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Long-term effect of lime application on the chemical composition of soil organic carbon in acid soils varying in texture and liming history

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Abstract

There is ample evidence to suggest that liming can regulate soil organic carbon (SOC) pools either directly through influencing the solubility of SOC or indirectly by altering total organic C input as crop residue and SOC loss via change in microbial activity. The aim of this study was to determine the long-term impact of lime application on the quantity and quality of SOC in acid soils. Soils were collected at depths of 0-10, 10-20, 20-30, 30-40 and 40-50 cm from four long-term lime trials with various lime rates (0-25 t ha⁻¹), lime histories (5-35 years) and soil textures (clay content 5-36%). Surface application of lime was effective in ameliorating both top and subsoil acidity at sites with low clay content. Liming decreased dissolved organic C (DOC) at 0-30 cm, but increased its aromaticity. Total SOC at 0-10 cm decreased or remained unchanged following long-term liming, depending on the rates of lime application and crop management. Changes in the contents of particulate organic C (POC) and humus organic C (HOC) predicted by mid-infrared spectroscopy (MIR) and partial least squares regression (PLSR) showed a similar trend to total SOC at all sites. Lime application had no significant effect on SOC below 10-cm layers and on the MIR-predicted resistant organic C (ROC) fraction. Solid-state ¹³C nuclear magnetic resonance (NMR) spectra indicated that the alkyl C content and alkyl/O-alkyl C ratio was lower in the limed than unlimed plots. Liming possibly had a marked effect on regulating the decomposition and preservation of certain C compounds. The apparent accumulation of alkyl C in the unlimed soil could indicate the potential ability of acid soils to store SOC.

Key words: carbon sequestration, farming practice, MIR-predicted SOC fractions, NMR, SOC composition

Introduction

Soil organic C (SOC) is formed through repeated recycling of new and existing plant and animal residues by soil microorganisms. The final product is a complex mixture of different types of organic C differing in decomposability, chemical recalcitrance and interactions with soil matrix. Some components of SOC (e.g. fragments of plant residue) are more susceptible to decomposition and some (e.g. charcoal) are less susceptible than the weighted average behaviour noted for total SOC. The dynamics of total SOC may thus not be a sensitive indicator of short- or long-term impacts of agricultural management practices on SOC stability and storage (Krull et al. 2003). Various chemical or physical fractionation schemes have been proposed to separate SOC into pools differing in solubility, particle size and density or biological stability, and to provide insight into the mechanisms accounting for changes in SOC due to factors such as land use, tillage or fertilization (Six et al. 2001; Skjemstad et al. