. In

soils, besides residue and manure inputs, sugar, amino acids, organic acids and phenols are being continuously supplied by root exudation, leaching and microbial activity (Hamer and Marschner 2002). Therefore, monitoring and understanding changes in SOC balance, due to substrates supplied to arable land, is very important in regard to SOC sequestration.

2.7.3 Initial soil properties and priming effect

Initial soil properties such as the nutrient status and C:N ratio of the existing soil organic matter, have a marked effect on carbon priming (Kuzyakov and Domanski 2000a). It is only the labile, endogenous SOC pool that is believed to be susceptible to priming effects, and not the stable pool (Jenkinson 1971). Thus, it seems that soils with SOC of higher degradability would lose more SOC through positive priming. Still, it is very difficult to predict how a certain added substrate will act in a certain soil (Hamer and Marschner 2005). According to microbial activation and co-metabolism theory, the addition of N-containing compounds would induce a greater priming effect in N-poor soils than in N-rich soils. The activity of indigenous microorganisms in N-poor soils would be limited by N-availability and hence they would be likely to increase upon the application of N-containing compounds (Hamer and Marschner 2005). Fontaine et al. (2003) also suggested that nutrient-poor soils are more prone to positive priming effects than nutrient-rich soils. This suggests that a strategic management plan for sustainable crop production should include specific nutrient management practices to minimize SOC losses.

2.7.4 Interactive effects of C substrate quality and nutrient availability on priming

Mineral N addition also has a marked effect on C priming. Proposed mechanisms responsible for the positive priming effect described above, include co-metabolism and microbial N mining that are strongly related to the influence of N on the C priming effect (Craine et al. 2007). Mineral N addition can either enhance or reduce the priming effect depending on which mechanism is dominant during substrate decomposition. According to the co-metabolism theory, N along with readily available C sources, would enhance the positive priming effect by stimulating microbial activity and enzyme synthesis for SOC degradation (Kuzyakov and Domanski 2000a). Alternatively, the N-mining mechanism would suggest that N addition would lift the N limitation of microbes, and reduce extra SOC losses due to mining N by microorganisms (Fontaine et al. 2011). Even though these two

mechanisms seem to have opposite effects, they can still occur alone or together, depending on the spatial and/or temporal availability of soil C and N (Cheng and Kuzyakov 2005).

The extent of N effect on priming is also affected by the chemical composition of added organic substrates. For example, Chen et al. (2014) demonstrated that the application of mineral N with easily-degradable sucrose enhanced the priming effect by 142%, while mineral N with less-degradable maize straw (C:N 42) did not change the priming effect in Stagnic Luvisol soils. Increases in the priming effect by mineral N application with sucrose can primarily be explained by the co-metabolism theory. If the application of C and N match the microbial demand, then microbial activity will be at a maximum, as would the synthesis of enzymes that degrade SOC (Hessen et al. 2004). Lack of N effects on the priming of the maize straw was attributed to the steady supply of available C combined with mineral N, which delayed the switch in the microbial utilization of added substrates to native SOC (Chen et al. 2014).

There are some contrasting results that report how N amendments with crop residues decreased the priming effect. For instance, there was a decrease in the priming effect, by an average of 9% after 200 days, following N application with a wide range of crop residues (Craine et al. 2007). He et al. (2016) observed substantial decreases in residue decomposition (38%) as well as a decrease in the priming effect (66%) when high rates of N (6 mg g^{-1} soil) was added to Typic Hapludult Ultisol soil. This decreased effect of N can be explained by the microbial N-mining theory. Nitrogen can also suppress the activity of SOC-degrading enzymes (Sinsabaugh et al. 2005) and ligninolytic enzymes such as phenol oxidase and peroxidase (Frey et al. 2014). Moreover, this decreased effect by N on priming might also be attributed to lowering soil pH by N and thereby reduce microbial activity, especially in high rates of N supply. Thus soil acidification might also have contributed to decreases in the priming effect (Compton et al. 2004).

It is likely that the above-mentioned interaction effect depends on the timing when measurements of the priming effect are made (Chen et al. 2014). In addition, the interpretation of any interaction between the C substrate and N additions (by adding N with crop residue) on the priming effect, can be problematic. This is because the N content in the crop residue can confound the results (Cassman et al. 2002; Trinsoutrot et al. 2000). The problem might be circumvented by applying N with pure C substrate, in order to determine the interaction between C substrates and N supply on the priming effect.

2.8 Effect of liming on C priming

Increases in soil pH by liming can have a marked effect on the C priming through their direct influence on microbial activity that drives mineralization of SOC (Rousk et al. 2015). For example, the addition of oilseed rape residues increased priming in 22-year-old limed soils, which was explained by the increase in microbial biomass C (23%) from liming (Bertrand et al. 2007). The beneficial effect of liming on microbial growth and activity by increasing soil pH and labile C availability to microorganisms in the longer term is well documented (Bezdicek et al. 2003; Filep and Szili-Kovács 2010; Fuentes et al. 2006; Rousk et al. 2009; Stenberg et al. 2000). Increasing plant and root biomass production due to liming can also increase the release of dissolved organic compounds from roots which are primary substrates for microorganisms (Jones et al. 2005). In addition, the increase in net negative charge at higher pH decreases the bonding strength between organic compounds and soil particles and so makes organic substances more available to microorganisms in limed soils (Curtin et al. 1998).

In contrast to these observations, the opposite results with reductions in C priming have been observed in the case of recently limed soils. Wachendorf (2015) reported that the application of lime along with root residue to a Gleyic Podzol decreased C priming effect by an average of 20% in 51-day incubation study. The SOC stabilization effect of liming was assumed to be responsible for this reduction in priming although the significant decrease of 17% in microbial biomass in the lime-amended soils compared to the control soils might also have contributed to this reduction effect. Decreasing microbial biomass and activity shortly after liming have also been reported earlier (Badalucco et al. 1992; Wilfredo A. Dumale Jr. 2011). Muhlbachova and Tlustos (2006) proposed that the short-term, immediate (~1 day) negative effect of lime on microbial biomass and activity had resulted from the strong exothermic reaction of soil water with lime. These observations suggest that the timing of the observation following the lime application needs to be taken into account when determining the effect of liming on C priming.

2.8.1 Interaction with C substrates

The quality of exogenous C substrates also controls the effect of liming on C priming via regulating soil pH. For instance, the application of low-molecular-weight organic substrates such as glucose did not significantly alter soil pH (Rukshana et al. 2011). Thus, the

application of such readily-available substrates might not affect the magnitude of priming induced by liming (Wang et al. 2015a). The addition of crop residues can increase soil pH through decarboxylation of organic anions (Tang and Yu 1999) and release of basic cations during residue decomposition (Butterly et al. 2011; Noble et al. 1996). Ammonification of organic N from plant residues can also increase soil pH by producing hydroxyl ions (Xu and Coventry 2003). Therefore, the priming effect in limed soils observed by Bertrand et al. (2007) could partly be due to the increased soil pH along with microbial activity following residue application. Nevertheless, very little is known about how liming and C substrate addition interactively affect the priming outcome. Moreover, the magnitude of pH changes after residue addition depends on the composition and the type of crop residues (Butterly et al. 2013). Further study is needed to understand the impact that the degradability of residues has on the priming effect of limed soils.

2.8.2 Interaction with N supply

Nitrogen fertilization of limed soils can also influence soil pH and therefore the priming effect. Nitrification following N fertilization is a well-known acidifying process (Gundersen and Rasmussen 1990; Bolan and Hedley 2003). The application of ammonium-based fertilisers to limed soils can decrease soil pH and hence the associated microbial activity (Lu et al. 2011), leading to a reduction in the priming effect. Moreover, N application might also satisfy a greater microbial demand for N in limed soils to some extent and thereby decrease the priming effect in concordance with the microbial N-mining phenomenon (Craine et al. 2007). This situation implies that liming should be accompanied by sufficient N application to minimize extra SOC loss in acid soils. Even though liming and N fertilization are very common practices in crop production on acid soils, the information on how they interactively affect C priming is limited.

2.9 Future outlooks

Soil acidification continues to increase globally, as does the area of land that is limed to ameliorate the acidity. It is important therefore to understand the impact of liming acid soils on SOC dynamics in order to maintain the soil C balance and for the mitigation of global warming. More detailed investigations are required to understand how liming can overcome soil acidity constraints without reducing the SOC content. The key role of farming practices

such as land cultivation, fertilization and residue management also need to be taken into account when determining the impact of liming on SOC dynamics.

More research is also required to gain insights into the complex processes of C priming that occur when acid soils are limed. Changes in microbial biomass and activity, together with shifts in microbial communities over time, need to be quantified when organic materials are added to limed acid soils. Furthermore, the interactive effects of liming and nutrient availability and the addition of residues of varying degradability on the dynamics of SOC mineralisation need to be better understood. Clearly, this area of research is complex. The complexity is illustrated in the schematic outline (Figure 2.4) of different processes affecting SOC mineralisation that are thought to occur when an acid soil is limed. Liming acid soil can exert either positive or negative impact on SOC and soil aggregate stability through various abiotic and biotic mechanisms. In abiotic ways, liming can contribute to atmospheric CO_2 concentration through lime dissolution. Liming can exert either beneficial or detrimental effect on soil aggregation depend on accompanying tillage system. In biotic ways, increase in microbial biomass and activity along with soil pH will also increase soil respiration and therefore SOC loss unless net biomass inputs can offset the net C respired by liming. The magnitude of SOC losses by liming will largely be controlled by amount of N fertilization. Hence, balanced N fertilization will minimize the SOC loss through priming effect in limed soils (Fig. 2.4).

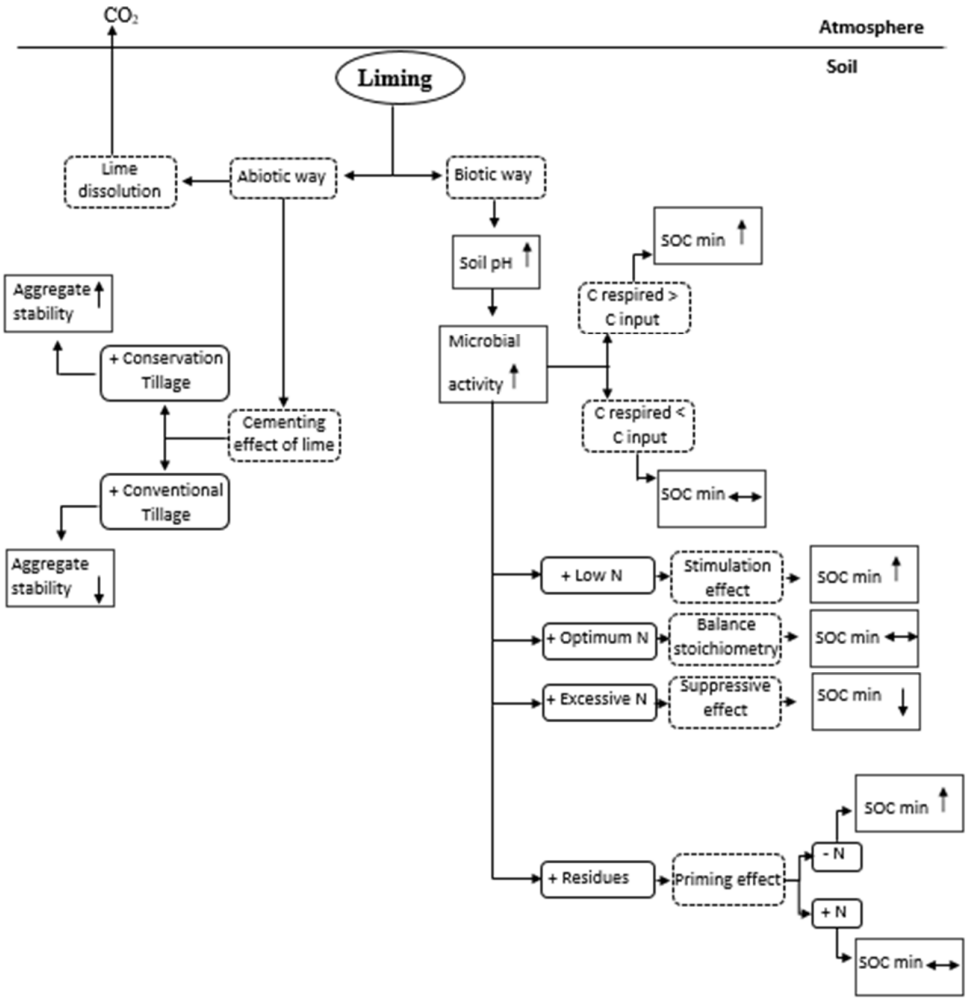

Figure 2.4: Proposed pathways of liming effects and interactions with farming practices on soil organic carbon mineralization (SOC min). Solid frame, dotted frame, up arrow, down arrow and double arrow represent management factors, mechanisms involved, increase, decrease and unchanged.

CHAPTER 3

The impact of long-term liming on soil organic carbon and aggregate stability in low-input acid soils

3.1 Introduction

Lime is widely applied to acid soils to counteract soil acidification and to minimize toxic effects of Al^{3+} and Mn^{2+} (Scott et al. 2003). However, there are concerns about the impact of liming on the stability of soil organic C (SOC) and on its contribution to CO_2 emissions (Paradelo et al. 2015). The application of lime to acid soils ameliorates soil acidity, but it can also contribute to soil CO_2 emissions due to chemical dissolution of lime and to changes in biological processes which enhance SOC mineralization in response to increased soil pH (Fuentes et al. 2006; Smolander et al. 1994). Given that soil acidification and lime utilization are increasing worldwide (Fisher et al. 2003), the effects of liming on soil CO_2 impact the global C cycle cumulatively. Studies have investigated the dynamics of SOC induced by liming (Ignacio Rangel-Castro et al. 2004; Staddon et al. 2003), however, contradictory results have been reported: liming decreased (Bertrand et al. 2007), increased (Johnson et al. 2005) or did not change (Lorenz et al. 2001) SOC concentrations in acid soils. Although there are many studies showing changes of SOC following liming, these studies mainly focused on changes in the quantity of SOC in the short-term while little is known how liming affects changes in net SOC in the long term.

It is well known that SOC can be protected from biodegradation within soil aggregates, where it can be stored in macro-aggregates in the short-term and micro-aggregates in the longer term (Howlett et al. 2011). Proposed mechanisms of SOC stabilization include its presence in recalcitrant stable form, by chemical reactions with mineral surfaces, and by the creation of physical barriers between substrates and decomposers (Christensen 2001). Ekschmitt et al. (2008) also proposed 'biologically non-preferred soil spaces' as major stabilization mechanisms of SOC such as the occlusion within aggregates (Lützow et al. 2006). Many studies have also reported a positive influence of aggregation on SOC accumulation and recognized soil aggregation as one of the major governing processes for soil C sequestration (e.g.Cambardella and Elliott 1993; Golchin et al. 1994; Jastrow et al. 1996; Six et al. 2000; 1998).

Applications of lime to acid soils have been known to improve soil aggregation and structural stability due to the cementing action of Ca^{2+} and carbonates with organic matter (Six et al. 1998). Increased inputs of crop residues can also increase aggregation as they serve as substrates for microorganisms which can promote soil aggregation by releasing polysaccharides (Oades 1984). Increases in pH as a result of liming can also increase clay flocculation in the long term by compressing the double layer between clay particles (Haynes and Naidu 1998) and precipitating hydroxy-Al polymers (Haynes 1984), which are excellent flocculating agents for clay particles (Rengasamy and Oades 1978). On the other hand, increased mineralization due to liming may deplete the labile SOC, the primary aggregate binding agent (Puget et al. 1998), and thereby have net negative effects on aggregate stability. Contradictory results have been reported on this issue with studies showing that lime additions increased clay dispersion and decreased aggregate stability (Castro and Logan 1991) while other studies showed decreased crust formation and increased water-holding capacity, aggregate stability and infiltration (Chan et al. 2007; Hoyt 1981). These contrasting results may be due to variations of initial soil pH, lime application rate, soil type, soil sampling time after liming, the cultivation system, the amount of organic matter input and climate conditions that had occurred in these different studies.

The aim of this study was to investigate the long-term effects of liming an acid soil on aggregation, aggregate-associated SOC and the total SOC. The study sites included two lime trials, with a wide range of lime application rates and time spans after liming (5 and 34 years since the initial application of lime). It was hypothesised that increasing the time following liming would increase the SOC content and aggregate stability, and thereby increase SOC that is protected within water-stable aggregates. This study elucidated that 34 years after liming decreased SOC and macro-aggregate stability in a low-input and cultivated system.

3.2 Materials and Methods

3.2.1 Site description

This study utilised two long-term lime trials initiated in 1979 and 2008, respectively, at the La Trobe University farm, Victoria, Australia (37°42’58.00”S 145°02'53.50”E and 83 m above mean sea level). The mean maximum and minimum air temperatures at the trial site are 20 °C and 9.6 °C, respectively, and the mean annual rainfall is 666 mm. The soil was classified as Sodosol (Isbell 2002) with the following characteristics: 10% sand, 61% silt, and 29% clay, electrical conductivity (EC) of 131 µS cm^{-1}, bulk density of 1.3 g cm^{-3}, total

organic C 20.5 mg g^{-1}, and total N 1.8 mg g^{-1}. The clay minerals of this soil were predominantly illite (70%) with some kaolinite (30%) (Wang et al. 2015).

The 34-year-old lime trial (initiated in 1979) was laid out in a completely randomised design, with lime rates of 0, 12.5, 25, 50, 75 and 100 t ha^{-1} in three replications. The 5-year-old trial (established in 2008) was laid on a randomized block design, at lime rates of 0, 3, 6, 12.5, 25 and 50 t ha^{-1} in three blocks. The size of the experimental lime plot was 2×1 m, bounded by a wooden frame of 20 cm height. Lime was incorporated once to surface 10 cm at the initialization of experimental sites. Prior to the establishment of the experiment, the entire site was under unimproved pastures. The 34-year-old lime trial had been under irregular rotations of fallow, cereal, pasture and grain legumes. These plots were cultivated with a hand hoe as land preparation before seeding. Crops were sown in each plot with 25-cm row spacing. Management practices such as no manure and minimal fertilizer application and termination of plant growth at vegetative stages were quite different from common agricultural practices. Annual C input from this lime trial was estimated less than 1 t C ha^{-1}. At the time of soil sampling in September 2013, these plots were under lentil (*Lens culinaris* Med.) and medic pasture (*Medicago truncatula* L.). The 5-year-old trial had been under unimproved pastures since the commencement of lime treatment. The estimated annual C input was about 1.3 t ha^{-1}.

3.2.2 Soil sampling

Soil samples were taken from lime treatments of 0, 12.5 and 25 t ha^{-1} from the 34-year-old site and 0, 3, 12.5 and 25 t ha^{-1} from the 5-year-old site to investigate the effect of liming on SOC dynamics. Five cores (5-cm diameter) were obtained from 0-10 and 10-20 cm depths from each plot to form a composite sample. Soil samples were transported to the laboratory where they were gently crushed and mixed, visible roots and residues removed, and sieved through a 10-mm sieve. Each soil sample was separated into two portions, one left field moist and the other air-dried. Moist soils were stored at 4 °C until microbial analysis. The air-dried soil was further subdivided two portions for aggregate fractionation and chemical analysis. The soil for chemical analysis was sieved to <2 mm.

3.2.3 Soil analysis

Soil moisture content of the samples was determined immediately after sampling by weighing the soil prior and after oven drying at 105 °C for 24 h. Soil pH was measured with a Thermo Orion pH meter (Thermo Orion 720A+, USA) after adding 0.01 M $CaCl_2$ to soil (1:5) and shaking on an end-over-end shaker for 1 h. Soil microbial biomass C (C_{mic}) was determined using field-moist soil immediately after sampling by 24-h fumigation with ethanol-free chloroform followed by 1-h extraction according to Vance et al. (1987). Eight grams of fumigated and non-fumigated soil samples were extracted with 40 mL 0.5 M K_2SO_4 and filtered through Whatman 42 filter papers (nominal pore size 2.5 µm) (Whatman International, Maidstone, England). Three blanks without soil were also filtrated to correct potential C contamination from the filter papers. Extracts were frozen at -20 °C until extractable C analysis by using chromic acid digestion and spectrophotometer method (Heanes 1984). The C_{mic} was calculated as the difference between extractable C from fumigated and non-fumigated samples, and incomplete extractability was corrected by a conversion factor (K_{EC}) of 0.45 (Jenkinson et al. 2004). The percentage of SOC present as C_{mic} (C_{mic}/C_{org}) was calculated by dividing C_{mic} by SOC multiplied 100 in order to better understand changes in microbial C-use efficiency due to liming. Organic C extracted from non-fumigated soils was denoted as K_2SO_4-extractable C, which is potential C substrates for microbial biomass.

Soil basal respiration was measured by quantifying CO_2 release from rewetted air-dried soil incubated in half-pint wide mouth Mason jars which have screwed caps and rubber septum to sample head space air with a syringe needle (Rukshana et al. 2014). Ten grams of air-dried soil were weighed into a small PVC core (37.6 cm^3) which was placed in the Mason jar. Then 2.3 mL Milli-Q water was added to each PVC core to create 60% water-filled pore space, which supported maximum microbial activity (Linn and Doran 1984). To maintain humidity during incubation, 2 mL deionised water was filled in the small plastic vial attached the jar. All the jars were then tightly closed by following exactly the same order of jar arrangement while undertaking CO_2 measurement and incubated at 25 °C. Headspace CO_2 release was measured by a gas analyser (Servomex 4210 Industrial Gas Analyser, Cowborough, UK) on 2, 4, 7, 10, 15, 21 and 31 days after incubation and soil respiration was calculated as described by Rukshana et al. (2014). The jars were opened and flushed with ambient air for 15 s after each measurement. The microbial metabolic quotient (qCO_2)

was calculated as a ratio of cumulative CO_2 efflux per unit of microbial biomass (μg CO_2-C per mg of C_{mic}).

Total SOC and total nitrogen (TN) of the bulk soil and individual aggregate-size classes were determined by the dry combustion method using a CHNS Analyser (PerkinElmer EA2400, Shelton, Connecticut, USA) after treatment of the soil with sulphurous acid (H_2SO_3) to remove inorganic C (Mackenzie et al. 2002). Briefly, 1 g of ground soil in a 50-mL beaker was treated with 1 mL of concentrated H_2SO_3 on a hot plate set to 100 °C and the sample was left to dry. The acid was continuously added until there was no fizzing. The samples were left to dry and cooled overnight, and re-ground with mortar and pestle for C and N analysis. In order to quantify inorganic C content from residual lime materials, total C content of both acid-treated and untreated soil samples were determined. Inorganic C content was in trace amounts and only in the 25 t ha^{-1} treatment of the 5-year-old trial (Wang et al. 2016).

3.2.4 Soil aggregate size fractionation

Aggregate size fractionation of the soil collected from 0-10 cm was performed by using the wet-sieving method to obtain water-stable aggregates (Clark et al. 2010). Twenty five grams of 10-mm sieved air-dried soils were placed on top of 2-mm sieve of a sieve-stack containing 2, 1, 0.25, and 0.053-mm opening sieves and immersed in deionized water for 10 min. The stack of sieves was subjected to automatic vertical movement for 15 min (70 rpm). Four fractions varying in aggregate sizes were collected: (1) large macro-aggregates (>2 mm), (2) small macro-aggregates (0.25-2 mm), (3) micro-aggregates (0.053-0.25 mm) and (4) silt and clay fraction (<0.053 mm) according to Six et al. (1998). The <0.053 mm fraction was allowed to settle overnight, decanted and dried at 60 °C. All aggregate fractions were oven-dried (60 °C), weighed and stored at room temperature prior to C and N analyses. The recovery of soil particles after sieving ranged from 93% to 99% of the initial soil mass.

3.2.5 Density fractionation and sand correction

It is well recognised that SOC exists in soils in forms with different turnover rates due to different degree of physical and chemical protections. The light fraction is labile and tends to be readily decomposable compared with the heavy mineral fraction when incubated. To make an appropriate comparison of SOC concentration in different aggregate-size classes,

it is important to quantify sand and undecomposed organic matter (light fraction) which are used to correct SOC content in aggregates (Stevenson and Elliott 1989).

All aggregate-size classes >0.053 mm were subjected to density separation to separate light and heavy fractions of organic material using NaI solution adjusted to a density of 1.7 g cm^{-3} (Strickland and Sollins 1987). The light fractions collected from all the aggregate sizes from 3 replicates of each lime treatment were combined to provide sufficient quantity for chemical analysis.

To correct for the sand in aggregate-size classes, the heavy fraction of the aggregate classes was dispersed with 0.5% sodium hexametaphosphate (SHMP) by shaking overnight (18 h) and washing through a 0.053 mm sieve (1:4 soil:SHMP) using Milli-Q water (Gijsman and Thomas 1995). The sand retained on the sieve and the aggregate passed through the sieve were collected separately and oven dried at 60 °C. The SOC and total N contents of all the sand-free heavy fractions and the light fractions were determined following ball milling. The aggregate-associated SOC reported is the SOC concentration of sand-free heavy fractions of aggregates. The recovery of soil particles after density fractionation ranged from 92% to 102% and the SOC recovery ranged from 93% to 102%.

3.2.6 Mean weight diameter

The mean weight diameter of the aggregates was calculated by using the following formula (Van Bavel 1950);

$$MWD = \sum_{i=1}^{n} (d)_i \, w_i$$

where MWD is mean weight diameter (mm); d is mean diameter of each fraction size i (mm); w is proportion of [total sample weight (g) – sand weight (g)] in the size fraction i; and n is number of size fractions.

3.2.7 Statistical analyses

All values are the arithmetic means of three replicates measured on an oven-dried soil basis. One-way analysis of variances (ANOVA) was conducted to investigate the effects of liming on the selected variables of each lime trial by using GENSTAT 16th Edition (VSN International, Hemel Hempstead, UK). To determine the significant differences (P<0.05)

among the treatment means, a Tukey's HSD multiple comparison within each depth was performed. Since the samples from three replicates were combined for the analysis of SOC content in the light fractions, no statistical analyses were performed on these data.

3.3 Results

3.3.1 Soil pH

After 5 and 34 years since the initial application of lime increased surface soil pH by up to 1.63 and 1.52 units and subsoil pH 0.39 and 1.72 units, respectively. The increase in subsoil pH was about 4 times greater at the 34-year-old site compared to that at the 5-year-old but the pH increase of surface soil was similar. These results highlight the progressive downward movement of surface-applied lime over time (Fig. 3.1).

3.3.2 Soil organic C and total N

After five years since the application, liming did not affect total soil organic C (SOC), total N, or C/N ratio in surface (0-10 cm) or subsoil (10-20 cm) layers, except a significant increase in C/N in surface soil with the lime rate of 25 t ha^{-1}. In comparison, after 34 years of an initial application, liming decreased total SOC and the C/N ratio and tended to decrease total N in both soil depths, particularly, in surface 0-10 cm (Fig. 3.2). The average concentration of SOC, total N, K_2SO_4-extractable C and C/N ratio of 34-year-old lime plots were lower than those of 5-year-old in both surface and subsoil layers. Differences in those between the two lime trials were greater in lime-treated plots than untreated control.

Increases in rate of lime application increased K_2SO_4-extractable C in the two depths of both trails. Liming increased the extractable C by up to 127 and 142% in surface and 67 and 93% in subsoil in 5- and 34-year-old limed trials, respectively (Fig. 3.2).

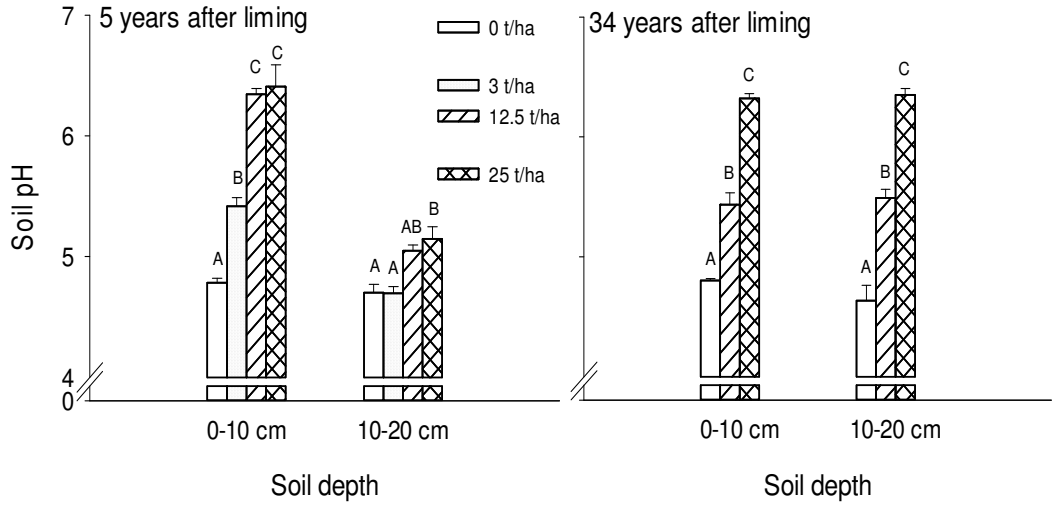

Figure 3.1: Effect of lime application rate on soil pH of surface (0-10 cm) and subsoil (10-20 cm). Lime was applied once 5 and 34 years ago. Different letters indicate a significant difference between treatment means of lime application rates within a depth at each lime site (Tukey's test, $P < 0.05$). Error bars indicate standard error (n = 3).

3.3.3 Aggregate size distribution and SOC contents

There were contrasting patterns of aggregate size distribution between the liming trials. In the 5-year-old lime trial, the higher rates of lime application increased large (>2 mm) and small (2-0.25 mm) macro-aggregates by up to 11% and 44%, but decreased micro-aggregates (0.25-0.053 mm) and silt and clay fractions (<0.053 mm) by up to 13% and 18%, respectively. However, in a 34-year-old lime trial, liming decreased large macro-aggregates by up to 30% and increased small macro-aggregates, micro-aggregates, and silt and clay fractions by up to 54%, 40%, and 72%, respectively, (Fig. 3.3). As a consequence, the mean weight diameter (MWD) (as a soil structural stability index) increased (P<0.05) with increasing lime rate in 5-year lime plots (from 1.18 ± 0.02 to 1.29 ± 0.02 mm) and decreased (P<0.002) MWD in 34-year-old lime plots (from 1.13 ± 0.01 to 1.01 ± 0.01 mm).

The distribution of all the aggregate-size classes between the two lime trials was similar in the unlimed control. The percentage of large macro-aggregate from the 25 t ha^{-1} treatment was about 28% lower in the 34-year-old than 5-year-old trial (Fig. 3.3).

The SOC concentration in the sand-free heavy fraction aggregates displayed a similar trend to SOC concentration of the whole soil, i.e., liming decreased the SOC concentration of the

aggregates <2 mm in 34-year-old trial but those decreases were only exhibited in small macro-aggregates in 5-year-old one (Fig. 3.3). Liming did not change the amounts and proportions of SOC in the light fraction, which represented <1.5 % of total SOC, in both lime trials (data not shown).

3.3.4 Microbial biomass and respiration

Irrespective of liming history, lime application markedly increased microbial biomass C (C_{mic}), and the percentage of SOC present as microbial biomass C (C_{mic}/C_{org}) in both surface and subsoil. Liming increased C_{mic} and C_{mic}/C_{org} up to 80% in surface 10 cm in both 5 and 34-year-old limed plots compared to those unlimed. However, the increase in subsoil varied widely between the two lime trials. In the 5-year-old trial, liming increased subsoil C_{mic} and C_{mic}/C_{org} by up to 155% and 209% while those in the 34-year-old were increased only 26% and 50%, respectively (Fig. 3.4). Microbial biomass C of surface 10 cm of any treatments did not differ between the two lime trials. However, C_{mic} of subsoil from 5-year-old plots was nearly double that from 34-year-old plots. Difference in C_{mic} between the two lime trials only exhibited in limed plots but not in the control.

Likewise, liming increased cumulative soil respiration (CO_2) by up to 13% in surface and 9% in subsoil in both lime trials (Fig. 3.4). Notwithstanding, the magnitude of increases in cumulative soil respiration due to liming was considerably lower than that of increase in microbial biomass C, resulting in decreased microbial metabolic quotient (qCO_2), by up to 38 and 34% in surface and 50 and 15% in subsurface in 5 and 34-year-old limed plots, respectively (Fig. 3.4). The cumulative CO_2 release, qCO_2 and the percentage of SOC present as C_{mic} (C_{mic}/C_{org}) from the surface soil were similar between the two lime trials. However, those of subsoil were greater in the 5-year-old lime plots.

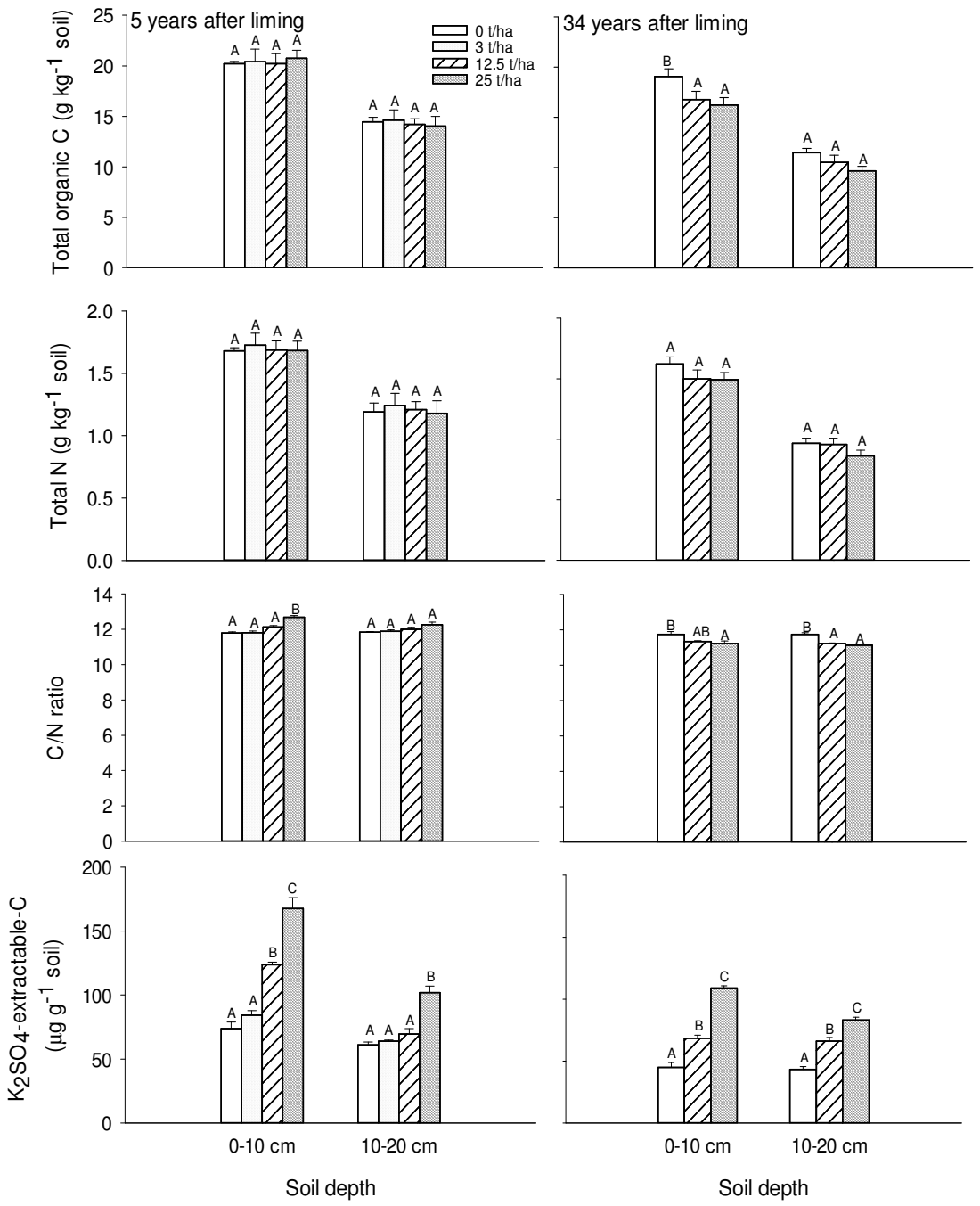

Figure 3.2: Effect of lime application on total soil organic C and total N, C to N ratio (C/N ratio), and K_2SO_4-extractable C of surface (0-10 cm) and subsoil (10-20 cm). Lime was applied once 5 and 34 years ago. Different letters indicate a significant difference between treatment means of lime application rates within a depth at each lime site (Tukey's test, $P < 0.05$). Error bars indicate standard error (n = 3).

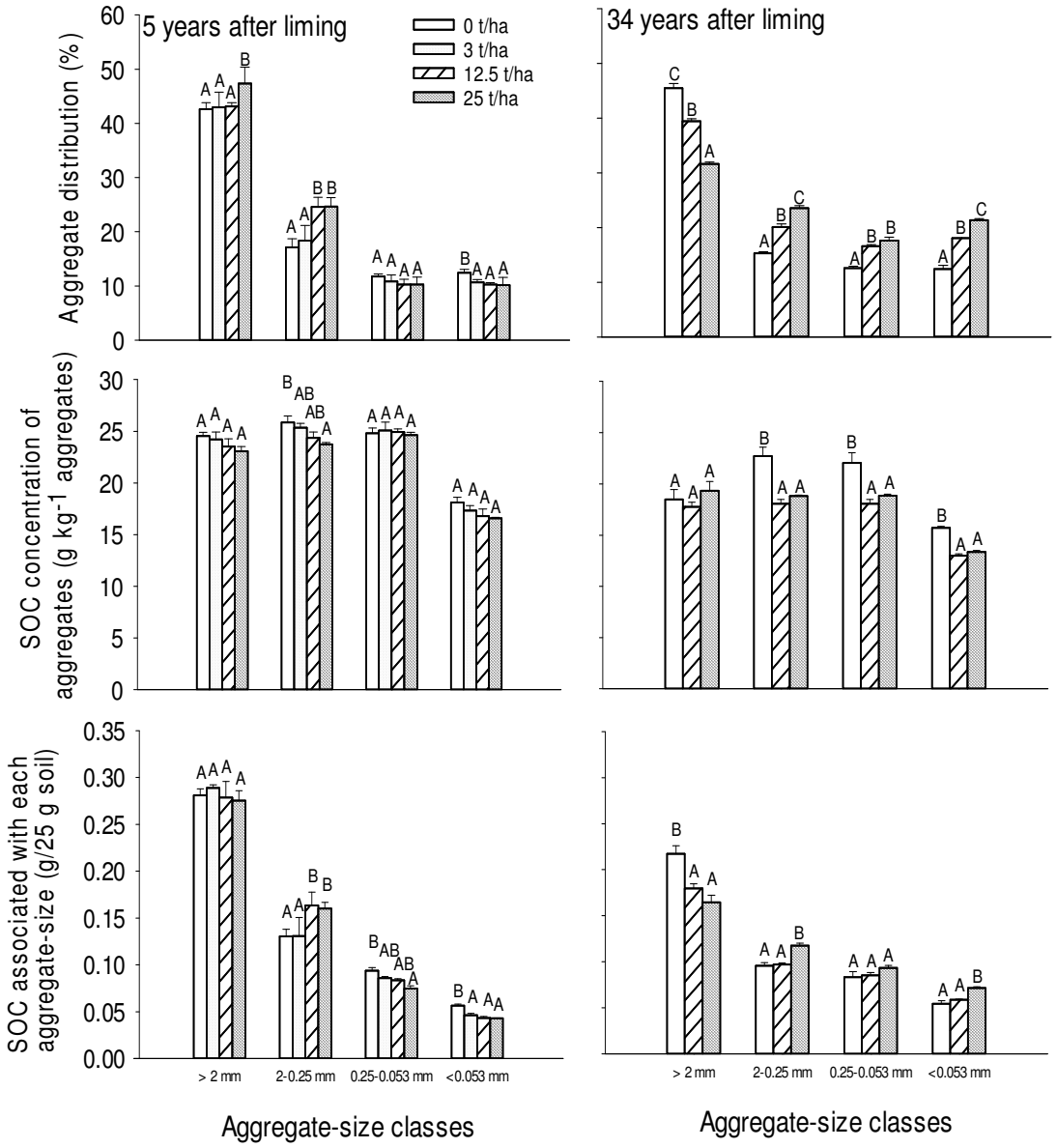

Figure 3.3: Effect of lime application on distribution of aggregate-size classes as large macro-aggregates (>2 mm), small macro-aggregates (0.25-2 mm), micro-aggregates (0.053-0.25 mm), and silt and clay fraction (<0.053 mm), concentration of soil organic C (SOC) in sand-free heavy fraction of each aggregate-size class, percentage of SOC distribution in each aggregate-size class in whole soils of surface (0-10 cm). Lime was applied once 5 and 34 years ago. Different letters indicate a significant difference between treatment means within a respective aggregate-size class at each lime site (Tukey's test, $P < 0.05$). Error bars indicate standard error (n = 3).

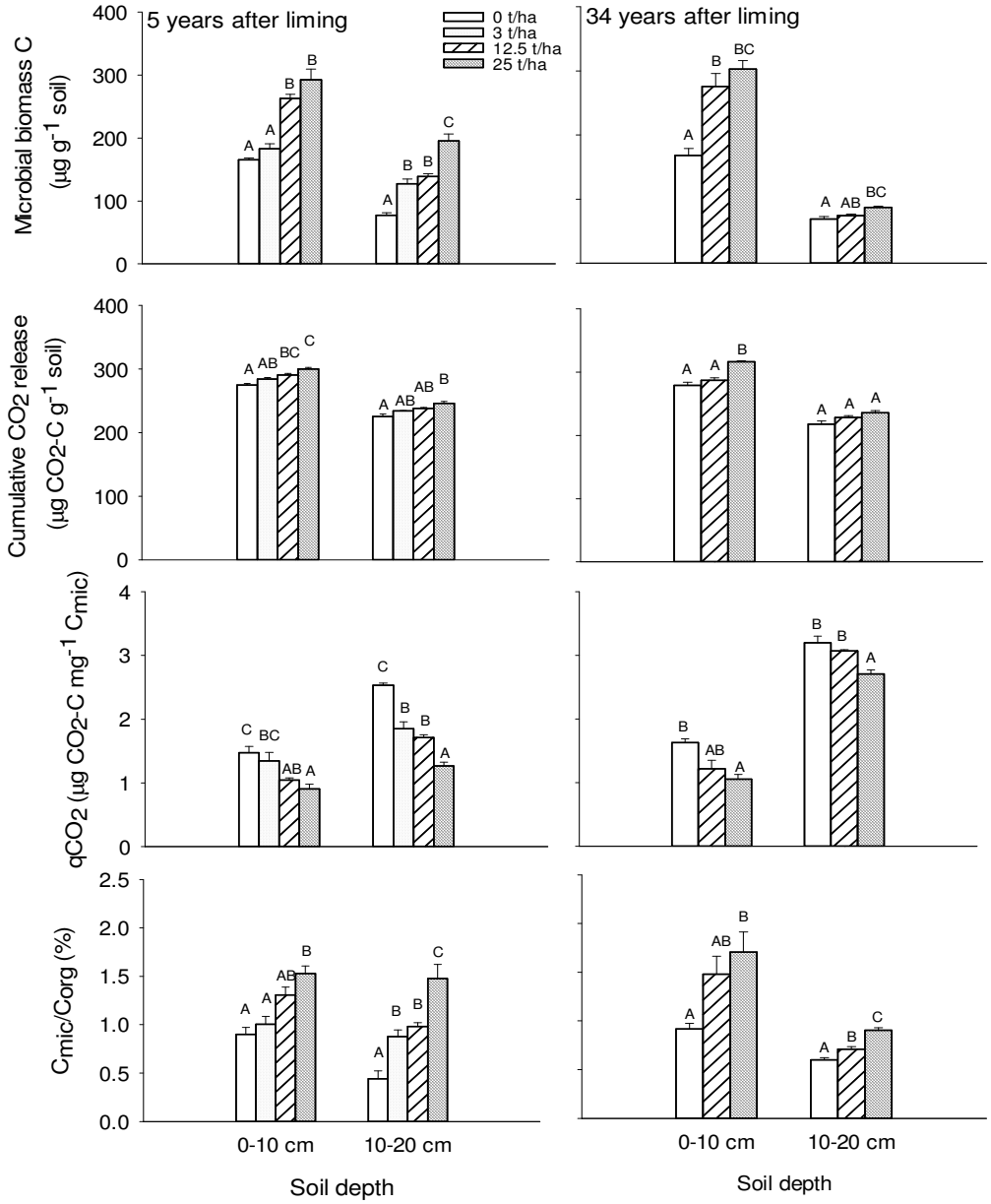

Figure 3.4: Effect of lime application on soil microbial biomass C (C_{mic}), cumulative soil respiration (CO_2) during 30-day incubation period, microbial metabolic quotient (qCO_2), and the percentage of SOC present as C_{mic} (C_{mic}/C_{org}) of topsoil (0-10 cm) and subsoil (10-20 cm). Lime was applied once 5 and 34 years ago. Different letters indicate a significant difference between treatment means of lime application rates within a depth at each lime site (Tukey's test, $P < 0.05$). Error bars indicate standard error (n = 3).

The increased cumulative soil respiration by liming is consistent with increased respiration rate (Fig. 3.5). The respiration rate was the highest at day 2 during the incubation, and decreased with time but the effect of liming tended to be persistent over the 31-d period at the two depths of both trials.

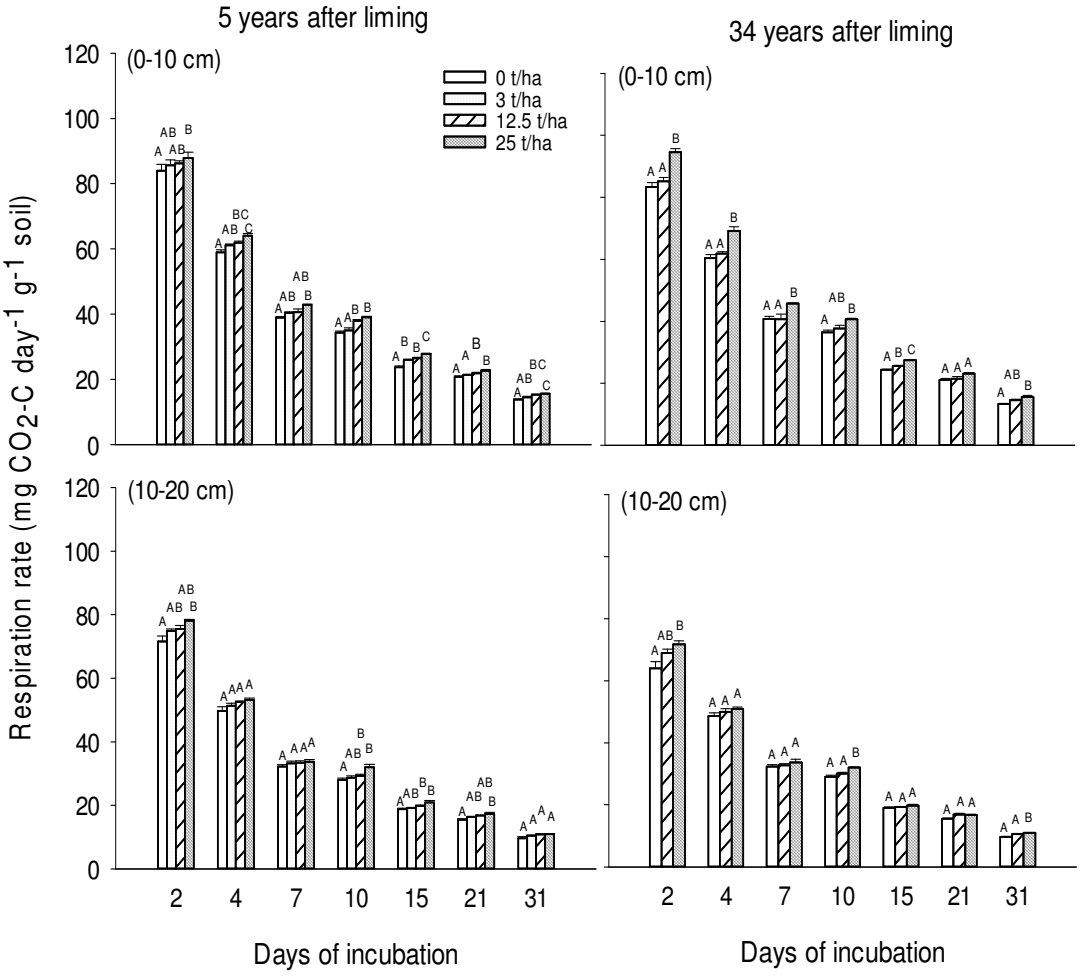

Figure 3.5: Effect of lime application on soil basal respiration of rewetted air-dried soils incubating under 25 °C of surface (0-10 cm) and subsoil (10-20 cm). Lime was applied once 5 and 34 years ago. Different letters indicate a significant difference between treatment means of lime application rates within a respective incubation period at each lime site (Tukey's test, $P < 0.05$). Error bars indicate standard error (n = 3).

3.4 Discussion

3.4.1 Effect of long-term liming on SOC of whole soil

Liming affected the SOC content and aggregate stability in the surface layer (0-10 cm) of the acid soil in this study. Thirty-four years after liming the acid soil that had received low organic matter inputs, there was a decrease in SOC content and the percentage of macro-aggregates in the soil. Increase in microbial biomass and activity in these limed soils would have been enhanced SOC mineralization. Combination effect of greater microbial biomass, low C input (<1 t C ha^{-1} yr^{-1}) resulting from lack of manure and fertilizer application and residue removal in this lime trial would probably have led to greater SOC loss. Cultivation for seeding annually would also have accelerated decomposition of SOC through reducing physical protection (Bronick and Lal 2005; Six et al. 2004). The lower SOC content in unlimed control plots of the 34-year-old than that from 5-year old reflecting soil disturbance and residue removal effects on SOC decomposition. In contrast, 5 years after liming a similar acid soil under unimproved pasture, there was no significant change in SOC content and aggregate stability had increased. In this trial, increase in plant biomass production (about 30%) would have been able to offset faster mineralization of SOC due to liming. Maintaining permanent vegetation without any soil disturbance since liming in this 5-year-old trial could also be a plausible reason for this maintenance of SOC in limed soils. However, liming did not increase SOC sequestration in either trial even it increased about 30% in plant productivity. Thus the results from this study did not support the hypothesis that the liming of this acid soil would improve SOC content and aggregate stability which in turn would improve physical protection of SOC within stable aggregates in the long-term.

The decreased SOC content in the surface soil 34 years after liming in this present study is in agreement with the results of studies in Australia and Brazil involving continuous cropping systems. Chan and Heenan (1999) reported an 11% decrease in SOC content at 0-10 cm depth 3 years after 1.5 t ha^{-1} of lime was applied to an acidic Ferrosol under continuous wheat with stubble burning. Similarly, the surface incorporation of 18 t ha^{-1} of dolomitic limestone in an acidic Ferrosol with continuous corn cultivation raised soil pH (1:1 water) from 4.7 to 6.5 in Southern Brazil but decreased SOC by 42% in the surface 0-17 cm layer over a 7-year period (Ernani et al. 2004).

In other studies, applications of lime to cropping soils did not significantly change the SOC content. Kemmitt et al. (2006) reported no increase in SOC despite large increases in crop

production (up to 250%) over 37 years in southern England, while Hati et al. (2008) reported similar results over 30 years in India. The long-term SOC maintenance was explained by the annual rates of organic matter input matching the CO_2 outputs. Briedis et al. (2012a) reported a 15% increase in SOC content at 0-27 cm depth 15 years after lime application to the surface of an acidic Red Latosols. This was ascribed to an increase (26 %) in annual C inputs from crop residues in limed soils relative to the control soils. It appears that increased C input as a result of liming treatment under no-till system would have been able to offset SOC mineralization and thereby increased SOC accumulation.

The inconsistent findings could have resulted from differences in initial and final pH after liming, plant biomass inputs and soil type between the studies (Chan and Heenan 1999; Kemmitt et al. 2006; Briedis et al. 2012a). Declining SOC contents due to liming could be related to increased microbial mineralization of SOC at favourable soil pH (Ahmad et al. 2014) which in turn accelerating SOC turnover rates. At the same time, if the increase in biomass inputs due to liming are not sufficient to offset the resulting faster SOC turnover rate, net C loss would occur. Increasing above- and below-ground plant biomass production due to liming acid soil has been well-documented (Hati et al. 2008; Kostic et al. 2015). In the present study, increases in the input of plant biomass due to liming would not have been able to offset the increased SOC mineralization in the frequently cultivated 34-year-old limed plots. These results suggest that lime application along with other management practices such as reduced tillage, residue retention, and balanced fertilization are important to increase crop biomass production and hence C sequestration in acid soils.

3.4.2 Microbial biomass C and carbon-use efficiency

The substantial increases (up to 80%) in microbial biomass C and respiration in limed soils in both lime trials reflect the persistence of the initial liming effect on soil pH, and on the size and activity of the microbial biomass. These results are similar to those reported in previous studies (Kemmitt et al. 2006; Rousk et al. 2010b). The increases have been attributed to proliferation of indigenous acid-intolerant bacteria (Rousk et al. 2010a) as bacteria exhibit a narrow optimal pH range for growth (Wheeler et al. 1991). However, the effect of liming on basal respiration was small relative to that on microbial biomass C (Fig. 3.4). This appears to be due to a higher efficiency of microbial C reutilization in limed soils which have led to decreased microbial specific respiration (qCO_2). The results were in agreement with findings of Webster et al. (2000). In addition, the subsoil respiration from

the two lime trails was similar although C_{mic} in the limed plots was halved in the 34-year-old trial. The reason for such a difference in C_{mic} between the lime trials is unknown. One possible explanation is that the soils from the 34-year-old trail were drier than those from the 5-year-old trial at the time of field sampling.

The large increase in K_2SO_4-extractable C in limed soils in the present study also suggests that lime-induced increases in soil pH increases the availability of labile C substrate to the microbial communities. These increases in biomass and activities of soil microbial communities increased mineralization of SOC in limed soils. In a previous study, increased substrate availability was the factor stimulating mineralization of SOC in limed Aridic Haploboroll soils (Curtin et al. 1998).

The lime-induced decrease in microbial metabolic quotient (qCO_2) and the increase in the percentage of SOC present as microbial biomass C (C_{mic}/C_{org}) in both trials indicate that historical liming increased microbial substrate-use efficiency, even though it increased soil basal respiration. This decrease in qCO_2 could be attributed to a lower maintenance energy requirement for the microbes in higher soil pH conditions as more energy is diverted from growth and production of the microbes to their maintenance under stressful, low pH environments (Odum 1985). These results are in good agreement with the work by Blagodatskaya and Anderson (1998) who found that more CO_2 was released per unit biomass under acid soils than neutral soils. They are also in accord with Anderson (1998) who revealed an increase in C_{mic}/C_{org} after 6 years of liming to an acid Norway spruce forest soil. However, even though the qCO_2 index had a strong negative relationship with soil pH, it can respond to some ecosystem development and perturbation in an unpredictable manner (Wardle and Ghani 1995). In addition, Nannipieri et al. (2003) also addressed the limitation of linking microbial biomass C and soil respiration to the soil functions as they can not differentiate batween active and inactive biomass.

The increase in C_{mic}/C_{org} in limed soils indicates that liming increased the conversion of a relatively large portion of SOC into the microbial biomass (Anderson and Domsch 1989). It has been proposed that the higher the amount of substrate C incorporated into microbial biomass, the lower the loss of microbial-derived organic C (Six et al. 2006). Therefore, in the 5-year-old lime trial, liming might increase labile C content through SOC incorporating into microbial biomass even though it did not change total SOC content. It is likely to overestimate the amount of liming-induced SOC losses unless C assimilated by microbial

community is taken into account. However, exact microbial community composition and shifting of fungal:bacteria ratios were not examined in this study. Further studies on the short- and long-term effects of liming on microbial community composition and activities are important to understand the mechanisms underpinning the SOC dynamics in such limed soils.

3.4.3 Aggregate stability

The long-term effects of liming on the percentage of macro-aggregates are inconsistent between the two trials. Liming decreased the percentage of large macro-aggregates (>2 mm) but increased those of small macro-aggregates (2-0.25 mm) and micro-aggregates (0.25-0.053 mm) in the 34-year trial whereas it increased those of large and small macro-aggregates in the 5-year trial.

Decreases in the percentage of large macro-aggregates following liming have been reported previously. For example, Muñoz et al. (2012) found that liming decreased the percentage of large macro-aggregates but increased small macro-aggregates (0.25-2 mm) and micro-aggregates (0.053-0.25 mm) in an acidic Andisol soil 13 years after annual application of 1 t ha^{-1} lime. The decrease in the percentage of large macro-aggregates in limed soils might be ascribed to (a) enhanced exposure of SOC to microbial degradation at higher pH leading to depletion of labile C which is the main source of binding materials for macro-aggregates in low-SOC soils (Tisdall and Oades 1982), and (b) preferential stabilization effects of Ca ions in the limed soil at the micro-aggregate level (Baldock et al. 1994).

In the 5-year-old trial, liming increased the stability of large and small macro-aggregates while maintaining SOC content. One plausible reason for this structural improvement was that the soil in this 5-year-old liming experiment was growing a continuous grass pasture with minimal disturbance, whereas the continually cropped 34-year-old trial was subjected to frequent soil disturbance. The results were in line with Briedis et al. (2012b) and Fornara et al. (2011) who reported that liming increased structural stability under no-till permanent pasture system. In another study, macro-aggregates were increased when the grasses were grown and the soils that were not disturbed (Oades 1984). Furthermore, at the same SOC level, limed soils tended to have higher aggregate stability (Chan and Heenan 1999) than those without. Therefore, liming effects on large macro-aggregate stability might be

strongly influenced by degree of soil disturbance, the level of addition of plant biomass shoots and roots to the soil and initial SOC content of the limed soils (Westerhof et al. 1999).

3.4.4 Aggregate-associated SOC

This study showed that liming decreased SOC concentrations in all the aggregate-size classes <2 mm in the 34-year-old lime trial whilst it decreased SOC concentrations only in the small macro-aggregates in the 5-year-lime trial. The results suggest that long-term liming with a low-input cultivation system depleted SOC occluded within aggregates, and that increases in physical protection of SOC within stable aggregates in limed soils is unlikely to occur unless lime-induced increases in biomass addition can compensate for the amount of SOC mineralized by microbial activity and soil disturbance. The increased microbial activities by liming are likely to increase SOC mineralization when the soils are cultivated and exposed to increased microbial activity (Six et al. 2000).

In spite of decreasing SOC concentration in the smaller aggregates (<2 mm), long-term liming increased the proportion of those in water-stable micro-aggregates and hence increased the total amount of SOC that is associated with these aggregates (Fig. 3.3). It seems that long-term liming increased the proportion of SOC which is better protected within micro-aggregates. The C associated with micro-aggregates has been known to be more protected from microbial decomposition and has longer residence time than those associated with macro-aggregates (Six et al. 2002). It may thus be expected that long-term liming increases the amount of better-protected C even though it decreases total SOC content. A further study is needed to explore chemical composition of the SOC in each aggregate-size class to better understand the effect of long-term liming on SOC stabilization.

3.4.5 Amelioration of surface liming on subsoil acidity

The initial surface application of lime at a relatively high rate gradually ameliorated subsurface acidity (Fig. 3.1), suggesting a gradual downward movement of alkalinity from lime and persistent of this positive effect in a long-term. Increases in soil pH due to liming that extend beyond the point of placement are in agreement with what shown by Wang et al. (2016). The fact that liming increased subsoil pH to lesser extent in the 5-year-old trial than in the 34-year-old trial in this present study, indicates that lime/alkalinity had moved slowly and progressively downward with time, as suggested (Conyers and Scott 1989).

Furthermore, it also suggests that surface liming at low application rates may not be effective in ameliorating subsoil acidity. For example, application of 3 t ha^{-1} lime did not increase subsoil pH over 5 years (Fig. 3.3) which could be explained as there was no excess alkalinity to move downward after neutralizing the surface acidity (Conyers and Scott 1989).

Extended amelioration of subsoil acidity suggests that surface liming could possibly increase C sequestration in subsoil through increasing below-ground biomass. Briedis et al. (2012b) demonstrated an increase in soil pH and macro-aggregate stability in the subsoil (10-20 cm) 15 years after surface application of 6 t ha^{-1} lime even though total SOC was not affected. This aggregate stability improvement was attributed to the greater production of plant biomass and C inputs into the system due to liming. Such an increase in aggregate stability would enhance SOC protection within aggregates in subsoil.

3.5 Conclusions

This study demonstrated that long-term liming (34 years since the initial application) decreased SOC and macro-aggregate stability in the low-input and cultivated system. In contrast, the liming of acid soils under undisturbed vegetation was able to maintain initial SOC content and improved soil structural stability in the shorter 5-year period. The results suggest that the impact of liming on SOC is strongly influenced by primary C inputs following liming and the capability of a system to compensate for extra C mineralization and respiratory losses by microbes at increased pH. The results from this study have important implications. To maintain or increase C balance and soil fertility, amelioration of soil acidity by liming should be followed by other management practices which maximize biomass input and minimize soil disturbance. Furthermore, the surface application of lime at relatively high rates could maintain its positive effect on soil pH, and over time ameliorate subsurface soil acidity, which should stimulate root proliferation and lead to the build-up of SOC in deeper soil layers. Further studies are warranted to explore the mechanisms that drive SOC dynamics following liming with the aim of acquiring integrated management strategies to protect SOC in limed acid soils and to understand whether surface liming increase C sequestration in subsurface layers.

CHAPTER 4

Residue addition and liming history interactively enhance mineralization of native organic carbon in acid soils

4.1 Introduction

Application of organic residues and their composts is increasing with the aim of enhancing soil organic matter content and fertility in farming systems. Plant residues are one of the major sources of nutrients and energy for soil heterotrophic microorganisms and strongly influence chemical, physical and biological processes in the soil. These residues are known to result in a short-term change in the turnover of native soil organic C (SOC), i.e. the priming effect (Bingeman et al. 1953; Kuzyakov et al. 2000b). Changes in microbial biomass and activity following addition of organic materials (Bell et al. 2003) can either increase (positive priming), decrease (negative priming) or have no effect on native SOC mineralization relative to non-amended soil (Blagodatskaya and Kuzyakov 2008; Kuzyakov 2002). However, the priming effect can vary widely, depending on the quality and quantity of organic materials added, soil pH, microbial biomass and activity, and the microbial community composition. Since there is a concomitant supply of fresh organic materials in agricultural fields, assessing and monitoring the impact of priming effect on the C balance are crucial in order to understand and predict soil C sequestration efficiency in the long-term.

Lime-induced increases in soil pH and associated changes in soil microorganisms and their growth through increasing plant productivity play an important role in SOC dynamics (Paradelo et al. 2015). The mineralization of native SOC in response to C substrate addition was greater in limed than unlimed calcareous soils (Bertrand et al. 2007). However, lime-induced priming effects can vary between soil types (Hu et al. 2012) and with the quantity and quality of added C substrate (Wu et al. 1993). Nevertheless, liming has been suggested to increase aggregate stability by means of Ca^{2+} ion bridging and thereby increase the physical protection of SOC against mineralization (Jastrow et al. 1996; Lützow et al. 2006). Nonetheless, to date, there is limited knowledge about the priming effect induced by crop residue addition in soils with different liming histories. Particularly, the role of soil pH in the priming effect remains poorly understood. Comparing the reported impacts of initial soil

pH on the priming effect from different studies performed on different soils will be ambiguous as soils from different sites vary greatly in their physical, chemical and biological properties besides soil pH. Therefore, investigating the influence of soil pH on priming in soils with minimal variation in factors other than pH, is essential to gain insight into the effect of soil pH on the C priming.

This study aimed to scrutinize the effect of soil pH and liming history on the priming of native SOC following addition of ^{13}C-labelled wheat and field-pea residues differing in C:N ratio. The soils had a wide range of pH gradient (4.7-7.4) resulted from long-term lime trials were used. Stable C isotopes were employed to provide further knowledge on SOC dynamics induced by newly-added organic materials. The study used two lime trials with 6 and 35 years of liming histories (lime only applied once) located 100 m apart. We hypothesized that a) limed soils would exhibit greater priming effect following residue addition as a consequence of increased soil pH and microbial biomass and activity, b) the priming effect would be greater with the residue of low C:N ratio (<30) during the early-stage of incubation (0-15 days) as it is readily degradable for microorganisms, whilst the opposite was true in the later stage (16-90 days) due to slower degradability of the residue of high C:N ratio.

4.2 Materials and Methods

4.2.1 Site description

Surface soil (0-10 cm) of a Sodosol (Isbell 2002) or Solonetz (WRB 2014) was taken from two adjacent long-term lime trials (100 m apart) initiated in 2008 (6-year-old) and 1979 (35-year-old) within the Agricultural Reserve of La Trobe University, Victoria, Australia (37°42′58″S 145°02′53.5″E). The experimental site has a temperate climate with an average air temperature of 16 °C and annual precipitation of 666 mm. The soils had native pH of 4.7-4.8, total SOC 15.9-20.4 g kg^{-1}, Total N 1.48-1.73 g kg^{-1}, C:N ratio 10.7-12.3, $\delta^{13}C$ -22.3 to -19.8‰ (Table 4.1), clay 29% and silt 61%, and an electrical conductivity (EC) of 131 µS cm^{-1} (1:5 water). The predominant clay mineral in this soil were illite (70%) with some kaolinite (30%) (Wang et al. 2015c). Prior to the commencement of the lime trials, the entire site was under unimproved pasture. The 6-year-old lime trial had volunteer pasture for five years and was recently cropped to lucerne (*Medicago sativa* L.), while the 35-year-old trial had been under irregular rotation since liming with above-ground residue removal. These different management practices had resulted in differences in C and N content

between the corresponding soil pH of each trial (Table 4.1). Both trials had surface lime applied (10 cm) only once at the commencement of treatment. The 35-year-old trial was laid out in a completely randomised design, with lime rates of 0, 12.5, 25, 50, 75 and 100 t ha^{-1} in three replications. The 6-year-old trial was laid on a randomized block design, with lime rates of 0, 3, 6, 12.5, 25 and 50 t ha^{-1} in three blocks. Details of the lime trials are described in Aye et al. (2016).

4.2.2 Soil sampling

Soil samples were collected to cover a wide range of pH from 4.7-7.4. In order to obtain the soils with comparable pH values between the two lime trials, soils were sampled from four lime treatments of each lime trial, i.e. 0, 3, 12.5 and 50 t ha^{-1} with corresponding pH of 4.8, 5.6, 6.5 and 7.2 from the 6-year-old trial and 0, 12.5, 25 and 50 t ha^{-1} with pH of 4.7, 5.8, 6.7 and 7.4 from the 35-year-old trial, respectively. Hereafter, for the sake of simplicity, average pH value of each pair will be used as initial pH for both lime trials, i.e. pH 4.8, 5.7, 6.6 and 7.3 (Table 4.1). Chemical characteristics of the soils are presented in Table 4.1. Five cores (5 cm ID, 10-cm deep) were collected from each of the 3 replicate plots of each lime treatment to form a composite sample. Soil samples were air-dried and crushed to pass through a 2-mm sieve. Roots and visible organic materials were carefully removed.

4.2.3 Crop residue production and $^{13}C/^{15}N$ labelling

Wheat (*Triticum aestivum* L.) and field pea (*Pisum sativum* L.) plants were grown under field conditions and repeatedly pulse-labelled with ^{13}C in a growth chamber until the full-maturity stage where the atmosphere was enriched with $^{13}CO_2$ (from $Na_2{}^{13}CO_3$ at 98 atom%, Sigma Aldrich, Miamisburg, USA) as described by Butterly et al. (2015). Plants were also labelled with ^{15}N by adding $Ca(^{15}NO_3)_2$ fertiliser (20 atom%, Shanghai Research Institute of Chemical Industry, Shanghai, China). After crop senescence, the plants were harvested, dried at 70 °C and the grain was removed. The remaining above-ground residues were ground and sieved (<2 mm) such that the particle size was between 0.5 and 2 mm. The C:N ratio of wheat and field-pea residues were 41.9 and 28.6, respectively (Table 4.1).

Table 4.1: Initial chemical characteristics of the soils and labelled wheat and field-pea residues. The soils were from two lime trials with 6 (New) or 35 years (Old) since liming. Values are means (n=3) with the standard error in parentheses.

Soil and treatment	Average pH of two trials	Total C (mg g^{-1})	Total N (mg g^{-1})	C:N ratio	^{13}C abundance ($\delta^{13}C$ PDB, ‰)
Initial pH, New					
4.8	4.8	20.2 (0.5)	1.68 (0.03)	12.0 (0.05)	-22.3 (0.8)
5.6	5.7	20.4 (1.0)	1.73 (0.04)	11.8 (0.09)	-20.1 (1.7)
6.5	6.6	20.2 (0.6)	1.69 (0.07)	12.0 (0.08)	-21.1 (0.9)
7.2	7.3	20.1 (0.7)	1.68 (0.07)	12.3 (0.10)	-20.6 (1.3)
Initial pH, Old					
4.7	4.8	19.1 (0.8)	1.62 (0.06)	11.8 (0.16)	-19.8 (1.0)
5.8	5.7	16.8 (0.8)	1.50 (0.07)	11.2 (0.04)	-21.0 (1.5)
6.7	6.6	16.2 (0.7)	1.49 (0.06)	10.9 (0.14)	-20.3 (1.4)
7.4	7.3	15.9 (0.3)	1.48 (0.06)	10.7 (0.07)	-20.3 (1.2)
Residue					
Wheat		407 (0.2)	9.7 (0.2)	41.9 (1.0)	431 (2.1)
Field pea		403 (3.6)	14.1 (0.3)	28.6 (0.3)	471 (15.2)

4.2.4 Incubation study

The decomposition of crop residues in soils with different liming histories was investigated in a 3-month incubation study. The experiment was laid out as a complete factorial of 2 liming history (6 and 35-year) × 3 residues (no residue, wheat and field-pea residues) × 4 initial pH in 3 replicates with a fully-randomized design. To perform pre-incubation of soils before residue amendment, sufficient amounts of each air-dried soil was adjusted to 50% water-filled pore space (WFPS), covered and incubated at 25 °C for 7 days to acclimate soil microbes. Forty grams (oven-dry equivalent) of pre-incubated soil were thoroughly mixed with 0.20 g (0.5% w w^{-1}, equivalent to 2 mg C g^{-1} soil) of either wheat or field-pea residue and placed in a PVC core. The same physical mixing of the non-amended control soil was also performed to eliminate disturbance effects. The soil was wet to 60% WFPS to maximize the activity of aerobic decomposers (Linn and Doran 1984), and placed in a 1-L Mason jar along with 8 ml of 1 M NaOH in a 50-ml vial to trap CO_2 and another vial containing 8 ml of water to maintain the humidity. The jars were incubated at 25 °C, in the dark, for 90 days. The NaOH traps were replaced at 7, 21, 49, and 90 days after incubation. A set of cores (n=72) were destructively sampled at 7, 30, and 90 days for soil analyses. Soil moisture content was determined at each observation by weighing the soil before and after a 24-h oven-drying at 105 °C to correct the results to an oven-dry basis.

4.2.5 Soil measurements

Soil pH was measured on moist soil with a pre-calibrated pH meter (Thermo Orion 720A+, USA) after extracting the soil with 0.01 M $CaCl_2$ (1:5) and shaking on an end-over-end shaker for 1 h followed by centrifugation at 492 × *g* for 10 min at each sampling time.

Carbon dioxide evolved was quantified by titrating a 2 ml aliquot of each NaOH trap with standardized 0.5 N HCl using a digital burette (Titrette, Germany) according to Zibilski (1994). To quantify the ^{13}C abundance of the CO_2 trapped, a 2 ml aliquot of each trap was mixed with 2 ml of 1.0 M $SrCl_2$ solution and 15 ml of Milli-Q water in a 50-ml conical flask to form $SrCO_3$ precipitate (Harris et al. 1997). The pH of the solution was neutralised by drop-wise addition of 0.5 M HCl into the flask in which pH probe had been immersed under magnetic stirring. The solution was transferred to a 50-ml tube and centrifuged at 1579 × *g* for 3 min and supernatant were discarded. The precipitate was resuspended and washed with 40 ml of Milli-Q water three times after centrifugation at 2808 × *g* for 6 min, 702 × *g* for 3 min and 274 × *g* for 3 min. Finally, each precipitate and 1 ml of Milli-Q water were vortexed, transferred to a glass vial and oven-dried at 60 °C. Determination of the ^{13}C abundance ($\delta^{13}C$ Pee Dee Belemnite, PDB) within precipitates was performed with Isotope Ratio Mass Spectrometry (SerCon 20-22, Crewe, UK).

Both microbial biomass C (MBC) and N (MBN) at each sampling time were determined according to the chloroform fumigation-extraction method described by Brooks et al. (1985) and Vance et al. (1987). Briefly, 8 g of fresh soils were fumigated with ethanol-free chloroform in a desiccator for 24 h at 25 °C. After removal of the fumigant, the soils were extracted for 1 h on an end-over-end shaker with 40 ml 0.5 M K_2SO_4 solution (1:5, w v^{-1}) and filtered through Whatman 42 (Whatman International, Maidstone, England). Another 8 g of non-fumigated samples were also extracted in the same way as formerly described at the time the fumigation commenced. Extracts were stored frozen at -20 °C before they were analysed. Total C within extracts was quantified colorimetrically following chromic acid digestion (Heanes 1984). Total N within extracts were determined by the phenol hypochlorite reaction and copperized-Cd reduction with a flow injection analyser (FIA) (QuickChem 8500, Lachat Instruments, USA) following an alkaline persulphate oxidation (Cabrera and Beare 1993). Microbial biomass C and N were computed as the differences between their respective concentrations, in the fumigated and non-fumigated samples. The extraction efficiency was adjusted by a factor of 0.45 for C and 0.54 for N (Brookes et al.

1985; Jenkinson et al. 2004). The extractable C from non-fumigated extracts was assigned as K_2SO_4-extractable C. Inorganic N (NH_4^+-N and NO_3^--N) from non-fumigated and non-oxidised extracts (3 ml) was also determined by FIA as previously mentioned.

4.2.6 Calculations and statistical analyses

The C priming effect of the residues was calculated by using the following equations (Cheng 1996):

$$C_R = C_T \times (\delta_T - \delta_C)/(\delta_R - \delta_C)$$

$$C_{SOC\text{-}AME} = C_T - C_R$$

$$PE = C_{SOC\text{-}AME} - C_{SOC\text{-}CON}$$

where $C_T = C_R + C_{SOC\text{-}AME}$, and is the total CO_2 release from residues and amended soil, C_R is CO_2 release from residues, δ_T is $\delta^{13}C$ value of C_T, δ_C is $\delta^{13}C$ value of CO_2 release from non-amended control soil, δ_R is $\delta^{13}C$ value of residues, $C_{SOC\text{-}AME}$ is SOC-derived CO_2 from amended soil, $C_{SOC\text{-}CON}$ is SOC-derived CO_2 from the control soil, and PE is priming effect of residues. As indigenous SOC contents varied between the lime treatments (Table 4.1), the magnitude of C priming was then normalised to per gram of SOC for Figure 4.2 and Table 4.3. Taking into account the possibilities of inorganic C contribution from the soils which once received 25 and 50 t ha^{-1} lime to the total CO_2 release, inorganic C content of these soils before and after incubation study were determined. The results showed that the potential contribution of inorganic C to the CO_2 release in the soils that received 25 (~0.2-0.7% total CO_2 released) and 50 t ha^{-1} lime (~0.6-1.0%) during the study was negligible and thus was unlikely to overestimate the priming effect of these limed soils.

The results presented are the means of three replicates and are expressed on an oven-dried basis (105 °C). All the data were checked for normal distribution and homogeneous variances. Three-way ANOVA were performed to scrutinize the main effects and possible interactions between treatment factors at each sampling time using GENSTAT 16th Edition (VSN International, Hemel Hempstead, UK). Where significant effects (P<0.05) were found, the least significant difference test (LSD) was used to compare the means. Curve fitting was employed to investigate the relationships between soil pH and residue decomposition, and between soil pH and C priming effect (Sigma 13.0, Systat Software Inc., Chicago, USA).

4.3 Results

4.3.1 Soil pH

Incorporation of both wheat and field-pea residues had a similar effect on soil pH in both lime trials. Residue addition increased the pH by 0.4 and 0.3 units in the soils with initial pH 4.8 and 5.7, respectively, but it had no significant effect in the soils with pH 6.6 and 7.3. Irrespective of residue treatment, in the soils with initial pH of 7.3, the pH was first decreased ($P<0.001$) up to 0.2 units during the first week of incubation and gradually increased back to values of initial pH by the end of incubation (data not presented).

4.3.2 Carbon dioxide (CO_2) release

Increasing initial soil pH significantly ($P<0.001$) increased CO_2-C efflux. The CO_2-C efflux was 9%, 21% and 29% greater at pH 5.7, 6.6 and 7.3, respectively, relative to the low pH (4.8) soils (Fig. 4.1). The most rapid CO_2-C efflux occurred during the first week, and then the rate declined over time.

The total CO_2-C emission was significantly greater (6%) in soils amended with wheat residue compared to the field-pea-amended soils. The dynamic pattern of CO_2-C efflux during the incubation period varied between the two residues. During the first week, the CO_2-C release was significantly higher (20%) with field-pea residue but thereafter it was on average 21% higher with wheat residue. However, the differences in total CO_2-C evolution between the two lime trials were not significant (Fig. 4.1 and Table 4.2).

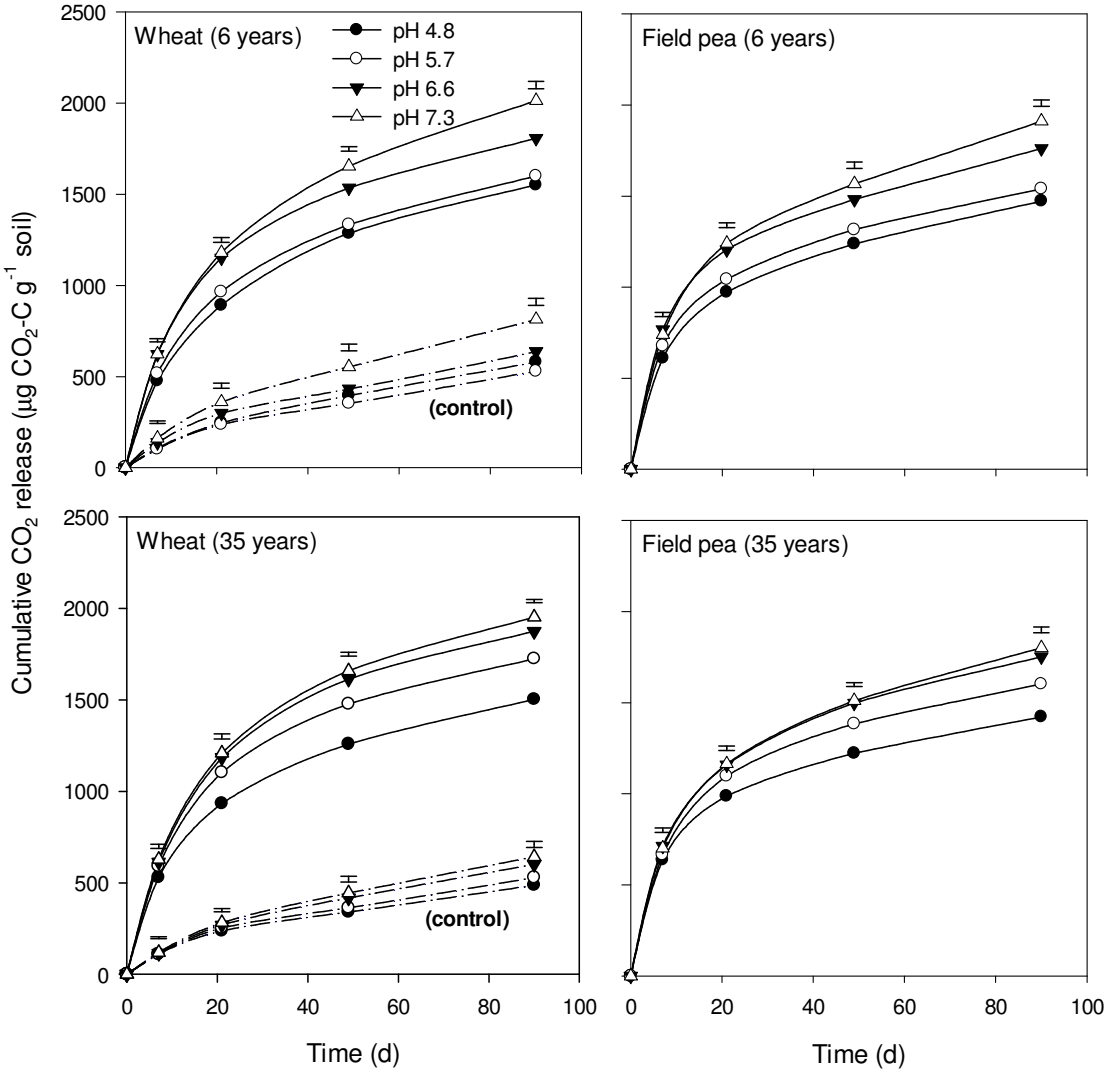

Figure 4.1: Cumulative total CO_2 release from soils incubated without residue (control) or with wheat (*left*) or field-pea (*right*) residue. The soils differing in initial pH were from two lime trials with either 6 (*top*) or 35 years (*bottom*) since liming. Vertical bars indicate the least significant difference (P=0.05) between means for each sampling time.

Table 4.2: The main effects of initial pH, residues and liming history on the cumulative CO_2 release, during the 90-day incubation.

Factor	Treatment	Cumulative CO_2 release (µg CO_2-C g^{-1} soil)			
		7 days	21 days	49 days	90 days
Initial pH	4.8	564	946	1251	1487
	5.7	613	1052	1377	1617
	6.6	679	1175	1532	1799
	7.3	673	1198	1598	1920
	LSD (*P*=0.05)	*10*	*12*	*11*	*13*
		***	***	***	***
Residue	Wheat	575	1077	1477	1753
	Field pea	689	1109	1402	1658
	LSD (*P*=0.05)	*7*	*8*	*8*	*9*
		***	***	***	***
Liming history	New	630	1081	1426	1707
	Old	635	1105	1453	1704
	LSD (*P*=0.05)		*8*	*8*	
		NS	***	***	NS

All values represent means (n=3). NS: not significant at *P*=0.05; ***: significant at the *P* value <0.001.

4.3.3 Primed soil organic C (SOC)

Like total CO_2 release, the cumulative SOC primed per gram indigenous SOC significantly (*P*<0.001) increased with increasing initial pH. However, it reached a maximum at pH 6.6, and then decreased with further increasing pH to 7.3. The greatest C priming was observed at pH 6.6 which was 37% and 20% greater than C priming at low (4.8) and high pH (7.3) (Fig. 4.2).

There was a highly significant (*P*<0.001) difference in cumulative C priming between the two residue treatments. Total C priming from soils with filed-pea residue was about 18% greater than those with wheat residue over 90 days of incubation (Fig. 4.2). However, there was a remarkable difference in the dynamic pattern of C priming between the two residues across the incubation time. Particularly, during the first week of the incubation, the C priming with field-pea was about 6-fold greater than with wheat residue, while that was about 34% greater in wheat-amended soils for the rest of the incubation period (Fig. 4.3). Moreover, the liming history also had a significant (*P*<0.001) effect on C priming. In limed soils (pH 5.5, 6.6 or 7.3) SOC primed was on average about 8% higher in the 35-year-old

than 6-year-old limed soils at the end of the study (Fig. 4.3 and Table 4.3). However, the temporal dynamic of SOC primed during the 90-day incubation period was considerably different between the two lime trials. In the first week of incubation, the SOC primed from the 6-year-old limed soils was significantly higher (16%) than its 35-year-old counterpart, whilst the latter was up to 18% greater during days 8-49. Nevertheless, there was no significant difference in those between the two lime trials at the last incubation phase of days 50-90 (Fig. 4.3 and Table 4.3).

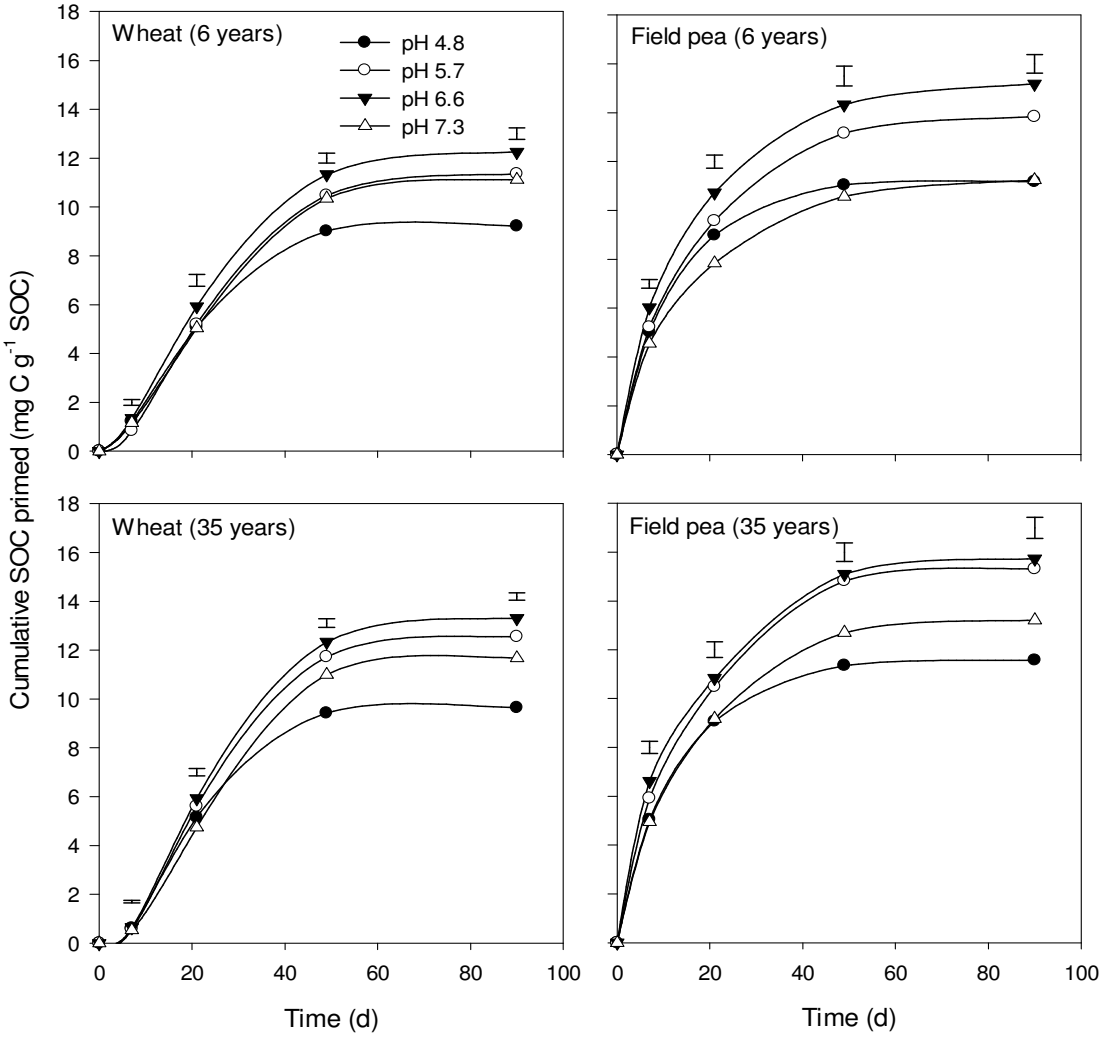

Figure 4.2: Cumulative amount of primed C per gram of indigenous SOC over 90 days in response to the addition of wheat (*left*) or field-pea (*right*) residue to soils differing in initial pH. The soils were from two lime trials with either 6 (*top*) or 35 years (*bottom*) since liming. Vertical bars indicate the least significant difference (P=0.05) between means for each sampling time.

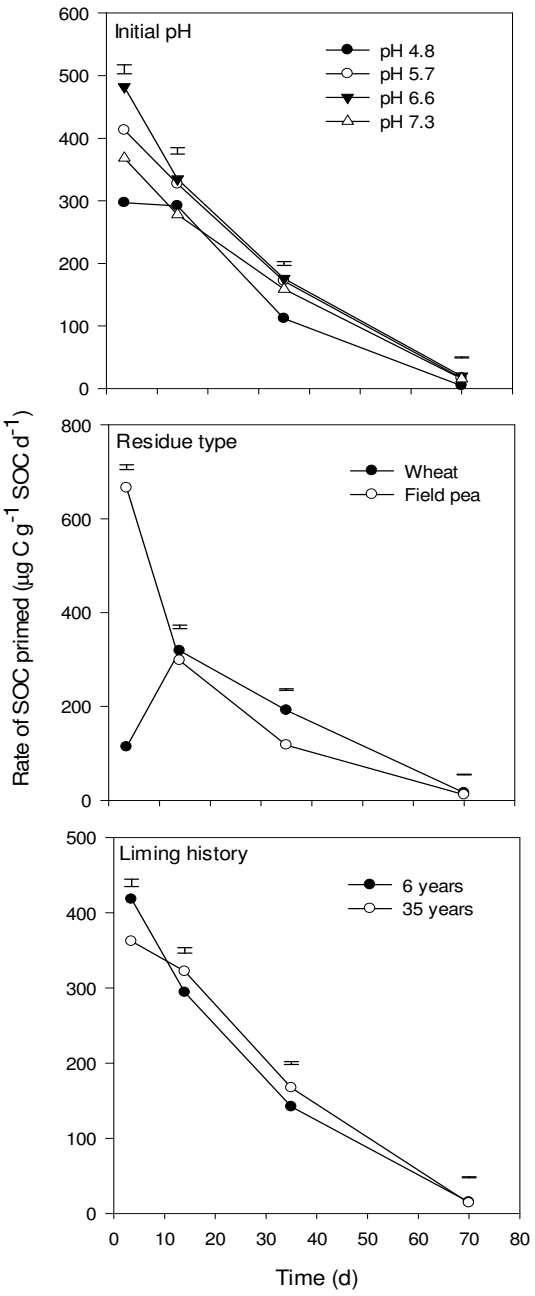

Figure 4.3: The main effects of initial pH, residue type and liming history on the rate of soil organic C mineralization (SOC primed), in response to the addition of wheat or field-pea residue to soils differing in initial pH. The soils were from lime trials with either 6 or 35 years since liming. Vertical bars indicate the least significant difference (P=0.05) between means for each sampling time.

Table 4.3: The main effects of initial pH, residue and liming history on the cumulative SOC primed during the 90-day incubation.

Factor	Treatment	Cumulative SOC primed (mg C g^{-1} soil C)			
		7 days	21 days	49 days	90 days
Initial pH	4.8	2.98	7.07	10.21	10.40
	5.7	3.14	7.72	12.54	13.26
	6.6	3.66	8.35	13.27	14.12
	7.3	2.80	6.70	11.15	11.81
	LSD (*P*=0.05)	*0.15*	*0.23*	*0.27*	*0.28*
		***	***	***	***
Residue	Wheat	0.87	5.34	10.70	11.39
	Field pea	5.42	9.58	12.88	13.41
	LSD (*P*=0.05)	*0.10*	*0.16*	*0.19*	*0.20*
		***	***	***	***
Liming history	New	3.18	7.30	11.28	11.92
	Old	3.11	7.62	12.30	12.87
	LSD (*P*=0.05)	*0.10*	*0.16*	*0.19*	*0.20*
		NS	***	***	***

All values represent means (n=3). NS: not significant at *P*=0.05; ***: significant at the *P* value <0.001.

4.3.4 Residue-derived CO_2-C

The residue-derived CO_2-C was markedly affected by the initial soil pH with a general trend similar to that of total CO_2 release. Overall, the decomposition of wheat was 15% greater than field-pea residue at the end of the 90-day study (Fig. 4.4). However, the dynamic of decomposition rate with incubation succession varied between the two residues. The decomposition rate of field-pea residue was significantly (*P*<0.001) greater (7%) than that of wheat residue during the first week, but was 6-14% lower thereafter (Fig. 4.5). Furthermore, the overall residue decomposition rate was 11% faster in the 35-year-old than the 6-year-old limed plots (Fig. 4.5).

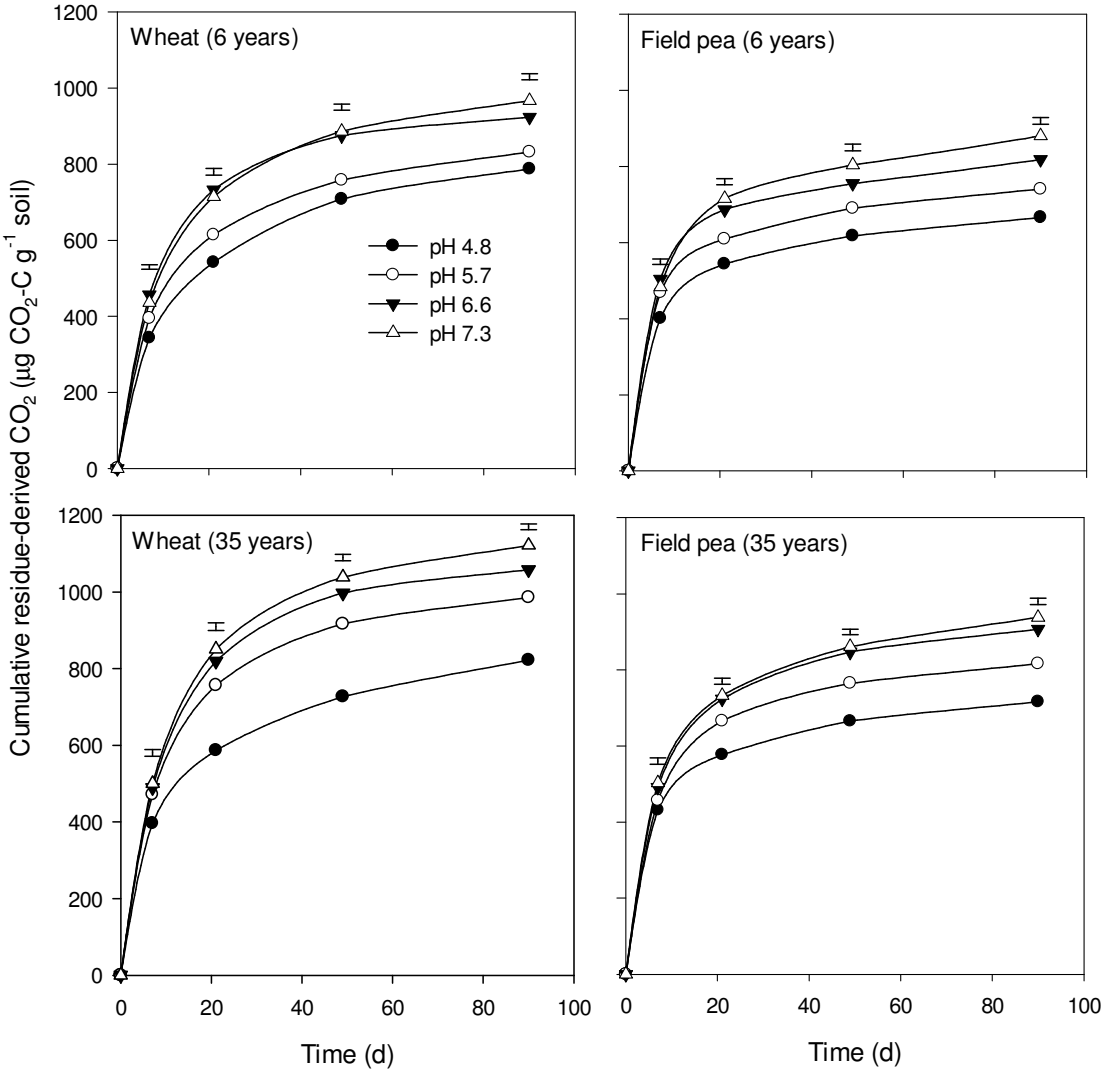

Figure 4.4: Cumulative residue-derived CO_2 release of soils differing in initial pH incubated with wheat (*left*) or field-pea (*right*) residue. The soils were from two lime trials with either 6 (*top*) or 35 years (*bottom*) since liming. Vertical bars indicate the least significant difference (P=0.05) between means for each sampling time.

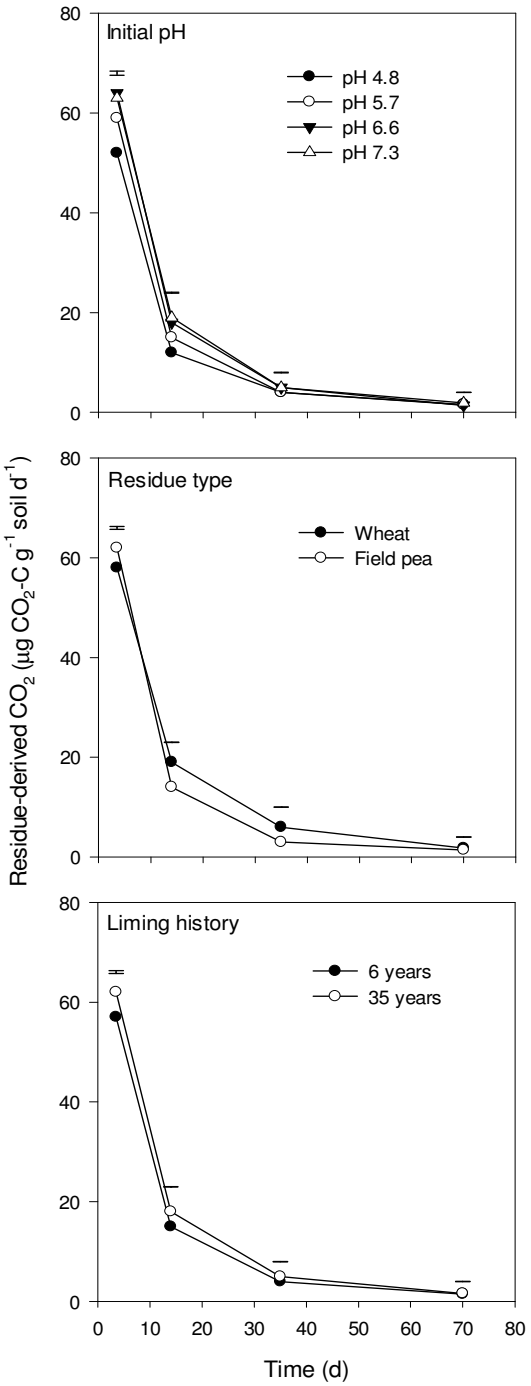

Figure 4.5: The main effects of initial pH, residue type and liming history on the rate of residue-derived CO_2 release in response to the addition of wheat or field-pea residue to soils differing in initial pH. The soils were from two lime trials with either 6 or 35 years since liming. Vertical bars indicate the least significant difference (P=0.05) between means for each sampling time.

4.3.5 Relationship between initial soil pH and cumulative CO_2 release from SOC and residues

The cumulative SOC primed during the 90-day incubation period increased with increasing initial pH, reaching the maximum at around pH 6.5, and then decreased with further increasing the soil pH. In comparison, the residue-derived CO_2-C increased linearly with initial pH (Fig. 4.6).

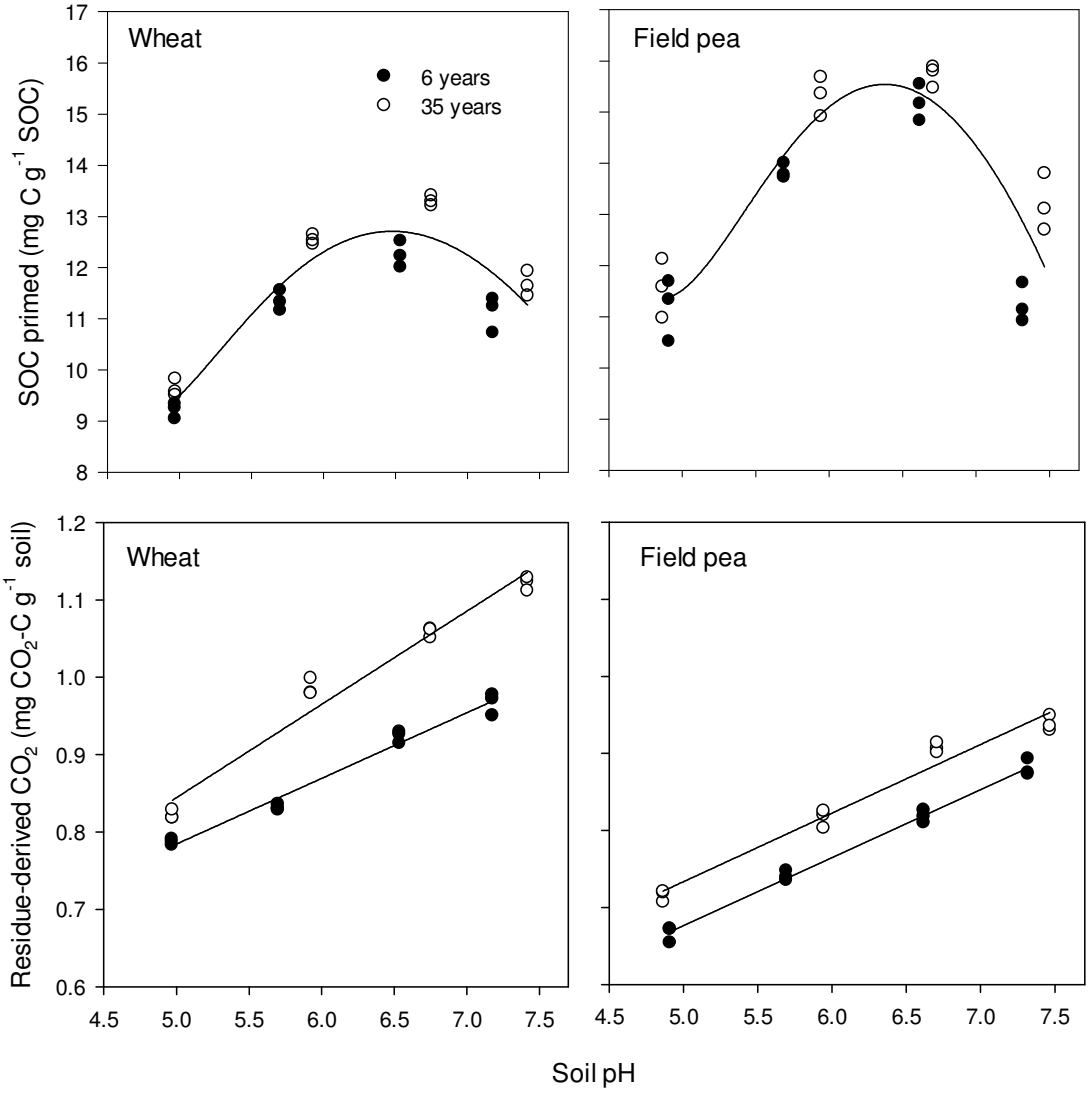

Figure 4.6: Relationships between initial soil pH and the amounts of soil organic C (SOC) primed in response to the addition of wheat or field-pea residue to soils from two lime trials with either 6 or 35 years since liming (*top*) and residue decomposed (*bottom*).

4.3.6 Microbial biomass C and N

Initial soil pH had a great impact on MBC and MBN (Table 4.4), and its effect generally followed the same pattern as the effect on SOC primed. Both MBC and MBN increased with soil pH up to 6.6 and then decreased at pH 7.3 The MBC and MBN decreased with time except that the MBN in the no-residue controls was the highest at day 30 (Table 4.4).

The residue type had some effects on MBC and MBN with this effect varying over time. Specifically, MBC and MBN of the two residue treatments did not significantly differ at day 7 but were 23% and 8% greater with wheat than with field-pea residue, respectively, at day 30. In contrast, the MBN with field-pea residue was 14% larger at day 90 (Table 4.4).

Liming history had only a minimal effect on both MBC and MBN. The 35-year-old limed soils had significantly (P<0.05) greater MBC (4-6%) and MBN (4%) during days 0-30, compared to the 6-year-old soils (Table 4.4). There was a significant (P<0.05) Liming history × Residue effect on MBC at all three sampling times and on MBN at days 30 and 90. The interaction on MBC resulted from differences in the MBC between the two lime trials which only occurred with wheat residue but not with field-pea residue. At days 30 and 90, MBN of wheat residue-amended soil was higher in 35-year than that of 6-year-old trial, whereas the MBN with field-pea residue was higher in the 6-year-old trial (Table 4.4).

Table 4.4: The main effects of initial pH, residue and liming history, and the effect of the residue × liming history interaction, on the C and N in the microbial biomass during the 90-day incubation.

Factor	Treatments	Microbial biomass C (µg g^{-1} soil)			Microbial biomass N (µg g^{-1} soil)		
		7 days	30 days	90 days	7 days	30 days	90 days
Initial pH	4.8	282	217	175	22.4	20.7	19.8
	5.7	311	240	188	26.9	23.3	21.1
	6. 6	350	272	209	31.3	26.4	23.6
	7.3	326	272	189	30.3	25.6	22.9
	LSD (*P*=0.05)	*16* ***	*14* ***	*14* ***	*1.1* ***	*0.8* ***	*1.8* ***
Residue	Control	217	201	172	18.5	19.8	19.3
	Wheat	366	303	198	32.0	27.3	21.5
	Field pea	368	246	201	32.6	24.9	24.6
	LSD (*P*=0.05)	*14* ***	*12* ***	*12* ***	*0.92* ***	*0.69* ***	*1.54* ***
Liming history	New	311	243	191	27.1	23.6	21.9
	Old	324	258	190	28.3	24.5	21.7
	LSD (*P*=0.05)	*11* *	*10* **	NS	*0.8* **	*0.6* **	NS
Residue × Liming history	Control - New	217	205	176		20.0	19.8
	Control - Old	217	197	169		19.6	18.9
	Wheat - New	348	276	188		25.5	20.0
	Wheat - Old	385	331	208		29.1	23.0
	Field pea - New	367	248	208		25.1	26.0
	Field pea - Old	369	245	193		24.8	23.2
	LSD (*P*=0.05)	*20* *	*17* ***	*17* *		*1.0* ***	*2.2* **

All values represent means (n=3). NS: not significant at *P*=0.05; *, **, and ***: significant at the *P* values <0.05, <0.01, and <0.001, respectively.

4.3.7 K_2SO_4-extractable C and inorganic N

Like the effect on total CO_2 release and residue decomposition, increasing the initial pH generally increased the concentration of K_2SO_4-extractable C with the magnitude decreased with incubation time (Table 4.5). There was a significant (P<0.05) difference in the concentration of K_2SO_4-extractable C between the two residue treatments with 9 and 14% greater with wheat than with field-pea residue at days 7 and 90. The K_2SO_4-extractable C was 9-23% greater (P<0.001) in 6-year-old than 35-year-old limed plots at all observations in 90 days (Table 4.5).

The treatment effects on inorganic N ($NH_4^+ + NO_3^-$) did not follow the same pattern as those on the extractable C (Table 4.5). The significant increase in inorganic N ($NH_4^+ + NO_3^-$) concentration with increasing initial pH exhibited at days 30 and 90 which reached the maximum at pH 6.6. It was the greatest in the non-amended control and smallest in the wheat-amended soils across the incubation study. The significant effect ($P<0.001$) of liming history on the concentration of inorganic N displayed only at day 30 and it was 13% greater in the 35-year-old than the 6-year-old limed plots (Table 4.5). There were Residue × Liming history and Initial pH × Liming history interactions (Table 4.5). The Residue × Liming history interaction was inconsistent with incubation time. In comparison, the old limed soils had greater concentrations of inorganic N at initial pH of 4.8 and 5.7 while the opposite was true at a higher initial pH, leading to the Initial pH × Liming history interaction.

Table 4.5: The main effects of initial pH, residue and liming history, and the interaction effects of residue × liming history and initial pH × liming history, on the concentrations of K_2SO_4-extractable C and inorganic N ($NH_4^+ + NO_3^-$) in soil, during the 90-day incubation.

Factor	Treatments	K_2SO_4-extractable C (µg g^{-1} soil)			Inorganic N (µg g^{-1} soil)		
		7 days	30 days	90 days	7 days	30 days	90 days
Initial pH	4.8	159	110	78	16.8	32.3	57.7
	5.7	173	118	84	16.3	30.5	59.9
	6.6	202	130	98	17.6	34.5	66.7
	7.3	237	151	121	16.1	33.3	66.2
	LSD (*P*=0.05)	*16* ***	*7* ***	*6* ***	NS	*2.2* **	*3.4* ***
Residue	Control	161	106	85	39.2	51.8	71.6
	Wheat	218	138	107	2.7	14.2	51.0
	Field pea	200	138	94	8.2	32.0	65.2
	LSD (*P*=0.05)	*14* ***	*6* ***	*5* ***	*0.7* ***	*1.9* ***	*3.0* ***
Liming history	New	211	140	99	16.4	30.1	62.9
	Old	175	114	91	17.0	34.4	62.4
	LSD (*P*=0.05)	*11* ***	*5* ***	*4* ***	NS	*1.5* ***	NS
Residue × Liming history	Control - New	170	107		39.3	47.6	
	Control - Old	151	106		39.0	55.9	
	Wheat - New	235	153		2.9	13.0	
	Wheat - Old	201	122		2.4	15.3	
	Field pea - New	227	159		6.9	31.8	
	Field pea - Old	173	116		9.5	32.2	
	LSD (*P*=0.05)	*19* *	*9* ***		*1.9* *	*2.7* ***	
Initial pH × Liming history	4.8 - New				15.5	26.5	53.2
	- Old				18.0	38.1	62.3
	5.7 - New				15.1	27.4	57.2
	- Old				17.4	33.7	62.6
	6.6 - New				18.2	35.3	71.7
	- Old				17.1	33.7	61.7
	7.3 - New				16.8	34.1	69.3
	- Old				15.4	32.4	63.2
	LSD (*P*=0.05)				*2.2* *	*3.1* ***	*4.8* ***

All values represent means (n=3). NS: not significant at *P*=0.05; *, **, and ***: significant at the *P* values <0.05, <0.01, and <0.001, respectively.

4.4 Discussion

4.4.1 Effect of initial pH

The initial pH of the soils greatly influenced the magnitude of priming effects induced by incorporation of crop residues. Importantly, this study revealed that the optimum pH for the priming occurred around pH 6.5. In contrast, residue decomposition increased linearly with initial pH up to the maximum of pH 7.4 (Fig. 4.6). The lower optimum pH observed for the priming effect than for residue decomposition is probably a function of the dominant decomposer organisms and associated metabolic capacity including the concentration of extracellular oxidative enzymes at the time of C substrate addition as suggested by Rousk et al. (2016) and their location within the soil. Ivarson (1977) observed that although total bacterial biomass increased with increasing pH up to 7.5, only fungal community composition was different at pH 5.5 and 7.5. Even though the direct effect of soil pH on fungal abundance was weaker than that on the bacteria community, indirect effects due to competitive interactions between bacteria and fungi at different pH values would have changed fungal community composition (Rousk et al. 2010a). Such changes in microbial composition could be partly responsible for this lower priming effect in the high initial pH (>7) relative to the moderately high pH (6.6) soils of the present study. Differences in competition for energy and nutrients between fresh organic matter-degrading and SOC-degrading microorganisms would also have contributed to this difference in priming among different pH levels as postulated by Fontaine et al. (2003). Moreover, variation in maintenance energy requirements of microbes among different soil pH would also have influenced microbial C-use efficiency, which plays a prominent role in C priming (Cotrufo 2013). However, relative importance of these mechanisms along with interdependence between bacteria and fungi in priming remains uncertain.

In other studies, greater positive C priming with increasing soil pH have been previously reported (Luo et al. 2011; Perelo and Munch 2005). Similarly, the decomposition of rice straw was greater in soils of high initial pH (6.3 and 7.1) than low pH (4.1) (Hu et al. 2012). Greater microbial biomass and microbial C-use efficiency associated with higher enzyme activity in soils of favourable pH (5-7) than in acidic environments are likely to be responsible for greater SOC and residue decomposition at high pH (Acosta-Martinez and Tabatabai 2000; Blagodatskaya and Anderson 1998; Dalenberg and Jager 1989).

However, lesser magnitude of C priming in slightly alkaline (7.3) than slightly acid (6.6) soil in this study was not in agreement with other studies. This could possibly be due to a negative impact of residual lime (0.1-0.2% inorganic C) remaining in the high pH soils (received 50 t ha^{-1} lime) on microbial growth and metabolic activity. There could be some free $CaCO_3$ (and high concentrations of HCO_3^-, Powlson and Jenkinson 1976), which may have negative effects on survival of some microorganisms (Bashan and Vazquez 2000; Farhangi et al. 2013). Yaganza (2009) suggested that osmotic stress causing an increased maintenance metabolism would have been partly responsible for the strong inhibition of bacteria growth by HCO_3^-. Garau et al. (2007) also observed a reduction in microbial population and β-glucosidase enzyme activity as soil $pH_{(H2O)}$ increased from 4.2 to 7.1 following liming. This reduction was proposed to be due to specific mineral deficiencies caused by carbonate. The lower (9%) microbial biomass in these slightly alkaline soils than slightly acid soils supports this hypothesis (Table 4.4). Similar to our findings, Aciego Pietri and Brookes (2008) also revealed that increases in microbial biomass with increasing soil pH reached maximum at around pH 6.7 and declined at higher pH in Chromic Luvisol soils with a liming history of more than 100 years.

Moreover, the 8% and 18% higher specific respiration rate (qCO_2) of our strongly acid (4.8) and slightly alkaline (7.3) than slightly acid (6.6) soils, respectively, (data not given) also indicates that microorganisms from both extremes in pH utilized more energy for maintenance (Anderson and Domsch 1993). The greater requirement of maintenance energy for microbes in these pH soils reflects its negative impact on the ratio of microbial growth to C uptake, i.e., microbial C-use efficiency (Manzoni et al. 2012). Lower microbial biomass and C-use efficiency in these pH soils might have been associated with a lower activity of extracellular enzymes (Dorodnikov et al. 2009) and consequently decreased the C priming compared to the slightly acid soils. The results highlight the important role of changes in pH following liming in microbial turnover of SOC.

4.4.2 Effect of residue type

The quality of plant residues added to the soils determined the magnitude of the priming effect and its dynamic with incubation time. The six-fold higher initial priming with field-pea (C:N 29) than with wheat (C:N 42) residue during days 0-7 (Fig. 4.3) could be ascribed to easier degradability of the field-pea residue, as indicated by Hobbie (2005). The results are consistent with previous findings that the application of easily degradable residues with

low C:N induced a greater priming effect than the application of high C:N residues during early stages of incubation (Conde et al. 2005; Thiessen et al. 2013; Wang et al. 2015a). The greater CO_2 release and decomposition of field-pea relative to wheat residue during the first week of incubation indicate the greater microbial activity with the former residue (Figs. 4.1 and 4.4). However, during this first week of incubation, even though the priming effect was considerably greater with field-pea relative to wheat residue, the residue type had little effect on microbial biomass C (Table 4.4). This indicated that microbial activity was more strongly influenced by residue C:N ratio than microbial biomass C as suggested by Nguyen and Marschner (2015).

The rapid decomposition of field-pea residue would have supplied a greater amount of C substrate to microorganisms than wheat residue in the first week of incubation. This energy-rich C source would have facilitated the synthesis of SOC-degrading enzymes (Fontaine et al. 2011; 2003) which are capable of breaking down the added residues and decomposing native SOC, enhancing the priming effect according to the co-metabolism theory (Kuzyakov et al. 2000b). Greater increase in microbial activity due to readily degradable immature wheat and vetch residues incorporation compared to the mature wheat residue at the beginning of incubation study was also observed (Moreno-Cornejo et al. 2015). Dilly et al. (2007) demonstrated that an increased production of enzymes after C amendment effectively degraded C polymers and caused a positive priming effect. During this process, microbes would also have obtained other essential nutrients, particularly N. However, the priming effect associated with field-pea residue declined sharply after the initial phase of incubation (days 0-7) in this present study. Such a diminishing priming effect could be partly due to an exhaustion of labile C substrate, and reductions in microbial activity, indicated by the 66% and 33% reduction in residue-derived CO_2-C, and in microbial abundance from the first to the second sampling time (Fig. 4.5 and Table 4.4). Besides, this decrease in C priming could also possibly be coupled with depletion of labile nutrients which are required for the microbes to produce enzymes for SOC decomposition (Koyama 2013). This assumption was in agreement with Joshi et al. (1993), who demonstrated that microbial abundance increased rapidly as litter decomposition progressed, and then decreased towards the end of the process. Increases in microbial abundance following incorporation of this field-pea residue would likely increase microbial assimilation of substrate C which then enhanced the contribution of microbial metabolites to SOC stabilization according to the

new paradigm of C stabilization proposed by Cotrufo et al. (2013). Flessa (2000) also observed that litter with high N contributed a higher leaching of C to the mineral soil.

The study showed considerably lower priming effect of wheat than field-pea residue during the first week of incubation (Fig. 4.3). This result indicated the existence of a lag phase in the priming effect of wheat residue which might be attributed to its low N content (9.7 g N kg^{-1}) compared to field-pea residue (14.3 g N kg^{-1}). A N shortage rather than C shortage in wheat residue-amended soils would have limited microbial activity at this early incubation stage (Merckx et al. 1987; Knapp et al. 1983). Jenkinson et al. (1985) also suggested that when soils contain plenty of fresh organic materials of wide C:N, microbial N demand cannot be met from inorganic N reserves. Besides, Henriksen and Breland (1999) also demonstrated that a residue N concentration below 12 g kg^{-1} markedly reduced its mineralization and the growth of soil microorganisms. Moreover, N limitation is also known to negatively impact on the production of extracellular enzymes necessary to degrade polymers contained within high C:N wheat residue (Jingguo and Bakken 1997). Generally, decomposer organisms have lower C:N ratios than most residues (Berg and McClaugherty 2003) and they immobilize inorganic N during the early phase of decomposition (Manzoni et al. 2008).

Furthermore, the approximate 3-fold lower concentration of inorganic N in wheat than field-pea residue-amended soils was also likely an additional cause of this one-week lag phase with wheat residue. The slower microbial activity in soils with wheat residue at this stage coincided with less CO_2 release and slower residue decomposition compared with field-pea residue (Figs. 4.1, 4.2, 4.4, Table 4.5). Notwithstanding, after this one-week delay, the priming effect with wheat residue caught up with that of field-pea residue and even surpassed from 3 weeks onwards (Fig. 4.3). This could be partially attributed to slow and progressive increases in labile C substrates and inorganic N for microorganisms from gradual decomposition of the wheat residue (Fig. 4.4 and Table 4.4). Such progressive decomposition of high C:N (>45) residues was also reported during days 45 onwards in a previous incubation study (Kriaučiūnienė et al. 2012). Increased availability of C and N substrate with progressive decomposition would have led to both co-metabolism and N-mining phenomena to occur simultaneously during days 8-21 when the priming effect reached the maximum. Microbial N-mining occurs whereby microbes acquire N from SOC to balance available C substrate in N-limited environments (Fontaine et al. 2003). These two

mechanisms are not mutually exclusive despite the fact that one mechanism could dominate at any given period depending on C substrate quality and nutrient availability (Cheng and Kuzyakov 2005). However, the low indigenous soil N (1.48-1.73 g kg^{-1}) (Table 4.1) and high N immobilization (Table 4.5) indicated that the predominant mechanism for this surged priming effect was mainly microbial N-mining while acknowledging the contribution of other proposed mechanisms (Kuzyakov et al. 2000b). This also corresponds well with Craine et al. (2007) who suggested that the priming effect caused by N-mining should be greatest when labile C is available and N is shortage.

A further driving mechanism behind this considerable increase in the priming effect with wheat residue after one week-lag phase could be accredited to changes in microbial community composition. Shift from fast-growing r-strategists to slow-growing K-strategists that are more competitive in decomposing substrates with low N can occur when N is limited (Blagodatsky et al. 2010). Substantially faster residue decomposition and a greater priming effect with wheat residue after day 7 (Figs. 4.3 and 4.4) might have partially resulted from increased N-mining by these K-strategists. This intensive C priming coincided with increased microbial respiration, residue decomposition and microbial biomass (Figs. 4.1, 4.3, 4.4, Table 4.5).

The different effects of microbial C-use efficiency and nutrient availability on the C priming highlight the importance of liming acid soils to maintain optimum soil pH (around 5.5-6.0) for crop production. An optimum soil pH would create a favourable environment for soil microbes and increase plant biomass production while minimizing SOC loss through priming. Further studies are needed to understand the complexation of C priming effects by integrating the effect of residue type on SOC decomposition with changes in microbial community composition and their functional capabilities.

4.4.3 Effect of liming history

The greater priming effect in 35-year-old than 6-year-old limed soils in this study might be partially ascribed to the effect of duration since liming on microbial biomass and community composition. The larger microbial biomass in the soils from 35-year-old limed plots would have utilized more C from decomposition of added residues as an energy source, leading to the greater priming effect through the mechanisms of co-metabolism and microbial N-mining relative to the 6-year-old limed soils (Figs. 4.2, 4.4, Table 4.5). The consistently

lower K_2SO_4-extractable C in the 35-year than the 6-year-old trial in the first 30 days despite faster decomposition of residues in the former indicates the greater microbial demand and utilization of labile C substrate in the former than the latter trial (Fig. 4.4 and Table 4.5). Greater decomposition of wheat residue in soils from the older than newer limed plots suggests that microorganisms were more efficient in utilizing the higher C:N wheat residue. Substantially greater microbial biomass C upon wheat residue addition in older than the newer limed soils could also be attributed to lower soil fertility (Table 4.1). An et al. (2015) demonstrated that the contribution of straw C to microbial biomass C was more effective in low fertility soil than the high fertility soil. This was also in line with the finding of Moreno-Cornejo et al. (2015) that soils with lower indigenous SOC content had a higher microbial turnover rate and greater priming effect than soils with higher SOC. The combination of greater microbial biomass and activity and utilization of labile C substrate from intensive decomposition of residue would have led to greater N-mining and hence the priming effect, in soils from the older limed plots.

Moreover, different management practices between the two trials could be another reason for this greater priming effect in the 35-year than 6-year-old limed soils. Continuous cultivation and cropping in the 35-year-old limed soils had led to decreased SOC and water-stable macro-aggregates (Chapter 3). Physical protection of SOC within aggregates is one of the proposed SOC stabilization mechanisms (Lützow et al. 2006) in which macro-aggregate stability plays a central role (Six et al. 1998). As such, decreased aggregate stability could be responsible for the greater priming effect in the 35-year-old limed soils. Furthermore, microbial C-use efficiency in the volunteer pasture of newer limed soils could have been greater than that of older limed soils. Microbial community composition is expected to be different between the two trials and greater substrate-use efficiency could have led to lower priming effect in the 6-year-old relative to the 35-year-old limed soils. Bölscher et al. (2016) also observed that a grassland ecosystem had a higher microbial substrate-use efficiency compared to arable land.

4.5 Conclusions

This study used soils from long-term lime trials to investigate the role of soil pH in the priming effect following addition of crop residues. It showed that increased pH resulting from long-term liming of acid soils favoured the decomposition of native SOC (priming effect). The magnitude of the priming effect depended largely on the quality of the added

residues. Although faster during the initial period, cumulative decomposition of field-pea residue (low C:N) during the 90-day study was lower than that of wheat residue (high C:N). It appears that the incorporation of residues of low C:N ratio would favour soil C storage and improve soil fertility through increasing microbial C-use efficiency in the long term.

This study demonstrated for the first time that the priming effect on the decomposition of SOC had a lower optimal pH (pH 6.6) than the decomposition of the crop residue (pH 7.3). The lower optimal pH range for the priming effect relative to respiration and residue decomposition highlights variation in residue-decomposing and SOC-decomposing microbial communities in soil with different initial pH. The study implies that over-liming of acid soils will accelerate the decomposition of crop residues as well as native SOC, and should be avoided. In practice, lime should be applied to acid soils at a rate that increases a soil pH sufficient to obtain the most cost-effective crop productivity and residue inputs (e.g. pH 5.5) but low enough to minimise C losses from the soil. The study clearly showed that not only C substrate, but also N availability to decomposer organisms strongly influence the magnitude of priming effect. Hence, further research addressing interactive effects of residue types on priming effect in soils differing initial pH is warranted.

CHAPTER 5

Non-linear response of soil organic carbon mineralization to nitrogen addition under different pH conditions

5.1 Introduction

The need of attention to soil organic C (SOC) management has now been recognized for improving soil fertility and productivity on the one hand and soil C sequestration on the other hand (Lal 2006). As the soil is the largest terrestrial C pool and stores about 75% of total terrestrial C stock (Stockmann et al. 2013), understanding the impact of agricultural practices on SOC dynamics is vital. The SOC pool size depends on the balance between formation of SOC from the plant litter decomposition and its mineralization to CO_2 (Cotrufo et al. 2015). Several biotic and abiotic factors such as soil texture and structure, temperature, moisture, soil pH, nutrient status, and soil microbial biomass and activity along others influence SOC mineralization (Campbell et al. 1994; Curtin et al. 1998; Wang et al. 2013).

One of the common agricultural practices which greatly influence changes in SOC content and CO_2 emission is liming (Kemmitt et al. 2006; Persson et al. 1989). Lime is frequently applied to acid soils with the aim of raising soil pH and ameliorating the negative effects of acidity for plant growth. It is well known that increases in soil pH following liming stimulate SOC mineralization through increasing microbial biomass, decomposition activity (Fuentes 2006; Rangel-Castro 2005) and increasing labile organic C release (Curtin et al. 1998). Such trends were primarily attributed to either the decrease in toxic effects of free Al^{3+} on microbial growth and activity or the increase in readily-available organic C to microorganisms (Chang and Alexander 1984; Rousk et al. 2009). Despite soil pH being recognized as an important factor regulating soil microbial biomass, activity and community composition (Page et al. 2010; Tate 2000), the mechanisms responsible for altering mineralization rate due to changes in soil pH by liming are not well documented. As the land area being limed is expanding, a deeper understanding of its impact on SOC dynamics is of the utmost importance for sustainable crop production.

Perhaps rather soil nutrient status, particularly N also plays a vital role in SOC mineralization. Decomposer organisms usually acquire necessary inorganic nutrients either from soils or added organic materials. Among the nutrients, N is in the greatest demand and

therefore is often the first element to limit microbial activity in soil (Blair et al. 1994). There are conflicting results regarding to the effect of N on SOC mineralization and general conclusion has not been drawn so far. Cleveland and Townsend (2006) reported that addition of N enhanced SOC mineralization through eliminating N limitation to the decomposer organisms. In contrast, negative effects of N amendment on SOC mineralization have also been revealed (Bowden et al. 2004; Ramirez et al. 2010; Wang et al. 2014) and are generally ascribed to reducing the biomass and activity of microbial communities. However, another study did not find any significant effect of N on the mineralization (Thirukkumaran and Parkinson 2000). These discrepancies in the literature would have been yielded from differences in soil properties such as initial soil pH, quantity and quality of indigenous SOC and size, activity and composition of microbial communities besides different amounts of added N (Fog 1988; Ramirez et al. 2012).

Despite soil pH and nutrient availability playing an important role in SOC mineralization, understanding of their interactive effects on this phenomenon remains poor. Decreased SOC mineralization due to mineral N addition has generally been reported to be more intense in slightly acidic (pH 5.0-6.0) soils than in moderate to strongly acidic (pH 3.7-4.6) soils (Bradford et al. 2008; Mo et al. 2008; Ramirez et al. 2010). Notwithstanding, these studies were conducted on different soil horizons so the inconsistent results between low and high soil pH might have been confounded by other initial edaphic characteristics and soil microbial properties. However, long-term annual N application to soils with a wide range of soil pH_{H2O} (5-7 resultant from initial liming 100 years ago) in Park Grass, UK showed that microbial biomass and soil respiration decreased with higher N addition and lower pH (Rousk et al. 2011). Clearly, in order to gain unequivocal insight into the way in which soil pH underpins SOC mineralization upon N availability, the same soil type which has a uniform management history apart from soil pH should be studied. Hence, this experiment aimed to address the combined effects of soil pH and mineral N availability on SOC mineralization. We hypothesized that i) N addition would reduce SOC mineralization regardless of the initial soil pH value through reducing microbial mining of organic nutrients and ii) this reduction 'effect' would be more pronounced in soils with higher pH and greater amount of indigenous SOC, associated with larger microbial abundance and activity, due to greater reduction in microbial nutrient mining upon N availability.

5.2 Materials and Methods

5.2.1 Soil sampling

Soil samples were collected (0-10 cm) from two acid soils (pH 4.6) with contrasting SOC content. The soil from the La Trobe University Farm, Victoria, Australia (37°42´S, 145°02´E) was a Sodosol (Isbell 2002) or Solonetz (WRB IWG 2014) with total SOC 20.6 g kg^{-1}, total N 1.89 g kg^{-1}, C/N 10.9, clay 26% and silt 62 % and pH buffer capacity 13 $mmol_c$ kg^{-1} pH^{-1}. The soil with a higher SOC content from Hamilton, Victoria (37°82´S, 142°07´E) was a Chromosol (Isbell 2002) or Luvisol (WRB IWG 2014) with total SOC 39.9 g kg^{-1}, total N 3.2 g kg^{-1}, C/N 12.5, clay 20% and silt 64 % and pH buffer capacity 40 $mmol_c$ kg^{-1} pH^{-1}. The Sodosol was under volunteer weeds and Chromosol was under pasture at the time of sampling.

5.2.2 Soil pH manipulation

In order to investigate the effect of soil pH on SOC mineralization with and without mineral N, while minimizing differences in other soil properties, the pH of two acidic soils were manipulated by acidifying (1 M H_2SO_4) or liming ($CaCO_3$) to achieve the target pH values of 4.0 (acidified), 4.6 (control) and 6.0 (limed) prior to N amendments. The amounts of acid and lime required to reach the desired pH values were determined according to their respective pH buffer capacities and validated by performing preliminary incubations. In the limed treatment, the amount of lime needed was thoroughly mixed with air-dried soil followed by addition of Milli-Q water adjusted to field capacity. For acidification, the soil was shaken as in the lime treatment and the required amount of acid was diluted with the amount of water needed to adjust the soil to field capacity, and mixed. Non-manipulated control soil was also subjected to mixing and adjusted to field capacity to maintain similar conditions of disturbance and water content. The soils were then incubated at 30 °C for 3 weeks allowing reactions to complete and stabilize before measuring soil pH. The actual soil pH values achieved were the same as the target pH of 4.0 (3.97 ± 0.006; average ± SE), 4.6 (4.63 ± 0.008), and 6.0 (5.98 ± 0.014). The ^{13}C abundance of the control and lime-amended soils were also determined to ensure that there was no residual lime remaining. Similar values of ^{13}C atom% between the control (1.12%, 1.56%) and limed soils (1.13%, 1.59%, respectively) indicated that reaction with lime had been completed.

5.2.3 Experimental design

The experiment was carried out as a 2 × 3 × 4 factorial design in a randomized complete block design and replicated three times. The first factor was 2 soil types, Chromosol (Isbell 2002) or Luvisol (WRB IWG 2014) and Sodosol (Isbell 2002) or Solonetz (WRB IWG 2014) with contrasting SOC content (39.9 and 20.6 g kg^{-1}). The second factor was soil pH with 3 levels; pH 4.0 (acidified), 4.6 (control) and 6.0 (limed). The third factor was N at 4 levels: 0 (control), 30 (low N), 100 (intermediate N), and 300 µg N g^{-1} soil (high N).

5.2.4 Incubation study

Soils were pre-incubated at 40% water-filled pore space (WFPS) for 14 days at 25 ˚C in the dark to stabilize soil respiration after manipulating soil pH (Butterly et al. 2010). The container was opened every 3 days during incubation to provide adequate aeration. Thirty grams of each pre-incubated soil were weighed into a 50-cm^3 polypropylene vial. The corresponding amount of N was added to each soil as aqueous NH_4NO_3 adjusted to about 60% WFPS (Linn and Doran 1984) and mixed well using a spatula. The N levels of 30, 100 and 300 µg N g^{-1} soil, were equivalent to the addition of approximately 60, 200 and 600 kg N ha^{-1}, 0-15 cm soil depth, respectively. These rates were also within the range of N amendment in many other studies (Mo et al. 2008; Nottingham et al. 2015; Ramirez et al. 2010). The non-amended control was also subjected to the same procedure to eliminate disturbance effects. The polypropylene vials were then placed in individual 1-L air-tight Mason jars and incubated at 25 °C for 30 days. Changes in soil pH and CO_2 release were monitored throughout the incubation. Soil water content and microbial biomass C and N and mineral N (NH_4^+-N and NO_3^--N) concentrations were determined at the end of incubation (day 30).

5.2.5 Soil pH monitoring

Observing the dynamics of soil pH during incubation was carried out to ensure that the manipulated initial soil pH values were maintained throughout the incubation and not significantly altered by NH_4NO_3 addition. To avoid disturbing the primary experimental units, four sets of additional sub-units (5 g soil) in duplicates, which received identical treatments as primary microcosm units, were prepared in a 50-ml polypropylene tube. Soil pH of these was measured at 2, 4, 7, 15 and 30 days after incubation.

5.2.6 Soil analyses

In order to express results on an oven-dry basis, soil moisture content was determined immediately after termination of the experiment by weighing the soils before and after oven-drying at 105 °C for 24 h. Soil pH in $CaCl_2$ was measured with a 1:5 soil-to-solution ratio. Moist soil (5 g) was shaken with 25 ml 0.01 M $CaCl_2$ on an end-over-end shaker at 200 rpm for 1 h, centrifuged at 492 × *g* for 10 min, then pH was determined with a pre-calibrated pH meter (Thermo Orion 720A+, USA). The CO_2 evolution from the soil was determined in the headspace of Mason jars at 1, 2, 3, 7, 15 and 30 days after incubation by using an infra-red gas analyzer (Servomex 4210 Industrial Gas Analyser, Cowborough, UK). The jars were flushed with ambient air for 15 s following each CO_2 measurement. Then the CO_2 concentration was calculated using a calibration curve developed with jars injected with known volumes of pure CO_2. Cumulative CO_2 evolution during the 30-d incubation was calculated from the summation of CO_2 release during the 6 periods observed. Then, the CO_2 release was normalized to per gram of SOC (Fig. 5.1 and Table 5.1) because the two soils had different indigenous SOC content.

Microbial biomass C and N were determined using the standard chloroform fumigation-extraction technique (Brookes et al. 1985; Vance et al. 1987). Two portions of moist soil (equivalent to 8 g oven-dry soil) were weighed. One of the portions was extracted immediately with 40 ml 0.5 M K_2SO_4 solution (1:5, w v^{-1}) for 1 h, while the other was fumigated with ethanol-free chloroform for 24 h followed by extraction in the same manner. The soil suspensions were passed through Whatman #42 filter papers (Whatman International, Maidstone, England). The extracts were kept frozen at -20 °C until analysis. Total organic C was analysed by a GE Sievers Innovox Laboratory TOC analyzer, Australia. Total N in the extracts was converted to free nitrate by digestion in an autoclave using an alkaline solution of potassium persulphate at 120 °C for 30 min (Cabrera and Beare 1993). The nitrate was then measured by a flow injection analyser (FIA) (QuickChem 8500, Lachat Instruments, USA). Microbial biomass C and N were quantified by subtracting the corresponding concentrations in the non-fumigated sample from the fumigated pair. To correct incomplete extractability of microbial biomass C and N, variable conversion factors calculated based on the C and N flushes during fumigation (Yevdokimov et al. 2006) were used for each N rate and soil pH combination. The conversion factors ranged from 0.26 to 0.31 in Chromosol and from 0.22 to 0.27 in Sodosol. Total inorganic N (exchangeable NH_4^+-

N and NO_3^--N) from non-fumigated and non-oxidized extracts was also determined by FIA through phenol hypochlorite reaction and copperised-Cd reduction as mentioned above.

5.2.7 Statistical analyses

Three-way analyses of variances (ANOVA) were performed in GENSTAT 17th Edition (VSN International, Hemel Hempstead, UK) to investigate the main effects of soil type, initial pH, N rate and their possible interactions on dependent variables. Prior to performing analysis, the normality and homogeneity of variance assumptions were checked by residual plots analysis. If there was a significant effect (P<0.05), the Least Significant Difference (LSD) test was applied for multiple comparisons. To determine the relationship (whether linear or non-linear) between N rate and CO_2 release, a two-way ANOVA (pH × N) with N rate polynomial contrast was performed (Fig. 5.1 and Table 5.3).

5.3 Results

5.3.1 Soil pH

Little change in soil pH in response to N addition was observed at 2, 4, 7, 15 and 30 days after incubation. Addition of NH_4NO_3 did not significantly alter the soil pH from each of the three initial pH levels of both Chromosol and Sodosol with the exception of decreased soil pH of about 0.2 units upon high N amendment (300 µg N g^{-1} soil) in the soils of pH 6 during the later period (day 15-30) of incubation (data not presented).

5.3.2 Soil organic C (SOC) minerlization

Mineralization of SOC, determined as CO_2 release, responded non-linearly to the increasing rate of N application. Generally, SOC mineralization increased (6%) at low rate (30 µg N g^{-1}) but decreased (7%) at high rate (300 µg N g^{-1}) and did not change at intermediate rate (100 µg N g^{-1}) of N amendment relative to the non-amended control. There was a significant (P<0.001) interaction between initial pH and N rate, where SOC mineralization was increased by both low and intermediate N rates at pH 4.0, while that was only increased by the low N rate at pH 4.6 and 6.0. The net increase in SOC mineralization by low rate of N was also greater at pH 4.0 than at pH 4.6 and 6.0 (Fig. 5.1 and Table 5.1).

At the end of 30-day incubation study, mean cumulative SOC mineralization per gram of SOC was 10% higher in the C-rich Chromosol compared to that of the C-poor Sodosol,

as expected. In both soils, increasing soil pH increased SOC mineralization (P<0.001). The SOC mineralization was on average 11 and 18% greater in soils with pH 4.6 and 6.0 than that in pH 4.0, respectively. There was a significant interaction (P<0.001) between soil × initial pH resulted from greater increase in CO_2 release with increasing initial pH in Chromosol than Sodosol (Fig. 5.1 and Table 5.1).

5.3.3 Microbial biomass C and N

Mineral N exerted suppressive effects on microbial biomass C (MBC) and positive effects on microbial biomass N (MBN). Nitrogen application decreased MBC on average by 8%, whilst MBN was substantially increased by up to 15%, 64% and 179% at low, intermediate and high rates of N, respectively (Table 5.1). As net decreases in MBC in response to N addition were much less than the increases in MBN, the C:N ratio of microbial biomass decreased drastically from 14.1 to 5.2 in Chromosol, and from 10.6 to 3.0 in Sodosol (Table 5.1).

The MBC, MBN and microbial C:N ratio increased with increasing initial soil pH in both soils (Table 5.1). The MBC in soils with pH 4.6 and 6.0 was 31% and 62% greater than that in strongly acidic (pH 4.0) soils, respectively (Table 5.1). Likewise, the MBN was 28% and 41% greater in soils with pH 4.6 and 6.0 compared to those with pH 4.0. However, there was no significant difference in microbial C:N ratio between pH 4.0 and 4.6, while that of pH 6.0 was 11% higher than that of pH 4.0. Nevertheless, there was a significant pH × N effect on MBN, whereby the increase in MBN due to N addition was more pronounced at pH 6.0, (14-300%) relative to the pH 4.0 and 4.6 (10-200%) (Table 5.1).

As previously observed for total CO_2 release, the MBC and MBN and microbial C:N were 81%, 17% and 41% higher in the Chromosol than in the Sodosol, respectively (Table 5.1). However, there was a significant interaction (P<0.001) between soil × initial pH in these microbial properties. Specifically, the increase in the MBC and MBN with increasing initial soil pH were more prominent in the C-rich Chromosol (24-79%) than the C-poor Sodosol (30-35%), whilst the microbial C:N ratio increased up to 19% with increasing pH in Chromosol but the change in pH did not affect the ratio in the Sodosol (Table 5.1).

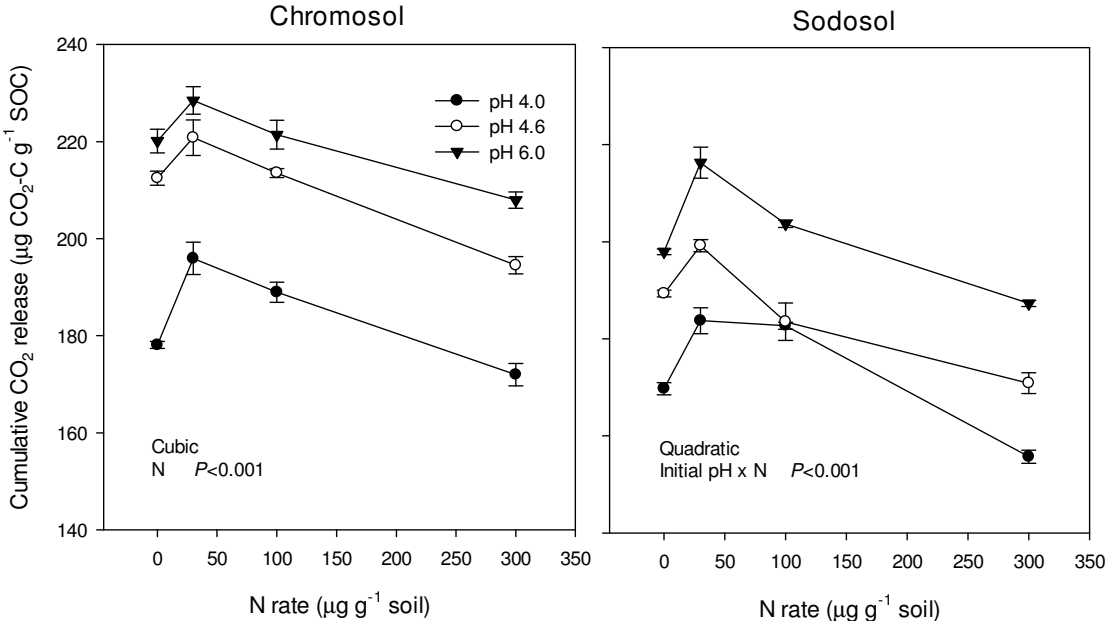

Figure 5.1: Cumulative CO_2 release per gram SOC of Chromosol (left) and Sodosol (right) in response to increasing N rate. CO_2 release increases compared to the control and peaks at 30 µg N g^{-1} and decreases at higher N rate with CO_2 release at 300 µg N g^{-1} is lower than the control. Error bars represent the standard error of the means (n=3). For each soil polynomial terms (cubic or quadratic) and the significance level of the regression are indicated.

There was also a strong significant interaction between soil type × N in MBC, MBN and microbial C:N ratio (Table 5.1). Any rate of N treatment in Chromosol decreased MBC, whereas it was only decreased by the high rate of N in Sodosol. The greater increase in MBN due to N addition in Sodosol than in Chromosol had led to this soil × N interaction. As a consequence, decreases in the microbial C:N ratio were much greater upon N amendment in Sodosol (106%) than in Chromosol (40%) (Table 5.1).

Moreover, there was a significant three-way interaction between soil type × initial pH × N on MBN and microbial C:N ratio. The greatest increase in MBN due to N application occurred at pH 4.6 in Chromosol, whereas that was lowest at pH 4.6 in Sodosol. Consequently, the greatest decrease in microbial C:N ratio due to N at pH 4.6 in Chromosol, where lowest decrease in that at pH 4.6 in Sodosol had caused this interaction (Table 5.1).

Table 5.1: The three-factor interactions (Soil type × Initial pH × N) on the cumulative CO_2 release, microbial biomass C (MBC) and N (MBN), and microbial C:N ratio (MBC/MBN), during the 30-day incubation.

Treatment	CO_2 (µg CO_2-C g^{-1} SOC)	MBC (µg C g^{-1} soil)	MBN (µg N g^{-1} soil)	MBC/MBC
Chromosol - pH 4.0				
Nil N	178	304	23.6	12.9
Low N	196	266	24.6	10.8
Medium N	189	264	32.9	8.0
High N	172	258	55.3	4.7
Chromosol - pH 4.6				
Nil N	212	376	25.6	14.7
Low N	221	361	29.6	12.2
Medium N	214	348	44.2	7.9
High N	195	338	70.5	4.8
Chromosol - pH 6.0				
Nil N	220	506	34.1	14.8
Low N	228	486	38.2	12.7
Medium N	221	473	50.5	9.4
High N	208	489	79.7	6.1
Sodosol - pH 4.0				
Nil N	170	175	16.5	10.6
Low N	184	165	19.8	8.4
Medium N	183	165	28.8	5.7
High N	156	162	56.9	2.8
Sodosol - pH 4.6				
Nil N	189	229	22.4	10.2
Low N	199	224	26.7	8.4
Medium N	184	226	38.2	5.9
High N	171	213	67.8	3.1
Sodosol - pH 6.0				
Nil N	198	235	21.3	11.1
Low N	216	229	25.8	8.9
Medium N	204	222	41.2	5.4
High N	187	216	70.1	3.1
LSD (*P*=0.05) for any two means	*7*	*17*	*2.1*	*1.2*
Significance level				
Soil	***	***	***	***
Initial pH	***	***	***	***
N	***	***	***	***
Soil × Initial pH	***	***	***	***
Soil × N	NS	**	***	***
Initial pH × N	**	NS	***	NS
Soil × Initial pH × N	NS	NS	***	***

All values represent means (n=3). NS: not significant at *P*=0.05; ** and ***: significant at the *P* values <0.01 and <0.001, respectively.

5.3.4 Total inorganic N (exchangeable NH_4^+-N and NO_3^--N)

Total inorganic N (exchangeable NH_4^+-N and NO_3^--N) increased dramatically with increasing N rate; i.e. 15, 54 and 150% increase in response to the low, intermediate and high N rates, respectively. However, the proportion of NO_3^--N decreased with increasing N rate (Table 5.2).

In general, the C-rich Chromosol had 59% greater exchangeable NH_4^+-N than the C-poor Sodosol despite the NO_3^--N concentrations being similar between the two soils. Even though soil pH only exhibited a weak effect on total inorganic N content, it showed strong negative and positive effects, respectively on exchangeable NH_4^+-N and NO_3^--N concentrations. Increasing soil pH significantly reduced (up to 5-fold) exchangeable NH_4^+-N concentration but substantially increased (up to 3-fold) NO_3^--N (Table 5.2). However, there was a significant (P<0.001) interaction between soil type and pH, whereby total inorganic N was lower with increasing pH in the Chromosol and opposite was true in the Sodosol. The interaction mainly occurred due to a much greater reduction in exchangeable NH_4^+-N with increasing pH in the C-rich Chromosol compared to the C-poor Sodosol, particularly at pH 6.0 (Table 5.2).

Table 5.2: The three-factor interactions (Soil type × Initial pH × N) on the NH_4^+-N, NO_3^--N and total inorganic N (NH_4^+-N and NO_3^-- N), during the 30-day incubation.

Treatment	NH_4^+-N (µg N g^{-1} soil)	NO_3^-- N (µg N g^{-1} soil)	NH_4^+-N and NO_3^-- N (µg N g^{-1} soil)
Chromosol - pH 4.0			
Nil N	130	18	148
Low N	136	31	167
Medium N	160	60	220
High N	204	120	324
Chromosol - pH 4.6			
Nil N	23	98	121
Low N	43	108	151
Medium N	80	122	202
High N	160	157	317
Chromosol - pH 6.0			
Nil N	1	143	144
Low N	1	152	153
Medium N	1	170	171
High N	70	191	261
Sodosol - pH 4.0			
Nil N	46	34	80
Low N	57	40	97
Medium N	93	75	168
High N	161	132	293
Sodosol - pH 4.6			
Nil N	2	101	103
Low N	2	117	118
Medium N	28	136	163
High N	125	161	285
Sodosol - pH 6.0			
Nil N	1	112	113
Low N	1	126	127
Medium N	12	156	168
High N	109	176	285
LSD (*P*=0.05) for any two means	*4*	*4*	*7*
Significance level			
Soil	***	***	***
Initial pH	***	***	***
N	***	***	***
Soil × Initial pH	***	***	***
Soil × N	***	***	***
Initial pH × N	***	***	***
Soil × Initial pH × N	***	***	***

All values represent means (n=3). *** represent significant at the *P* value <0.001.

5.4 Discussion

5.4.1 SOC mineralization

Mineral N amendment showed divergent effects on SOC mineralization which varied with the rate of N applied in this 30-day incubation study. Generally, cumulative mineralization of SOC was stimulated by the low rate of N (30 µg N g^{-1}), was not significantly changed by the intermediate rate (100 µg N g^{-1}), while it was suppressed by the high rate (300 µg N g^{-1}). Therefore, our first hypothesis that the addition of mineral N would reduce SOC mineralization through lifting N limitation to the decomposer organisms can only be partially accepted. The trend of SOC mineralization in response to the rate of mineral N application was similar among all pH values of both SOC-rich (Chromosol) and SOC-poor (Sodosol) soils (Fig. 5.1). As such, the results did not support our second hypothesis, that mineral N-induced reduction in SOC mineralization would be more prominent in the soils with higher initial pH and greater indigenous SOC.

The different responses of SOC mineralization to the various rates of N application observed in this present study is not unexpected, reflecting the inconsistent effects of mineral N on SOC mineralization previously reported such as stimulatory (Tu et al. 2013), no effect (Yoshitake et al. 2007) or inhibitory (Mo et al. 2008). These divergent effects of N on SOC mineralization could be partially ascribed to different responses of different soil organic matter fractions and their relative decomposition rates at different levels of N as proposed by Neff et al. (2002). However, our findings confirm that the influence of N on the direction and magnitude of SOC mineralization mainly depends on the rate of N applied.

An increase in SOC mineralization for the low rate of N (30 µg N g^{-1}) might be attributed to the stimulation effect of N on decomposer organisms as microbial activity might have been limited by N availability (Wardle 1992). A significant increase in MBN due to N addition (Table 5.1) indicated that microbes were N limited in the non-amended soil of our study. A N-stimulated increase in respiration was also revealed by Westerman and Kurtz (1973) for a relatively low rate of N (30 µg N g^{-1}) application. Similarly, Jiang et al. (2016) demonstrated that application of 20 µg N g^{-1} as NH_4NO_3 into a grassland soil of Colorado increased SOC mineralization of 16% even though the MBC was not changed. The increase in SOC mineralization was attributed to an increase in microbial enzymatic activity due to N which promote decomposition of complex C forms (Bowden et al. 2004). Zhu et al. (2016) also reported that addition of 30 µg N g^{-1} as urea enhanced SOC mineralization (18%) in

semiarid grassland in China. They explained the effect of this low rate of N as alleviation of microbial N limitation by this low rate of N resulting stimulation of soil microbial activity (Wei et al. 2013; Xia and Wan 2008). A possible explanation for this stimulation effect is that the amount of N applied was sufficient to stimulate microbial activity but not to fulfil the consequent increase in microbial N demand for growth. Consequently, microbes would have acquired N from pre-existing soil organic matter to balance their stoichiometry, which in turn led to faster SOC mineralization through microbial N-mining mechanisms of C priming (Fontaine and Barot 2005).

In the case of the intermediate N rate, the amount of N supplied might have been able to better satisfy the increasing microbial N demand associated with microbial growth and activity and consequently lower SOC mineralization compared to the low N rate (Fig. 5.1). This argument is supported by the observation of Fontaine et al. (2011) that C priming decreased when sufficient N was available for microbes. Our results are also in accordance with those of Allison et al. (2008) and Wang et al. (2014) that application of approximately 100 µg N g^{-1} as NH_4NO_3 did not significantly alter SOC mineralization (Fig. 5.1). Craine et al. (2007) suggested that despite low amounts of N increasing microbial N-mining, it could be suppressed by high rate of N supply. In comparison with the low N treatment, this intermediate rate might also have enabled the decomposers to preferentially decompose cellulose-like C sources rather than mine N by providing ample amounts of N for them as postulated by Fontaine et al. (2003). Consequently, a lesser N demand of microbes in this treatment could have been partly responsible for this lower SOC mineralization relative to the low N rate.

In the high N treatment (300 µg N g^{-1}), however, SOC mineralization was considerably reduced relative to the control (Fig. 5.1 and Table 5.1), which is in line with previous findings. Ramirez et al. (2012) showed that application of N as high as 250 µg N g^{-1} to soils from 28 different sites of North America consistently decreased respiration on average by about 12%. Foereid et al. (2004) also observed that excessive amounts of mineral N (500 µg N g^{-1}) decreased SOC mineralization about 50%. They attributed this reduction to the suppression of the decomposition of native SOC. Even though such a reduction in mineralization has often been attributed to the suppressive effect of excessive N supply on the activity of certain enzymes, the mechanisms behind this are not clearly understood (Carreiro et al. 2000). The suppressive effect of excessive N supply on soil microorganisms and their activity in the present study can be clearly confirmed by significantly lower MBC

and proportion of NO_3^--N in total N with this high-N treatment compared to the control (Table 5.1 and 5.2). Shen et al (2010) also demonstrated a 15-50% decrease in nitrification with increasing rate of N fertilization from 150-300 µg N g^{-1} soil in an Anthrosol of vegetable land in China.

The suppressive effect of excessive N supply is also in agreement with the conclusion of Fog (1988) in a review of over 60 N-amendment studies that application of exogenous N retarded decomposition of SOC. In this current study, the following reasons might have been responsible for this marked reduction in SOC mineralization for the high N rate amendment. First, direct inhibition of N compounds on enzymes required to decompose recalcitrant C could have primarily reduced overall microbial activity (Gallo et al. 2004; Keyser et al. 1978). Gallo et al. (2004) showed that application of N (~100 µg N g^{-1}) decreased the activity of phenol oxidase and peroxidase up to 20-50% depending on soil type. The large increase in MBN with this excessive N application (Table 5.1) in our study might have been associated with lower enzymatic activity as the enzymatic activity is generally negatively correlated with MBN (Piotrowska-Długosz and Wilczewski 2014). Second, C availability could have been reduced due to a marked increase in condensation of N-rich C compounds (Haider et al. 1975). In addition, rapid formation of recalcitrant humic compounds resulting from reaction of excess N with phenolic compounds could slow SOC mineralization (Berg and Matzner 1997). However, this process is not likely to play a significant role in our study as these are slow humification reactions relative to the length of the study. Lastly, osmotic stress caused by high concentrations of N ions might result in a partial sterilization effect (Broadbent 1965). Müller et al (2006) revealed that the harmful effect of ammonium to bacteria was not due to direct ammonium-specific toxicity, instead the enhanced osmolarity or increased ionic strength of the medium. Decreased respiration by the high rate of annual N (80 µg g^{-1} soil) application for more than 100 years in a Park Grass experiment has been attributed to a decrease in SOC quality due to the high rate of N application (Rousk et al. 2011). Such divergent effects of N rate on SOC mineralization suggests that there would be an optimal amount of N to apply for a specific soil to reduce extra SOC loss by insufficient N supply and condensation and detrimental effect of excessive N on microorganisms.

5.4.2 Microbial biomass C and N

Increasing N application decreased microbial biomass C (MBC) (Table 5.1). Similar results have also been reported in the previous studies (Bowden et al. 2004; Compton et al. 2004; Gallardo and Schlesinger 1994; Li et al. 2016). Černý et al. (2008) showed a decline in MBC by 13% following annual application of 60 µg N g^{-1} to rotation crops for 8 consecutive years in a Luvisol. In most of the studies, the decrease microbial biomass due to NH_4^+ application has been ascribed to decreased soil pH through nitrification process of added NH_4^+ (Biederbeck et al. 1996; Zhang et al. 2008). This might not have been the main reason in the present study since soil pH did not significantly decline during the course of incubation. Instead, in our study, a shortage of C substrate relative to N and the direct inhibitory effect of N compounds on microorganisms, particularly in the high N-treated soil, would have been the major contributing factors for reduced MBC. Loss of competitive ability in several species of basidiomycetes and declined ectomycorrhizal community richness following N application was observed (Edwards et al. 2004; Lilleskov et al. 2002). Such changes in microbial community due to N addition (Treseder 2008) could have also resulted in reduced capacity of the community to degrading SOC. Decreasing synthesis of lignin-degrading enzymes by white-rot-fungi in the presence of a high rate (10 mM NH_4) of low-molecular-weight N was also reported (Tien and Myer 1990). In the current study, greater NH_4^+ accumulation in N-amended soils might have also exerted a harmful effect on particular groups of microorganisms (Von Wiren and Merrick 2004), thereby retarded overall microbial growth. Moreover, the proportion of total N present as NO_3^--N decreased with increasing N rate (Table 5.2) and this could indicate reduced nitrification due to the inhibitory effect of N saturation on nitrifying bacteria. These are in line with the observation of Shen et al. (2010) and Hynšt and Šimek (2012). All these arguments are strongly supported by recent findings that excess application of N beyond a certain threshold decreased microbial abundances as well as community diversity (Han et al. 2017; Li et al. 2016), which are likely to change C cycling.

Nonetheless, our finding that mineral N effected MBC is contrary to some other studies showing that mineral N addition either had no effect (Banerjee et al. 1999; Sarathchandra et al. 2001) or a positive effect on MBC (Kanazawa et al. 1988; Lynch and Panting 1982). These conflicting results could possibly be explained as not all soil microbial properties are affected equally by N fertilizer (Lupwayi et al. 2012) in addition to variation in conditions

among the studies such as soil properties, added N form and the presence of plants. Ding et al. (2010) suggested that N fertilization was more favourable for those microorganisms that use easily degradable organic matter, but less favourable for those that use recalcitrant organic matter.

The decrease in microbial C:N ratio with increased N rate (Table 5.1) is in agreement with Allison et al. (2008) who reported that application of N (100 µg N g^{-1}) increased MBN approximately 3-fold and decreased microbial C:N ratio from 16.0 to 5.2. It also agrees well with Changhui et al. (2014) who delineated that application of 50 µg N g^{-1} enhanced MBN by 9% and decreased microbial C:N ratio by up to 11%. The decrease in microbial C:N ratio with increasing N rate (Table 5.1) could be ascribed to the shortage in available C when there was excess available N as suggested by Friedel and Gabel (2001). This argument was supported by much greater decrease in this ratio in C-poor Sodosol (20-300%) than C-rich Chromosol (16-63%) in this current study (Table 5.1). This could also be due to luxury assimilation of N by microbes beyond their metabolic requirement or changes in microbial community composition from high microbial C:N fungi (~ 10) to low C:N (~ 4) bacteria (Paul and Clark 1998). This assumption is also supported by the findings of De Vries et al. (2006) that increase in bacterial growth rate with higher N rate and are consistent with the recent observation of microbial communities adapting biomass stoichiometry by altering the microbial community composition (Fanin et al. 2013).

5.4.3 Effect of soil pH

The SOC mineralization, measured as CO_2 release, increased with increasing initial soil pH in both Chromosol and Sodosol. Greater CO_2 release could be partly explained by the substantial increase (up to 62%) in microbial biomass with increasing soil pH (Table 5.1). Increased labile C availability with increasing soil pH could occur via decreased bonding strength between organic compounds and soil particles with increasing net negative charge (Curtin et al. 1998) and this would have contributed to the greater SOC mineralization in higher pH soils (pH 4.6 and 6.0) relative to low pH soils (pH 4.0). Similar results have been well documented (e.g., Chapter 3; Ahmad et al. 2014; Kemmitt et al. 2006). The net increase in SOC mineralization due to increasing initial soil pH was about 6% greater in C-rich Chromosol compared to the C-poor Sodosol (Fig. 5.1 and Table 5.1), reflecting a greater increase in labile C supply from the former soil. However, the increase in CO_2 (11-18%) due to increasing initial soil pH was much less than that increase in MBC (31-62%). This

indicated a lower energy demand for cellular maintenance by microbes in the higher pH soils relative to the low pH soils due to favourable environment for microbes in the moderately and slightly acidic soils (pH 4.6 and 6.0) (Nsabimana et al. 2004). There is reported evidence that the microbial biomass concentrations and their active contributions to ecosystem processes are loosely connected (Hartman and Richardson 2013; Kemmitt et al. 2008). This can also be explained as microbes diverting energy from growth to maintenance in response to environmental stress (Odum 1985).

The response of CO_2 release to the rate of mineral N application also varied largely among different pH soils. The net increase in CO_2 release due to the low and intermediate N rates were greater in the strongly acidic soils (pH 4.0) than the moderately and slightly acidic soils (pH 4.6 and 6.0) (Fig. 5.1 and Table 5.1). This could be attributed to more stressful environment for microorganisms in the low pH soils which in turn led to higher respiration (Aciego Pietri and Brookes 2008). This argument can be confirmed since the greater net increase in CO_2 release in the low pH soils due to the low and intermediate N rate occurred despite the large net decrease in MBC in low pH soils (Fig. 5.1 and Table 5.1). Greater CO_2 release from the strongly acidic soils could also be, in part, explained as changes in C availability and microbial community composition due to acidification prior to N amendment. Changes in soil communities from fungal dominant to bacterial dominant with increasing soil pH have been reported (Rousk et al. 2009). Initial acidification decreased MBC about 19% and 23% in Chromosol and Sodosol, respectively (Table 5.1), and it is likely that by products of the dead microbial biomass would also contribute to this greater CO_2 release in the low pH soils. Such influence of acidification on the N effect on SOC mineralization might be transient as microorganisms might not have had sufficient time to acclimatize the disturbance effect within a relatively short period of time. Application of N to a Chromic Luvisol with pH gradient of 3.3-7.4 which had been manipulated by liming and fertilization a long time ago (46 years) in the Park Grass site showed that soil respiration increased linearly with increasing soil pH (Rousk et al 2011). Reduced magnitude of CO_2 release relative to microbial biomass in the C-rich Chromosol than C-poor Sodosol (Table 5.1) suggested that microbes in the former soil had greater C-use efficiency due to the higher C availability (Cheng 2009). This finding highlights that maintaining high C content in agricultural land is important for nutrient availability and C sequestration.

Soil pH also greatly influenced the response of microbial C- and N-use efficiency to N addition. A greater decrease in MBC due to N application in acidified soils (pH 4.0)

compared to that in control (pH 4.6) and limed (pH 6.0) soils (Table 5.1) suggested that the detrimental effect of N addition on soil microorganisms would be more pronounced in strongly acidic soil. In addition, greater net assimilation of applied N occurred with increasing soil pH (Table 5.2), indicating greater microbial N-use efficiency at higher pH. Furthermore, greater accumulation of NH_4^+-N (decreased proportion of NO_3^--N) in acidified soils compared to the control and limed soils (Table 5.2) again suggests that the adverse effects associated with NH_4^+ toxicity would likely to be more intense in strongly acidic (pH 4.0) soil in which nitrification process was much lower than the moderate to slightly acidic soil (pH 4.6-6.0) (Table 5.2). Notwithstanding, the net increase in NH_4^+-N accumulation upon acidifying soil was noticeably greater in Chromosol than Sodosol (Table 5.2). This could be attributed to either the two soils having different indigenous nitrifying bacteria or the C-rich Chromosol had greater indigenous fungal:bacterial ratio than C-poor Sodosol as NH_4^+ accumulation of even control soil (pH 4.6) was higher in the former than the latter (Table 5.2).

5.5 Conclusions

This study demonstrates that the addition of inorganic N to the soil can have positive, neutral or negative effects on SOC mineralization, depending on the amount of N applied. Nevertheless, this current experimental approach did not allow to separate SOC-derived CO_2 from microbial respiration to quantify the magnitude of indigenous SOC losses. Thus, in order to gain insight into the mechanistic effect of N application on SOC dynamics, further studies exploring the interactive effect of N and ^{13}C-labelled organic materials on SOC mineralization is needed. The findings also indicate that N fertilization of strongly acidic soils would likely result in greater SOC loss and highlight the importance of liming acid soils for improving crop productivity and C balance. This study also suggests that application of N fertilizer must be adequate but not excessive, so as to maximize the economic profitability of crop production while minimizing either microbial oxidation of native SOC or negative impacts on soil microbes. Under-fertilization would not satisfy crop N demand and would trigger SOC loss through microbial N mining. Excessive accumulation of NH_4^+-N at high N levels would induce ammonium toxicity to plants. Therefore, future studies should closely examine the effects of various amounts of added N, in relation to microbial stoichiometric ratios, on SOC mineralization to better understand the underlying mechanisms as how N supply affects SOC mineralization.

CHAPTER 6

Influence of initial pH and nitrogen status on soil organic carbon priming by ^{13}C-labelled glucose and lignocellulose

6.1 Introduction

Soil organic carbon (SOC) represents about 60% of terrestrial C stocks (Lal 2004b) and thus understanding the impact of agricultural management practices on soil C balance is of utmost importance. Changes in SOC content can have a strong impact not only on nutrient availability, water-holding capacity, soil structural stability and soil health, but also on atmospheric CO_2 concentration (Baldock et al. 2012). The amount of SOC in cropland depends on the balance between C inputs and losses. Carbon inputs are regulated by biomass production and its return to the soil through deposition which is mainly controlled by climate, soil type and management practices (Kuzyakov and Domanski 2000a). Agricultural practices such as conventional tillage, crop residue removal and/or burning favour SOC loss and reduce SOC stocks (West and Marland 2002). In addition, the application of crop residues and other organic materials with the purpose of improving soil fertility and SOC status can also promote native SOC decomposition via the 'priming' effect (Bingeman et al. 1953). The magnitude and direction of this priming effect largely depend on the quantity and quality (C:N ratio) of the added C and soil properties such as nutrient availability, pH, and the size, activity and community composition of microbial biomass (Blagodatskaya and Kuzyakov 2008; Qiao et al. 2016). Therefore, a deeper understanding of how initial soil pH, and C and N availability interactively drive SOC mineralisation is needed to sustain long-term soil C balance.

Numerous studies have implicated the quality of C substrates and their availability to decomposers as a major controller of the magnitude and direction of the priming effect (De Graaff et al. 2010; De Nobili et al. 2001; Fontaine et al. 2007; Mary et al. 1993). It has generally been recognized that addition of labile C substrate to soil favours stoichiometric decomposition of native SOC by microbes through the production of extracellular enzymes (Hessen et al. 2004). Recalcitrant C substrate, with a higher C:N ratio (>25), also enhanced native SOC decomposition mainly through microbial-N mining processes (Moorhead and Sinsabaugh 2006). However, reports on the magnitude and direction of the priming effect

induced by C substrates with different quality are inconsistent. Many studies have revealed that addition of labile C substrates such as glucose yielded a higher priming effect than more recalcitrant substrates (Mary et al. 1993; Nottingham et al. 2009) at least in the short-term (days). However, others observed the reverse (Mondini et al. 2006; Wu et al. 1993) and clear trends have not been drawn so far. and clear trends have not been drawn so far. These inconsistencies could be due to variation in the amount of added C substrate in relation to the existing SOC pool (Blagodatskaya and Kuzyakov 2008), quality of added substrates (C:N) and other background soil physical and chemical properties among the studies.

Soil pH has also been shown to greatly influence soil microbial biomass and activity which are central to SOC decomposition and thus the priming effect. However, very few studies have focused on the role of soil pH on C priming. The general trend drawn by Blagodatskaya and Kuzyakov 2008 based on 12 studies was that C priming increased with increasing pH (e.g., Bell et al. 2003; Hamer and Marschner 2005; Perelo and Munch 2005; Šantrůčková et al. 2004). Nevertheless, linking soil pH with observed priming effects from different studies conducted on different soils is confounded as each soil has unique properties other than pH. Therefore, it is important to investigate the role of initial soil pH on priming effects within the same soil matrix.

Even though the role of substrate C in priming has been intensively studied, priming effects have not been successfully linked with nutrient availability (Blagodatskaya et al. 2009). Nitrogen availability to microorganisms is another important factor which underpins the magnitude and direction of the priming effect (Blagodatskaya et al. 2007; Liljeroth et al. 1994). The reported results of the role of N in priming effects are controversial. For example, N decreased (Craine et al. 2007; He et al. 2016), enhanced (Chen et al. 2014; Moran et al. 2005) or did not affect (Liljeroth et al. 1994) SOC priming. Rousk et al. 2016 and Murphy et al. 2015 showed that labile C replaced microbial use of C from soil organic matter, while N was selectively mineralized from soil organic matter due to distinct N-mining response of the microbial biomass. However, the responsible mechanisms of priming induced by N availability were not clearly identified. These discrepancies might be due to variations in C substrate quality, the rate of N applied, indigenous soil N content, and other physical, chemical and biological properties of soils (Cheng 2009; Craine et al. 2007). Among several theories which have been proposed to explain the priming effect (Kuzyakov et al. 2000b), microbial N-mining is strongly related to nutrient availability (Craine et al. 2007).

Moreover, to elucidate the effect of N on the priming from the studies that added N to crop residue-amended soils could be ambiguous as the residue itself contains various forms and amounts of organic N depending on its biochemical composition and C:N ratio (Trinsoutrot et al. 2000). The effect of added mineral N on priming would be confounded by the availability of residue N to microbes during decomposition. Therefore, pure C substrates combined with inorganic N were used to investigate the effect of initial soil pH and mineral N and their interactions on the magnitude and direction of the priming effect in this study. The amount of C substrate added (0.5 mg C g^{-1} soil) was based on about double that contained within the microbial biomass C of the soil used to ensure that the added C substrate was sufficient not only to arouse microbial activity but also to stimulate C priming (Blagodatskaya and Kuzyakov 2008). This amount of C was also within the rate reported by others (Chen et al. 2014; Hartley et al. 2010; Wu et al. 1993). The amount of N added (0.05 mg N g^{-1} soil) was based on a C:N ratio 10 with the added C substrates, which is comparable to the C:N ratio of the soil and its microbial biomass so that N would not be a limiting factor in the +N treatments enabling to examine the role of N in C priming. We hypothesized that 1) the application of the labile C substrate, glucose, would induce greater SOC mineralisation than the more recalcitrant C substrate, lignocellulose; 2) N addition would stimulate microbial activity, promote C-substrate mineralisation and consequently decrease SOC mineralisation; and 3) this reduction in C priming with N addition would be greater in the slightly acidic soils (pH 6.6) which were associated with larger microbial biomass compared to the moderately (pH 4.7) and strongly (pH 4.1) acidic soils.

6.2 Materials and Methods

6.2.1 Soil sampling and processing

Surface (0-10 cm) soils were collected from a 35-year lime trial at the Agriculture Reserve, La Trobe University, Victoria, Australia (37°72´ S, 145°05´ E). The soil was classified as Sodosol based on the Australian Soil Classification (Isbell 2002) and Solonetz in the WRB system (WRB IWG 2014). It had a silty clay loam texture comprised of 9% sand, 60% silt, and 31% clay, 20.5 mg C g^{-1}, and 1.9 mg N g^{-1}. Soil samples were taken from each of the 3-field replicate lime plots that had received 0 and 25 t ha^{-1} lime ($CaCO_3$) 35 years ago, resulting in pH_{CaCl2} of 4.7 and 6.6, respectively. After homogenizing and removing visible plant materials, the soils were air-dried and sieved (≤2 mm).

6.2.2 Soil pH manipulation

Soil pH was manipulated prior to the commencement of the experiment with the aim of covering a wide range of soil pH (4.1-6.6) (i.e., from strongly to slightly acidic). The soil samples from 0 and 25 t lime ha^{-1} had pH values of 4.7 and 6.6 and fell in the categories of moderately and slightly acidic soils. The soil from non-limed plots was manipulated with acid to obtain a strongly acidic (pH 4.1) soil. Specifically, the amount of 1 M H_2SO_4 required to lower soil pH from 4.7 to 4.1 was calculated based on the soil pH buffer capacity (22 $mmol_c\ kg^{-1}\ pH^{-1}$). To minimize the drastic effect of H_2SO_4 on the microbial community, a quarter of the acid required was mixed with the amount of Milli-Q water required to adjust the soil to 90% field capacity and applied uniformly with a 1-ml pipette once a week for one month. After adding the acid solution, the soil was covered and incubated at 25 °C for 4 days to allow equilibration and then air-dried for 2 days before the soil pH was re-measured. After the final acid addition, the soils were allowed to equilibrate for 1 month and pH was measured again to ensure the manipulated pH value was stable. For the sake of clarity, the pH of 4.1, 4.7 and 6.6 (range ± 0.03 pH units) referred to hereinafter as the strongly, moderately and slightly acidic soils, respectively.

6.2.3 Experimental design and incubation conditions

The experiment was a 3 × 3 × 2 factorial designed laboratory incubation, with 3 initial pH levels (4.1, 4.7 and 6.6), 3 C substrates (non-C-amended control, glucose and lignocellulose) and 2 N levels (with and without N). The amount of C substrate and mineral N added was 0.5 mg C g^{-1} soil and 0.05 mg N g^{-1} soil. These combinations led to 18 different treatments, each of which had 3 replications.

Sufficient amounts (~ 1.2 kg) of each soil were pre-incubated (50% field capacity) at 25 °C for 14 days prior to C and N treatments in order to allow microbial stabilization (Butterly et al. 2010). During the pre-incubation, the containers were opened every 3 days to ensure the maintenance of aerobic conditions. Pure extracts of uniformly ^{13}C-labelled D-glucose (99 atom%, Sigma Aldrich, Missouri, USA) and ^{13}C-labelled lignocellulose (high degree of polymerization derived from maize, 97 atom%, Isolife, Wageningen, The Netherlands) were used as labile and recalcitrant C substrates, respectively (Table 6.1). Initial characteristics of the soils are presented in Table 6.1.

Table 6.1: Physicochemical characteristics of soils and substrates used in the experiment.

Soil and treatment	Total C (mg g^{-1})	Total N (mg g^{-1})	C/N ratio	Sand %	Silt %	Clay %	$\delta^{13}C$ (‰)	Atom % ^{13}C
Initial soil pH *(*$CaCl_2$)								
4.1	20.9	2.0	10.6	10	62	28	-19.0	
4.7	20.6	1.9	10.9	9	60	31	-19.0	
6.5	19.2	1.8	10.7	10	60	30	-19.8	
Substrate								
Glucose	395	0.3	-					99
Lignocellulose	415	1.7	-					97

Six treatments were imposed on each soil pH level: non-C-amended control with N [C+N] and without N [C-N], glucose with [G+N] and without N [G-N] and lignocellulose with [L+N] and without N [L-N]. Each experimental unit was set up by weighing 30 g (oven-dry equivalent) of pre-incubated soil into a 50-ml PVC core and either ^{13}C-labelled glucose or lignocellulose substrate was added and thoroughly mixed. The same mixing procedure was subjected to the non-C-amended controls to maintain uniform disturbance across all treatments. Inorganic N was added as aqueous NH_4NO_3 (2 ml) in the volume of water required to adjust soil to 60% field capacity and to maximize the activity of aerobic decomposer microorganisms. The non-N treatment (-N) received the same volume of solution as pure Milli-Q water. The liquid was applied evenly to the surface, allowed to settle for 15 min and then mixed with a spatula. Each core was placed in a 1-L air-tight Mason jar with a screw-cap lid containing a vial with 8 ml of water to reduce drying during the incubation. An open wide-mouth scintillation vial containing 8 ml of 1 M NaOH was also placed in the jar to absorb CO_2 released during the subsequent incubation. Three jars containing only the NaOH traps and water vials were also incubated as blanks. The jars were sealed and incubated at 25 °C for 30 days. The NaOH traps were replaced on days 3, 10, 20, and 30 to analyse $^{12}CO_2$ and $^{13}CO_2$. These sampling times were chosen according to the peaks in CO_2 release from each substrate identified during a preliminary experiment. Before replacing a new trap and resealing, each jar was thoroughly flushed with ambient air to ensure that the air in each jar was consistent. A set of experimental units (54 cores) were destructively harvested at 10 and 30 days after incubation to determine soil pH, microbial biomass C and N, and mineral N ($NH_4^+ + NO_3^-$) contents.

6.2.4 Soil analyses

Soil pH of each sample was measured prior to incubation and at each sampling time with a pre-calibrated Thermo Orion pH meter (Thermo Orion 720A+, Beverly, USA) after extracting soil with 0.01 M $CaCl_2$ (1:5) by shaking on an end-over-end shaker for 1 h following centrifugation at 492 × *g*.

To quantify the total CO_2 evolved, a 2-ml aliquot from each NaOH trap was precipitated with 8 ml of 0.25 M $BaCl_2$ solution and then titrated with standardized 0.5 N HCl against the phenolphthalein indicator using a digital burette (BRAND Titrette, Wertheim, Germany) according to Zibilski (1994). using an Elemental Analysis-Isotope Ratio Mass Spectrometer (SerCon Hydra 20-20, Crewe, UK). Concisely, to form $SrCO_3$ precipitates, a 2-ml aliquot from each trap was placed into a 50-ml conical flask, mixed with 2 ml of 1.0 M $SrCl_2$ solution and 15 ml Milli-Q water. A pH probe was then immersed in the solution and 0.5 M HCl was added drop-wise under magnetic stirring until the pH was neutralized. The solution was then transferred to a 50-ml tube and centrifuged at 1579 × *g* for 3 min and supernatants were discarded. The precipitate was subjected to series of resuspension with 40 ml Milli-Q water followed by centrifugation at 2808 × *g* for 6 min, 702 × *g* for 3 min and 274 × *g* for 3 min and discarding the washes. Finally, each precipitate was vortexed with 1 ml Milli-Q water and collected into a glass vial and oven-dried at 60 °C.

Soil microbial biomass C (MBC) and N (MBN) were determined by the chloroform fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987). Eight grams of moist soil was fumigated with about 50 ml ethanol-free chloroform in a desiccator at 25 °C for 24 h in the dark. Another 8 g of non-fumigated soil was extracted with 40 ml of 0.5 M K_2SO_4 (1:5, w v^{-1}) by shaking on an end-over-end shaker for 1 h followed by filtering through Whatman #42 (Whatman International, Maidstone, England). Fumigated soils were extracted the same as non-fumigated soils following the evacuation of chloroform. Extracts were stored at -20 °C before total organic C analysis (GE Sievers Innovox Laboratory TOC analyser, Boulder, USA). Total N was determined by a flow injection analyser (FIA) (QuickChem 8500, Lachat Instruments, Loveland, USA) after oxidising with alkaline potassium persulphate at 120 °C for 30 min (Cabrera and Beare 1993). Microbial biomass C and N were expressed as the difference between organic C and total N from fumigated and non-fumigated soil, respectively. Potential incomplete extraction of C and N was corrected by a factor of 0.45 (Jenkinson et al. 2004). Mineral N (NH_4^+-N and NO_3^--N) from

non-fumigated and non-oxidised extracts (3 ml) was also determined by FIA through phenol hypochlorite reaction and copperized-Cd reduction. To express all the results on mass-basis of oven-dry soil, gravimetric moisture content of the incubated soil at each sampling date was determined immediately after harvesting by weighing the soil prior and after oven drying at 105 °C for 24 h.

6.2.5 Calculations and statistical analyses

The C priming effect (PE) was quantified according to Cheng (1996) as follow:

$$C_S = C_T \times (\delta_T - \delta_C)/(\delta_S - \delta_C)$$

$$C_{SOC\text{-}AME} = C_T - C_S$$

$$PE = C_{SOC\text{-}AME} - C_{SOC\text{-}CON}$$

where $C_T = C_S + C_{SOC\text{-}AME}$, and is the total CO_2 release from added C substrates and C-amended soil, C_S is CO_2 release from substrates, δ_T is $\delta^{13}C$ value of C_T, δ_C is $\delta^{13}C$ value of CO_2 release from non-amended control soil, δ_S is $\delta^{13}C$ value of substrates, $C_{SOC\text{-}AME}$ is SOC-derived CO_2 from amended soil, $C_{SOC\text{-}CON}$ is SOC-derived CO_2 from the control soil, and PE is priming effect of substrates.

All the statistical analyses were performed with the GenStat 17th Edition (VSN International, Hemel Hempstead, UK) after evaluating homogeneity of variance by plotting the residuals vs. the fitted values. No data transformation was necessary. A three-way analysis of variance (ANOVA) with a *post-hoc* least significant difference test (LSD) was carried out for each time point to investigate the main effects of initial pH, C substrate and N and the potential interactions between the dependent variables. With the aim of determining changes in soil pH during incubation within the same initial pH soil and the effect of C substrate and N on soil pH within each observation, the data were also subjected to one- and two-way ANOVA with least significant difference test (LSD) (Table 6.2).

6.3 Results

6.3.1 Soil pH

Changes in soil pH in response to the initial application of C and N substrate varied among different initial soil pH. In the strongly acidic soil (pH 4.1), irrespective of treatments, soil

pH increased with incubation succession up to 0.2 units (Table 6.2). In the moderately acidic soil (pH 4.7), addition of N decreased soil pH (by 0.2 units), but application of lignocellulose or glucose alone (-N) increased the pH up to 0.08 and 0.23 units, respectively (Table 6.2). Nevertheless, in the slightly acidic soil (pH 6.6), regardless of the treatments, the soil pH decreased by up to 0.36 units during incubation, except for a slight increase in pH (0.07 units) with the lignocellulose only treatment (L-N) and no change with the glucose only (G-N) treatment (Table 6.2).

Table 6.2: Dynamics of pH of the strongly acidic (pH 4.1), the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils incubated for 10 and 30 days with control, glucose and lignocellulose with (+N) and without N (-N).

Treatments	pH 4.1			pH 4.7			pH 6.6		
	Time			Time			Time		
	Initial	10 days	30 days	Initial	10 days	30 days	Initial	10 days	30 days
Control + N	4.13 **a**	4.18 **b**	4.27 **c**	4.66 **c**	4.44 **a**	4.54 **b**	6.60 **b**	6.25 **a**	6.31 **a**
Control - N	4.13 **a**	4.17 **b**	4.25 **c**	4.66 **b**	4.66 **b**	4.62 **a**	6.60 **b**	6.52 **a**	6.51 **a**
Glucose + N	4.13 **a**	4.30 **b**	4.31 **b**	4.66 **b**	4.66 **b**	4.58 **a**	6.60 **b**	6.40 **ab**	6.24 **a**
Glucose - N	4.13 **a**	4.33 **b**	4.36 **c**	4.66 **a**	4.89 **c**	4.77 **b**	6.60 **a**	6.67 **a**	6.57 **a**
Lignocellulose + N	4.13 **a**	4.14 **a**	4.20 **b**	4.66 **b**	4.53 **a**	4.53 **a**	6.60 **c**	6.42 **a**	6.47 **b**
Lignocellulose - N	4.13 **a**	4.21 **b**	4.28 **c**	4.66 **a**	4.74 **c**	4.71 **b**	6.60 **a**	6.67 **b**	6.66 **b**
LSD (P=0.05)		*0.07*	*0.05*		*0.03*	*0.03*		*0.06*	*0.15*
Significance level									
C substrate		***	***		***	***		***	*
N		NS	*		***	***		***	***
C substrate × N		NS	*		NS	***		NS	NS

For each row, means with the same letter are not significantly (P=0.05) different between sampling times within each pH treatment. NS, * and *** indicate not significant at P=0.05, and significant at the P<0.05 and P<0.001, respectively.

6.3.2 Carbon dioxide release

Initial soil pH had a weak but significant effect on total CO_2 release. The CO_2 release during the 30-day incubation from the strongly acidic soil (pH 4.1) was only 7% lower than that of the moderately and slightly acidic soils. There was no significant difference in CO_2 release between the moderately and the slightly acidic soils (pH 4.7 and 6.6) (Fig. 6.1).

Carbon substrate amendment significantly increased total CO_2 release with contrasting dynamic pattern between the two substrates throughout the incubation period (Fig. 6.1). The CO_2 release from the C-amended soils was on average 52% greater than that of control at the end of the 30-day incubation. Nevertheless, during the first 20 days of incubation, total CO_2 release was greater in glucose-amended soils than lignocellulose-amended soils with the magnitude of this difference declining as the incubation progressed (Fig. 6.1).

Nitrogen also showed a minor but significant effect ($P<0.05$) on CO_2 release with only 3% less total CO_2 with N amendment relative to that without N during the 30-day study. This effect of N was only exhibited in the C-amended soils and not in the control soils throughout the incubation (Fig. 6.1). Nonetheless, there was a significant ($P<0.05$) initial pH × N interaction in which the effect of N on CO_2 release occurred only in the slightly acidic soils (pH 6.6) (Fig. 6.1).

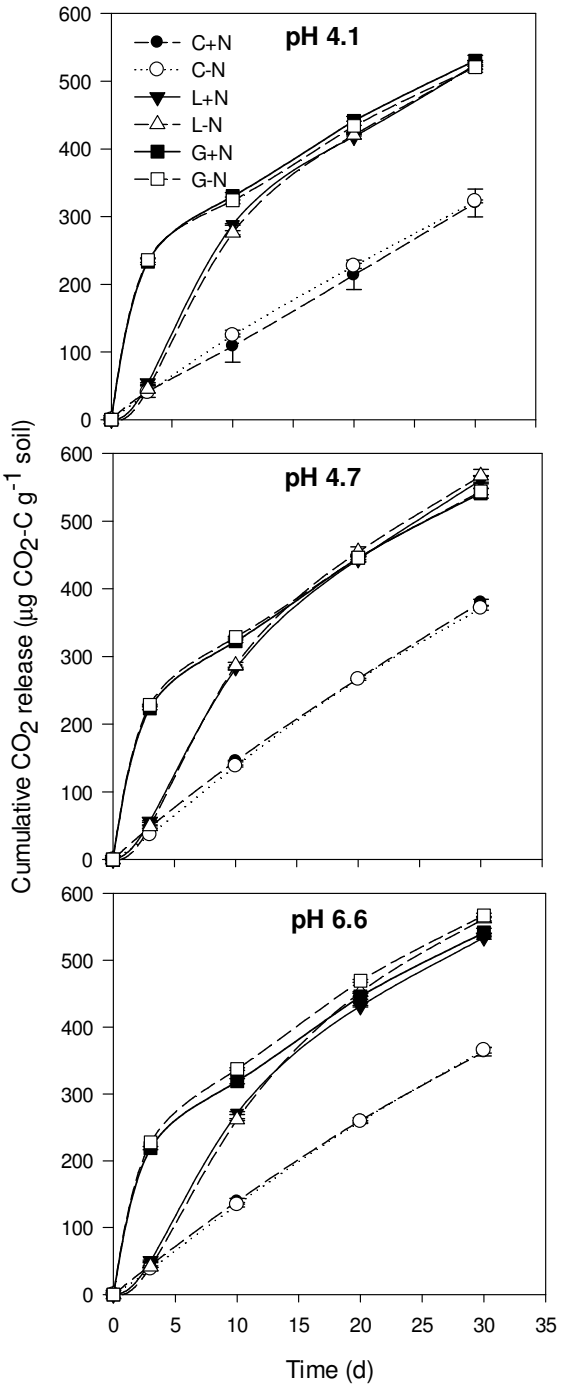

Figure 6.1: Cumulative CO_2 release from control with (C+N) and without N (C-N), glucose with (G+N) and without N (G-N), and lignocellulose with (L+N) and without N (L-N) in the strongly acidic (pH 4.1) (top), the moderately acidic (pH 4.7) (middle) and the slightly acidic (pH 6.6) (bottom) soils over 30 days. Error bars represent the standard error of the means (n=3). Where error bars not shown, symbols are larger than error bars.

6.3.3 Substrate derived CO_2-C

Initial soil pH had a marked effect on substrate-derived CO_2-C which varied among different periods of incubation (Fig. 6.2). Mineralisation of C substrate, as measured by $^{13}CO_2$ evolution, in the strongly acidic soils (pH 4.1) was about 7% greater than that in the moderately and slightly acidic soils (pH 4.7, 6.6) during days 0-10 and the reverse trend was true for the rest of the incubation period (days 11-30), in which C substrate mineralisation in the moderately and slightly acidic soils was on average 9% greater than those of the strongly acidic soils (Fig. 6.2). Thus, cumulative CO_2-C derived from substrate was about 6% greater in the moderately and slightly acidic soils compared to the strongly acidic soils (Fig. 6.2 and Table 6.3).

Mineralisation of the two C substrates was very different during the initial phase but the difference diminished as the incubation progressed (Fig. 6.2). Glucose mineralisation was about 808% and 14% higher than its counterpart lignocellulose during the early 0-3 and 4-10 days of incubation, where the glucose mineralisation peaked. About 66% of the total amount of glucose mineralized was released within the first 3 days, irrespective of initial soil pH (Fig. 6.2). Nevertheless, from day 10, mineralisation of lignocellulose was about 7% higher than that of glucose (Fig. 6.2). Lignocellulose mineralisation peaked during days 4-10 and at least 51% of total lignocellulose derived CO_2-C was released in this period (Fig. 6.2). However, the overall mineralisation of C substrate (% of the total amount added) during the study was similar between the two substrates, i.e. 34% for glucose and 37% for lignocellulose. Nonetheless, the difference in the mineralisation between the two substrates was greater in the moderately acidic soils (12%) than the strongly and slightly acidic soils (6%) (Fig. 6.2 and Table 6.3).

The addition of NH_4NO_3 only exhibited a slight effect on substrate-derived CO_2-C in which N addition decreased the mineralisation of both C substrates by 2% (P<0.05) throughout this 30-day incubation study (Fig. 6.2). However, the effect of N on substrate mineralisation differed between initial soil pH. At pH 4.1, N enhanced CO_2-C derived from glucose but diminished that from lignocellulose, whereas at pH 4.7, N did not significantly affect C-substrate mineralisation. Nevertheless, N addition decreased the mineralisation of both substrates at pH 6.6 (Fig. 6.2).

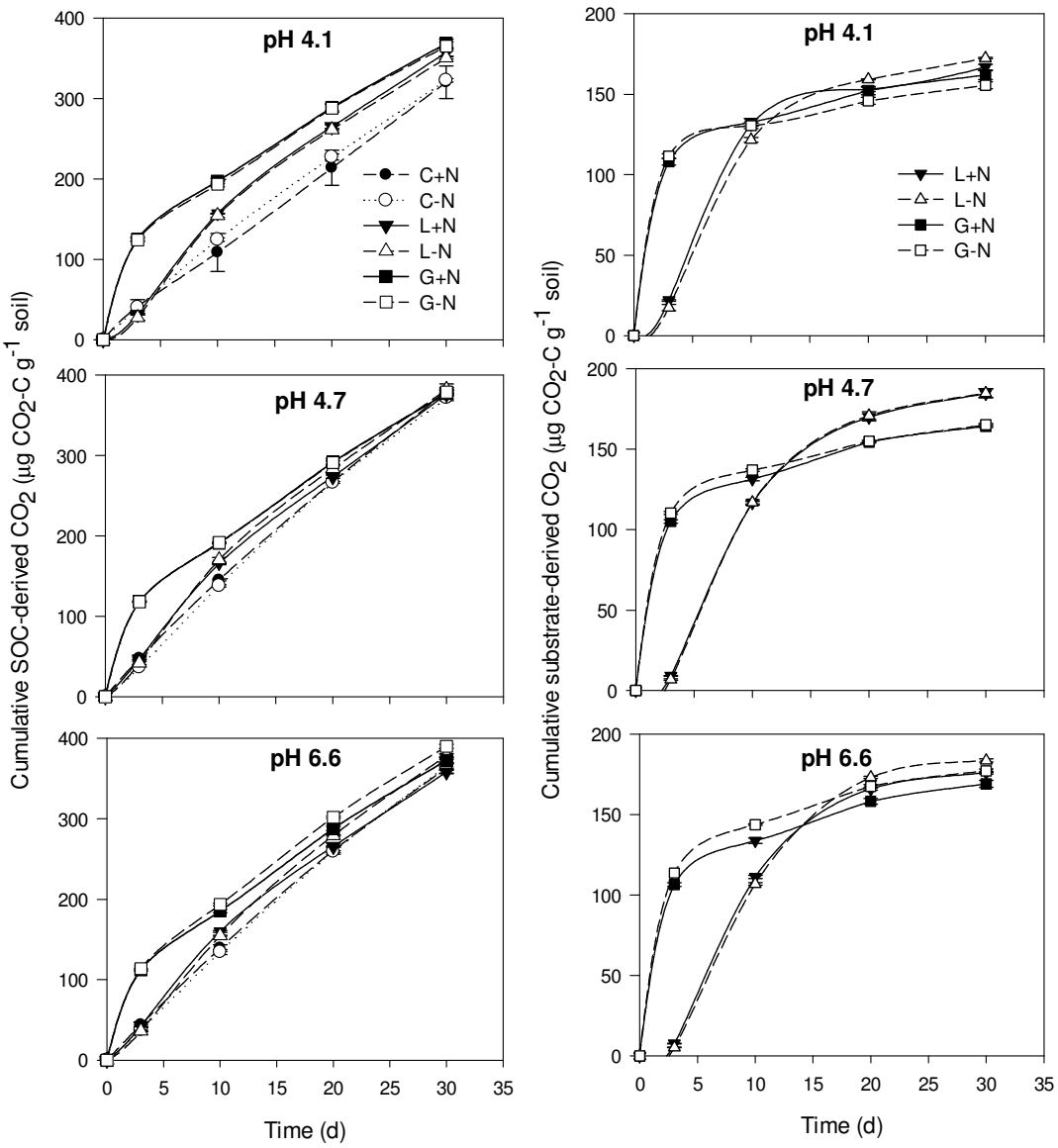

Figure 6.2: The cumulative SOC-derived CO_2 (left) and substrate-derived CO_2 (right) from control with (C+N) and without N (C-N), glucose with (G+N) and without N (G-N), and lignocellulose with (L+N) and without N (L-N) in the strongly acidic (pH 4.1) (top), the moderately acidic (pH 4.7) (middle) and the slightly acidic (pH 6.6) (bottom) soils over 30 days. Error bars represent the standard error of the means (n=3). Where error bars not shown, symbols are larger than error bars.

6.3.4 C priming effect

Initial soil pH had a marked effect on native SOC mineralisation induced by the C addition (priming effect). Cumulatively, the priming effect was greatest at pH 4.1 which was approximately 20- and 3-fold greater than that at pH 4.7 and 6.6, respectively after 30 days (Fig. 6.2 and Table 6.3).

The direction and magnitude of C priming was also significantly influenced by the type of C substrate. The addition of labile C substrate, glucose, yielded greater loss of native SOC compared to that of recalcitrant C, lignocellulose. The differences in magnitude of SOC primed between the two substrates diminished with incubation, for example from 80 µg CO_2 g^{-1} soil at days 0-3 to about 8 µg CO_2 g^{-1} soil at days 21-30 (Figs. 6.2, 6.3 and Table 6.3). In addition, the pattern of SOC primed differed between the two C substrates throughout the incubation. In glucose-amended soils, a strong positive priming effect was observed during days 0-3, followed by negative priming. On the other hand, in lignocellulose-amended soils, there was negative priming during days 0-3 which turned to positive priming during days 4-10 and followed by negative priming again during days 11-30 (Fig. 6.3). At the end of incubation, overall priming with glucose was about 8 µg CO_2 g^{-1} soil (69%) greater than that with lignocellulose (Fig. 6.2 and Table 6.3).

There was an interaction ($P<0.05$) between initial pH and C substrate, where the differences in priming effect between the two C substrates was highest at pH 6.6 and lowest at pH 4.7. In addition, the prominent negative priming effect in lignocellulose-amended soils during days 0-3 was observed only in the strongly acidic soils (pH 4.1) (Figs. 6.2, 6.3 and Table 6.3).

Mineral N addition showed a significant effect on native SOC mineralisation irrespective of the type of C substrate (Fig. 6.3 and Table 6.3). The overall reduction of SOC priming in response to N addition in this 30-day incubation study was 8 µg CO_2 g^{-1} soil (69%) (Fig. 6.3 and Table 6.3).

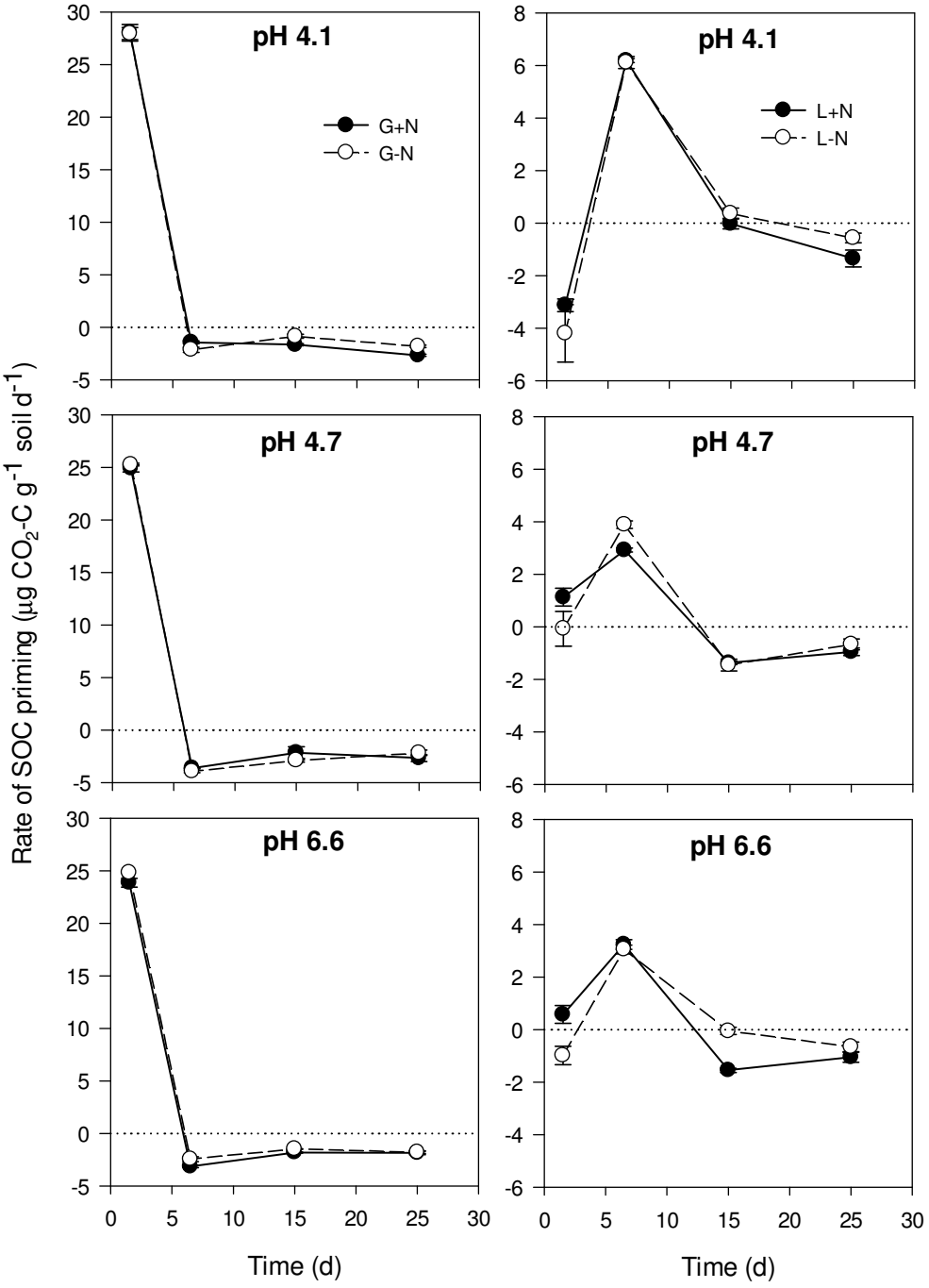

Figure 6.3: The rate of SOC priming from glucose with (G+N) and without N (G-N) (left), and lignocellulose with (L+N) and without N (L-N) (right) in the strongly acidic (pH 4.1) (top), the moderately acidic (pH 4.7) (middle) and the slightly acidic (pH 6.6) (bottom) soils over 30 days. Error bars represent the standard error of the means (n=3). Where error bars not shown, symbols are larger than error bars.

Table 6.3: Basal soil-derived C, primed-C and substrate-derived C in the strongly acidic (pH 4.1), the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils during the 30-day incubation of glucose and lignocellulose and with (+N) or without N (-N).

Treatments	Basal soil-derived C (µg CO_2-C g^{-1} soil)	Primed-C (µg CO_2-C g^{-1} soil)	Substrate-derived C (µg CO_2-C g^{-1} soil)
pH 4.1			
Glucose + N	369	31.3	162
Glucose - N	365	42.7	156
Lignocellulose + N	357	20.2	167
Lignocellulose - N	351	28.2	173
pH 4.7			
Glucose + N	377	1.5	164
Glucose - N	378	-1.9	165
Lignocellulose + N	376	0.7	185
Lignocellulose - N	382	5.9	185
pH 6.6			
Glucose + N	372	13.1	169
Glucose - N	390	24.7	177
Lignocellulose + N	358	-1.6	176
Lignocellulose - N	379	11.4	184
LSD (*P*=0.05) for any two means	*13*	*12.6*	*5*
Significance level			
Initial pH	***	***	***
C substrate	**	**	***
N	*	**	*
Initial pH × C substrate	*	*	***
Initial pH × N	**	NS	**
C substrate × N	NS	NS	NS
Initial pH × C substrate × N	NS	NS	*

NS, *, ** and *** indicate not significant at *P*=0.05, and significant at the *P*<0.05, *P*<0.01 and *P*<0.001, respectively.

6.3.5 Microbial biomass C and N

Unlike CO_2 release, initial soil pH exerted a substantial effect on both MBC and MBN. The MBC and MBN concentrations in the slightly acidic soils (pH 6.6) were on average 100% and 14% greater than those of the strongly (pH 4.1) and the moderately (pH 4.7) acidic soils (Table 6.4).

The addition of C substrates increased the MBC and MBN concentrations by 55% relative to the control soils. The MBC and MBN concentrations were about 10% and 3% greater with glucose than with lignocellulose at days 10 and 30. However, MBC and MBN

concentrations with glucose amendment were higher than those with lignocellulose at pH 4.1 and pH 6.6 but the reverse applied at pH 4.7, resulting in significant pH × C substrate interactions at both sampling times (Table 6.4).

Nitrogen addition showed a significant (P<0.001) suppressive effect on MBC and positive effect on MBN concentrations (Table 3). It decreased MBC by about 10%, but increased MBN by up to 26%. There was a significant (P<0.01) C substrate × N interaction at day 30, whereby the suppressive effects of N on MBC was 15% greater in the lignocellulose-amended soils than in the glucose-amended soils (Table 6.4).

6.3.6 Microbial metabolic quotient, qCO_2

The qCO_2 decreased with increasing initial soil pH in which the qCO_2 of moderately and slightly acidic soils were 43% and 51% lower than that of strongly acidic soils (Table 6.4). Interestingly, at day 10, the qCO_2 with the glucose amendment was 63% lower than the non-amended control, while that with lignocellulose was about 13% higher than the control. However, by day 30, the qCO_2 of both C-amended soils was lower than the control. Overall, there was a significant pH × C interaction (P<0.001), in which the difference in qCO_2 between the two C substrates was greatest in the strongly acidic soils and lowest in the moderately acidic soils (Table 6.4).

Nitrogen addition also exhibited a prominent effect on the qCO_2. The average qCO_2 across C types was 30% and 17% higher with N than without N addition at day 10 and 30, respectively (Table 6.4). The N-induced increase in qCO_2 was greater at pH 4.1 compared to that at pH 4.7 and 6.6, leading to a significant pH × N interaction (P<0.05) (Table 6.4).

Table 6.4: Microbial biomass C and N, and microbial metabolic quotient (qCO_2) in the strongly acidic (pH 4.1), the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils incubated for 10 and 30 days with control, glucose and lignocellulose with (+N) and without N (-N).

Treatments	Microbial biomass C (µg g^{-1} soil)		Microbial biomass N (µg g^{-1} soil)		qCO_2 (µg CO_2-C µg MBC d^{-1})	
	10 days	30 days	10 days	30 days	10 days	30 days
pH 4.1						
Control + N	48	96	10.5	19.3	0.450	0.111
Control - N	62	128	8.5	10.7	0.385	0.080
Glucose + N	180	169	25.4	29.5	0.077	0.053
Glucose - N	188	195	18.6	18.6	0.067	0.045
Lignocellulose +N	158	144	23.7	25.7	0.263	0.074
Lignocellulose - N	150	159	16.6	16.7	0.230	0.065
pH 4.7						
Control + N	192	205	26.7	24.0	0.074	0.055
Control - N	218	202	20.7	20.7	0.069	0.052
Glucose + N	252	238	36.8	48.2	0.056	0.040
Glucose - N	250	254	25.6	25.6	0.057	0.039
Lignocellulose + N	270	259	37.4	35.0	0.124	0.045
Lignocellulose - N	319	337	30.4	30.4	0.107	0.034
pH 6.6						
Control + N	196	202	30.7	30.4	0.070	0.051
Control - N	208	230	24.1	32.2	0.067	0.046
Glucose + N	368	322	43.7	60.4	0.039	0.030
Glucose - N	386	336	28.9	30.4	0.041	0.029
Lignocellulose + N	190	282	46.0	40.8	0.198	0.037
Lignocellulose - N	276	335	32.4	33.0	0.117	0.033
LSD (P=0.05) for any two means	*71*	*25*	*2.0*	*1.5*	*0.220*	*0.012*
Significance level						
Initial pH	***	***	***	***	***	***
C substrate	***	***	***	***	***	***
N	*	***	***	***	NS	***
Initial pH × C substrate	***	***	***	***	**	***
Initial pH × N	NS	NS	***	***	NS	*
C substrate × N	NS	**	***	***	NS	NS
Initial pH × C substrate × N	NS	**	*	***	NS	NS

NS, *, ** and *** indicate not significant at P=0.05, and significant at the P<0.05, P<0.01 and P<0.001, respectively.

6.3.7 Inorganic nitrogen (NH_4^+-N and NO_3^--N)

Contrary to the previously described parameters, inorganic N concentration was negatively correlated with initial soil pH with this trend more prominent for NH_4^+-N (Table 6.5). The concentration of NH_4^+-N in strongly acidic soils (pH 4.1) was almost 5- and 15-fold greater than those in the moderately acidic (pH 4.7) and slightly acidic (pH 6.6) soils whereas those differences in NO_3^--N were only about 33 and 50%.

Both glucose- and lignocellulose-amended soils had 26% lower inorganic N concentration relative to the control soils, particularly during days 0-10 (Table 6.5). At the end of the study, however, inorganic N in glucose-amended soils was approximately 5% more than the control, whereas that in lignocellulose-amended soils was 28% less than the control. In addition, there was a significant (P<0.001) interactive effect of Initial pH × C substrate on inorganic N in which the differences in inorganic N concentrations between the two substrates was greatest in the moderately acidic soils.

Table 6.5: Concentrations of NH_4^+-N, NO_3^--N and total inorganic nitrogen (NH_4^+-N + NO_3^- - N) in the strongly acidic (pH 4.1), the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils incubated for 10 and 30 days with control, glucose and lignocellulose with (+N) and without N (-N).

Treatments	NH_4^+-N (µg g^{-1} soil)		NO_3^-- N (µg g^{-1} soil)		NH_4^+- + NO_3^-- N (µg g^{-1} soil)	
	10 days	30 days	10 days	30 days	10 days	30 days
pH 4.1						
Control + N	39.5	39.4	83	87	122	126
Control - N	10.7	16.4	65	70	76	86
Glucose + N	2.3	34.2	84	89	107	123
Glucose - N	8.1	16.6	56	70	64	87
Lignocellulose + N	17.4	22.7	80	86	98	109
Lignocellulose - N	3.9	10.4	51	59	55	70
pH 4.7						
Control + N	17.7	18.8	73	75	91	94
Control - N	1.7	1.0	34	46	36	47
				93		
Glucose + N	2.0	2.2	72	93	74	95
Glucose - N	1.3	1.4	34	73	35	74
Lignocellulose + N	1.5	1.5	57	71	59	72
Lignocellulose - N	0.8	1.4	16	28	17	29
pH 6.6						
Control + N	1.4	2.2	76	85	78	87
Control - N	0.7	1.6	35	47	36	48
Glucose + N	1.0	1.1	51	71	52	72
Glucose - N	0.8	2.0	17	54	17	56
Lignocellulose + N	1.2	1.3	52	66	53	67
Lignocellulose - N	1.4	1.6	16	28	17	30
LSD (P=0.05) for any two means	*1.5*	*1.8*	*3*	*3*	*4*	*4*
Significance level						
Initial pH	***	***	***	***	***	***
C substrate	***	***	***	***	***	***
N	***	***	***	***	***	***
Initial pH × C substrate	***	***	***	***	***	***
Initial pH × N	***	***	NS	***	***	**
C substrate × N	***	***	***	***	**	***
Initial pH × C substrate × N						

NS, ** and *** indicate not significant at P=0.05, and significant at the P<0.01 and P<0.001, respectively.

6.4 Discussion

The study provides new insights into the priming effect induced by C substrates as affected by initial pH and soil N status. We demonstrated that the priming effect was greatest in strongly acidic (pH 4.1) soil, followed by slightly acidic (pH 6.6) soil and it was lowest in moderately acidic (pH 4.7) soil in a 30-day incubation period. The findings from this study also verify that labile C substrate (glucose) yielded a greater priming effect compared to the recalcitrant C substrate (lignocellulose) which support the first hypothesis. Although 63-66% of the substrates remained in the soil at the end of the study (324-335 μg C g^{-1} soil), this was less than the total CO_2-C derived from SOC (361-379 μg C g^{-1} soil), indicating a negative C balance. For the first time, we showed that addition of mineral N reduced both substrate mineralisation and the priming effect regardless of C substrate quality (availability) and initial soil pH. This finding did not fully support our second hypothesis that N was expected to increase substrate mineralisation but decrease the priming effect. Furthermore, the greatest net reduction in C priming due to N addition occurred in the moderately acidic (pH 4.7) soil which was contrary to our third hypothesis.

6.4.1 Soil pH effect on priming

This study showed that the priming effect in response to exogenous C addition was largely controlled by initial soil pH, consistent with previous work (Chapter 4; Luo et al. 2011; Perelo and Munch 2005). The influence of initial soil pH on the priming effect could be attributed to variation in abundance and community composition of decomposer microorganisms among different pH soils (Fierer and Jackson 2006) as the priming effect is expected to be mediated by decomposer organisms (Rousk et al. 2009). In addition, the variation in the proportion of added substrate C relative to the indigenous MBC among different pH soils might also have played a significant role in priming (Blagodatskaya and Kuzyakov 2008). The different MBC concentrations across the initial pH range (Table 6.4) meant that the amount of C added (0.5 mg g^{-1} soil) was about 6 times higher than indigenous MBC at pH 4.1, while that was only 2.5 and 2 times higher than the MBC at pH 4.7 and 6.6 (Table 6.4). Consequently, the net increase in MBC in response to C-substrate amendment at pH 4.1 was much larger (69%) relative to that at pH 4.7 (29%) and 6.6 (48%) (Table 6.4). Such a surge in microbial biomass and activity in this strongly acidic soil could have been responsible for the greatest mineralisation of native SOC (Fig. 6.2) through co-metabolism

and microbial N-mining mechanisms (Kuzyakov et al. 2000b). However, it is noteworthy that not all the extra C mineralisation due to substrate C addition should be assumed as accelerated non-biomass SOC mineralisation because it can be contributed from CO_2 released by rapid microbial respiration upon receiving labile C substrates (Dalenberg and Jager 1981; Wu et al. 1993), which is known as apparent priming effect (Blagodatskaya and Kuzyakov 2008).

The manipulation of soil pH to obtain the strongly acidic (pH 4.1) soil would have altered C availability and likely changed the functional capacity of the microbial community. Microbial biomass was reduced by half (~110 μg C g^{-1} soil) when the pH was reduced from 4.7 to 4.1. It is likely that some of the dead microbial biomass contributed to the primed C after glucose and lignocellulose were added and that this labile C pool may not be present in soils with a more static soil pH. This may indicate that pH effects on priming occur via its effect on C availability since soil microbes had the capacity to utilise this C in the strongly acidic soil once the other substrates were added.

The effect of initial soil pH on the priming effect differed between the two C substrates. Even though the magnitude of the priming effect in glucose-amended soils was consistently higher in the strongly acidic soils throughout the study, the pattern in lignocellulose-amended soils was more dynamic (Fig. 6.3). A negative priming effect occurred in lignocellulose-amended-strongly acidic soils during the early incubation stage (days 0-3) which coincided with faster mineralisation of this substrate (Fig. 6.2). This could be ascribed to a greater abundance of microbes adapted in strongly acidic soils which were more efficient in degrading and utilizing the high polymer lignocellulose in a short time (Fontaine et al. 2011). As a consequence, microbes would have utilised the abundant labile C that was added rather than mining from soil organic matter, in turn caused the negative priming in accordance with the preferential substrate utilization mechanism (Cheng 1999).

The priming effect was greater in the strongly acidic soil (pH 4.1) than the two higher pH soils (pH 4.7 and 6.6) even though lignocellulose mineralisation was lower in the former soil after day 3 (Fig. 6.2). This could possibly be explained by the greater net increase in microbial biomass during the initial stage following C-substrate amendment in this strongly acidic soil (Table 6.4). Greater biomass would have prolonged their activity by using the labile C substrates from the early stages of incubation as an energy source according to the

'microbial activation' theory (De Nobili et al. 2001), in the strongly acidic soil, which in turn decomposed a larger amount of native SOC than the two higher pH soils either indirectly (glucose) or through the co-metabolism (lignocellulose) phenomenon (Kuzyakov and Bol 2006).

Nevertheless, if the priming effect was compared only between the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils, the priming effect was greater in the latter soils (Fig. 6.3). This could also be attributed to a greater net increase in microbial biomass upon substrate supply in the slightly acidic soils compared to the moderately acidic soils (Table 6.4), which is consistent with our previous study (Chapter 4). Similar results have also been reported (Bell et al. 2003; De Nobili et al. 2001; Hamer and Marschner 2005; Perelo and Munch 2005) despite most of these studies being conducted in different soil matrixes with very narrow range of pH.

6.4.2 Effect of C substrate on SOC priming

Overall, the application of both glucose and lignocellulose substrates induced a positive priming effect in all pH soils during this 30-day incubation study. Generally, C priming was largest through the first 3 days with glucose and 10 days with lignocellulose, which was also the period of highest substrate mineralisation. After these peaks, the priming effect of both substrates diminished and negative values remained until the end of the study (Fig. 6.3). Consequently, the priming effect of both C substrates in this current study was much lower than that reported in previous studies using comparable substrates and observation periods (Bastida et al. 2013; Fontaine et al. 2004b). The very low cumulative SOC primed in this present study could be partially ascribed to the large amount of C substrate added which was 2-5 times the microbial biomass C. The results agree with Blagodatskaya and Kuzyakov (2008) who reported that when such high amounts of substrate C were applied, the priming effect tended to be close to zero or even negative. Preferential substrate utilization was assumed to be the major process involved in this phenomenon with the switch of substrate conditions for decomposer organisms from energetically-expensive soil organic matter to easily accessible added substrates (Cheng and Kuzyakov 2005; Kuzyakov 2002). The increase in C-use efficiency (decrease qCO_2) in C substrate-amended soils compared to the control at the end of the study (Table 6.4) also indicates that a less stressful environment for microbes in the former soils, which might have led to this negative priming effect (Odum 1985).

On the whole, the greater priming effect in the soils amended with glucose relative to those with lignocellulose might be partly explained by the greater net increase in MBC in the glucose-amended soils than in the lignocellulose-amended soils (Fig. 6.3 and Table 6.3). Similar results have also been revealed earlier (Mary et al. 1993; Nottingham et al. 2009). Even though the duration of positive priming induced by glucose lasted only 3 days, the cumulative native SOC loss was greater in glucose-amended soils than in lignocellulose-amended soils (Fig. 6.2). The large dose of labile glucose amendment provided sufficient labile C for microbial growth as indicated by greater net increases in MBC (Table 6.4) and this 'activation' of the soil microbes could have facilitated the production of extracellular enzymes able to degrade native SOC. Although indirectly, the effect of glucose addition may have facilitated SOC mineralisation during the first 3 days of incubation similar to the co-metabolism mechanism (Kuzyakov and Bol 2004), whereby the additional enzymes produced degrade SOC. The abundance and/or activity of extracellular enzymes are positively correlated with microbial population as long as fresh substrate is not limiting (Kshattriya et al. 1992; Joshi et al. 1993).

In the case of lignocellulose, there was a lag phase of about 10 days to reach maximum substrate mineralisation and priming compared to glucose (Fig. 6.2). Such a delay in the mineralisation of both substrates and native SOC might be accredited to initial poor adaptation of the microbial community to decompose polymeric lignocellulose substrate, particularly in the moderately and slightly acidic soils (Torres et al. 2014). Furthermore, mineralisation of lignin-rich recalcitrant organic compounds requires a depolymerization step to produce soluble components for microbial absorption and metabolism (Fontaine et al. 2003), while glucose can be directly assimilated by microbes (Jagadamma 2014).

Such differences in mineralisation between the two substrates could be a reason for the different lag phase of negative priming, 4-10 days with glucose and 11-20 days with lignocellulose (Fig. 6.3). Preferential utilization of labile C source rather than more recalcitrant indigenous SOC resulted in the negative priming effect. With glucose amendment, such preferential substrate utilization occurred earlier than that with lignocellulose since glucose is readily assimilated by microbes, while lignocellulose is more recalcitrant and requires a period of depolymerization prior to the products being preferentially utilized as labile C.

6.4.3 Effect of N on SOC priming

The lower amount of SOC primed in soils amended with N than without, reflects that N addition alleviated N limitation and consequently reduced microbial N-mining. This result is in line with many other studies (Blagodatskaya et al. 2007; Foereid et al. 2004; Fontaine et al. 2004a; He et al. 2016; Henriksen and Breland 1999). The greater inorganic N concentrations in N-amended soils (Table 6.5) indicate that N availability was not limited, decreasing the need for microbes to acquire N from soil organic matter. Reduction in inorganic N due to microbial N immobilisation following C substrate amendment, especially within 10 days after incubation was more prominent with lignocellulose than glucose (Table 6.5), suggesting that more N was needed by microbes while degrading the high polymeric substrate (Vahdat et al. 2011). In another study, Szili-Kovács et al. (2007) observed that the application of sucrose and sawdust in soils reduced the concentrations of inorganic N by 6 and 20%, respectively. Differences in N demand between the two C substrates might have modified stoichiometry of soil N, which in turn affects the growth and activity of decomposers and the SOC mineralisation processes as suggested by Allison et al. (2008) and Bowden et al. (2004). Kirkby et al. (2014) also demonstrated that augmenting the residues with supplementary nutrients decreased SOC mineralisation and suggested that the application of additional nutrients beyond that required for crop production is needed to minimise extra SOC losses. Moreover, decreased activity of SOC-degrading enzymes due to mineral N addition (Sinsabaugh et al. 2005) would have also contributed to the decreased priming. Nevertheless, many other studies reported alternative results that the application of mineral N enhanced the C priming effect (Conde et al. 2005; De Graff et al. 2006). Such inconsistency among the studies could possibly be due to different biotic and abiotic characteristics of soils, variation in the amount and quality of added substrate C and N relative to stoichiometry of indigenous microorganisms. Moreover, soils are composed of a complex mixture of organic molecules that vary in N content and the energy required by microbes to break them down. As such, addition of inorganic N is likely to increase the mineralisation of some SOC fractions but decrease that of others (Neff et al. 2002).

The more pronounced reduction in the priming effect with added N in soils amended with lignocellulose than glucose could be ascribed to the greater decrease in MBC and soil pH in the former treatments (Fig. 6.3 and Table 6.4). The influence of decreasing soil pH by N

addition on microbial biomass and activity was demonstrated earlier (Compton et al. 2004; Foereid et al. 2004; Fog 1988; Frey et al. 2004; Kuzyakov et al. 2000b). It was postulated that N application might exert a direct negative effect on microbial biomass through increased solute concentrations and/or indirectly by decreasing the soil pH via nitrification of added N. Therefore, the results confirmed that N was less important in priming in soils amended with glucose compared to those with lignocellulose. This indicated that the application of sufficient amounts of N would provide not only better crop yield, but may also minimise indigenous SOC loss upon crop residue returning to the soil.

6.5 Conclusions

To our knowledge, this is the first study to investigate the impact of pure C substrates and N on priming at a range of initial pH levels. The effect of initial soil pH on priming was non-linear such that priming was greatest in the strongly acidic (pH 4.1) soils, followed by the slightly acidic (pH 6.6) soils and lowest in the moderately acidic (pH 4.7) soils. The greatest net increase in MBC due to the largest proportion of added C substrate relative to indigenous C pool at pH 4.1 would be mainly responsible to this greatest priming effect. Further studies with examination of shifts in microbial community composition and functional activity upon C and N amendment at different initial pH levels are needed.

The addition of N decreased the amount of SOC primed, and this effect was larger with lignocellulose than glucose. The supply of adequate (optimal) N in the agricultural field is likely to be essential to minimise SOC loss due to priming and microbial N mining. Moreover, the more prominent effect of N on decreasing SOC priming in slightly acidic soils (limed soils) than strongly acidic soils suggests that liming acid soils should be accompanied with N application.

CHAPTER 7

General Discussion

This thesis has examined the impacts of lime application on the dynamics of soil organic carbon (SOC) in acid soils, and carried out detailed investigations into the mechanisms underpinning these impacts. The thesis also reports on investigations into the impact of liming on soil aggregate stability, the interactions between liming, carbon (C) substrates and mineral N on SOC mineralization. The key findings are that:

i. Liming can have either a positive or a negative effect on SOC content and soil aggregate stability, depending on associated management practices such as tillage, crop residue addition and nutrient management.
ii. Increases in soil pH by liming enhance native SOC loss via the C priming effect, with this effect being greater with addition of the lower C:N field-pea residue compared to the higher C:N wheat residue.
iii. Liming increased SOC mineralization with additions of mineral N, but the mineral N had a non-linear effect on SOC mineralization that was mainly driven by the amount of N applied, with liming having minor effect on the N effect.
iv. The application of mineral N can reduce the magnitude of extra native SOC loss caused by exogenous C substrate application (i.e. priming) in limed soils.

Liming acid soils, followed by a 34-year cropping history with above-ground residue removal and minimal biomass inputs, significantly decreased both SOC content and aggregate stability (Chapter 3). The decreasing SOC content in this cultivated limed soil could be attributed to a deficit of biomass C inputs that was unable to compensate for the larger rate of C mineralization by the increased and more active microbial population in the limed soil (Inagaki et al. 2016). Depleting the labile C pool, because of the higher rate of mineralization in the limed soil would also likely to be mainly responsible for degrading soil aggregate stability, as labile C contributes to aggregate binding (Christensen 2001). It is also likely that cultivation, which increases the breakdown of soil aggregates and surface areas exposing to microbial decomposition (Merante et al. 2017) would also contribute to this negative effect of liming that lowered the SOC content in this soil.

On the other hand, liming did not significantly reduce the SOC content, 5 years after its application to native vegetation (Chapter 3). Liming improved soil aggregate stability in this soil. Above- and below-ground biomass C inputs from this no-tillage and no-cropping lime trial would have been able to offset the accelerated C mineralization by liming. The balance between net biomass inputs and net C respiration induced by liming would determine the direction and magnitude of SOC dynamics following liming. This suggests that liming can exert either a beneficial or detrimental effect on the SOC stocks and soil structural stability depending on the cropping system and on the management practices used such as cultivation, plant residue addition and soil fertility management, all of which influence the SOC balance (Follett 2001). There are several reports that lime application in conjunction with minimum cultivation and balanced fertilization increased SOC storage (Briedis et al. 2012a; Fornara et al. 2011). This implies that in order to improve crop productivity in acid soils while maintaining SOC stocks and a good soil structure, lime application should be followed by balanced nutrient management, crop residue retention and a conservation tillage system. Haynes and Naidu (1998) predicted that increasing plant biomass production by liming would increase SOC content in the long-term. However, to achieve this goal, it would be necessary to increase biomass production and biomass C inputs into the limed soil.

Liming acid soils increases soil pH and this can lead to increased microbial biomass and activity. This can also enhance the C priming effect (Chapter 4) which is the phenomenon where extra native SOC is lost in response to an exogenous C substrates supply (Bingeman et al. 1953). This phenomenon can be one of the mechanisms which increases SOC loss in limed soils, as there is a concomitant increase, as a result of liming, in the supply of C substrates from decaying of above- and below-ground plant biomass, root exudates and microbial production of organic acids in the agricultural field (Chandrasekaran 1969). Greater microbial activity in limed soils would be more efficient in degrading C substrates and using labile C as an energy source to produce energetically-expensive enzymes which are able to decompose recalcitrant SOC (Hamer and Marschner 2005) compared to the non-limed soils. Such an abundance of C substrate availability will also create stoichiometric imbalances between resources and soil microbial communities (Mooshammer et al. 2014), which in turn can increase microbial N demand, as microbes are largely homeostatic in terms of their biomass C:N ratio (Cleveland and Liptzin 2007). Mining of N by microbes from soil organic matter to maintain their stoichiometry would lead to extra losses of SOC.

Such losses of SOC induced by the microbial N-mining phenomenon is likely to be greater in N-limited environment.

Nevertheless, the magnitude of native SOC loss via priming is controlled by the chemical composition (C:N ratio) of available C substrates. Application of lower C:N field-pea residue (C:N 29) induced greater priming than that resulting when higher C:N wheat residue (C:N 42) were added in the 90-day incubation study that is documented in Chapter 4. However, there was contrasting temporal dynamics in the priming for the two residues. The priming effect was higher with field-pea residue during the early decomposition (0-7 days) stage, while the reverse occurred for the wheat residue during the later stages of the incubation (21-90 days). These early and late peaks in priming coincided with maximum substrate mineralization as revealed in the study by Mason-Jones and Kuzyakov (2017). Thus, the nature of the priming effect can change over time following substrate application, according to C substrate availability to soil microorganisms. Nevertheless, the proportion of added residues remained in the soil after 90 days was greater with field-pea residue than wheat residue, indicating that lower C:N field-pea residue is likely to have a larger C storage potential relative to wheat residue in the long term. This assumption needs to be validated by long-term observations on the effect of adding C substrates with varying C:N ratios, on the various SOC pools. The finding suggests that the long-term impact on SOC dynamics will depend on the type of crop that is growing, and on the quality of its residues that are retained in the limed soils.

Chapter 4 showed that the higher the lime rate that was applied, the greater the loss of SOC (pH_{CaCl_2} increased up to 6.5). This indicates that over-liming acid soils might not only have a negative effect on micronutrient availability (Fageria and Baligar 2008) and on the economics of the liming operation, but also enhance native SOC loss via the priming effect. Thus lime addition to acid soils should target a post-liming pH value that maintains the availability of essential micronutrients to plants and minimizes extra SOC loss from over-liming.

Nutrient availability to microorganisms can also regulate SOC decomposition. Non-linear responses in SOC mineralization to mineral N application is described in Chapter 5. Low rate of N (30 µg N g^{-1}) increased SOC mineralization, while intermediate rates (100 µg N g^{-1}) did not significantly affect SOC mineralization whereas high rates (300 µg N g^{-1}) decreased SOC mineralization (Chapter 5). Liming and the indigenous SOC content in the

soil did not significantly influence the effect of N on SOC mineralization. This indicates that routine N fertilization in the field would be likely to enhance SOC loss by stimulating the effect of N on microorganisms (Ren et al. 2016) as the low rate of N applied in this study would be closer to the rate of mineral N routinely applied in the field. Nevertheless, the response of SOC mineralization to mineral N would also be driven by indigenous soil N content and microbial demand of N (Meyer et al. 2017). For instance, the application of N to a N-limited environment would trigger increases in microbial activity and consequently increase SOC mineralization (Zhu et al. 2016). On the other hand, if N is applied to an N-sufficient environment, such impacts are unlikely to occur. It seems that the intermediate N rate applied in this study would have matched, more-or-less, the N demand by decomposer organisms, and so there was no effect of N on SOC mineralization for this treatment. Decreases in SOC mineralization in the high rate of N addition indicates the detrimental effect of excessive N application on microbial activity due to a direct osmotic effect of high ionic concentrations and/or indirectly due to decreasing soil pH by the nitrification process (Compton et al. 2004). Moreover, excessive N applications can also result in environmental risks such as eutrophication, NO_3^- leaching, and ammonia toxicity (Jones et al. 2014). Thus excessive N application should be avoided in order to optimize N use efficiency and economic returns from fertilizer-N, and minimize environmental risks such as nitrate leaching.

The results reported in Chapter 6 show that extra loss of SOC by the C priming effect, following the addition of exogenous C substrates, can be reduced by applications of mineral N. Applications of N decreased the priming effect with the addition of both ^{13}C-labelled glucose and lignocellulose, irrespective of initial soil pH value (Chapter 6). Reductions in the priming effect by an average of 9%, following N application with a wide range of crop residues has also been reported by Craine et al. (2007). However, the effect of N on priming was more prominent with lignocellulose than glucose. This can be attributed to a significant reduction in soil pH and in microbial biomass due to N application with lignocellulose, while there was no significant N effect on soil pH and microbial biomass with glucose (Chapter 6). The reduced activity of some of the SOC-degrading enzymes, by mineral N addition (Sinsabaugh et al. 2005) is also likely to have been responsible for this N effect. However, other researchers (Conde et al. 2005; Liljeroth et al. 1994) reported mixed results where N application enhanced C priming, or did not affect priming. This suggests that applications of N can have different effects on priming depending upon the amount of added

N, the microbial N demand, and the soil N status and type of available C substrates (Wang et al. 2014). The magnitude of the priming effect would also depend on the amount of added C and N substrates relative to the stoichiometry of indigenous microorganisms (Chen et al. 2014). Further studies should investigate the effect of N on priming that relates to the microbial stoichiometric ratio, to better understand the underlying mechanisms as to how N supply affects C priming in soil.

As the area of acid soils is increasing, a strategic management plan for soil acidity should include liming followed by balanced fertilization, residue retention and conservative tillage to maintain sustainable crop production in acid soils. Application of lime to raise soil pH to levels where micro-nutrient availability is adequate and where there is minimal loss of extra SOC, would increase both profitability and soil health. Balanced fertilization along with liming is very important as it would produce greater plant biomass which in turn would offset the net increase in C respiration resulting from liming, and this would decrease native SOC mineralization in the long term. Applications of fertilizer N at appropriate rates can also provide benefit by reducing extra SOC loss through the priming effect. Adoption of these beneficial cultural practices in limed soils will be important in order to maintain sustainable plant production, and to reduce atmospheric CO_2 emissions from the acid soils.

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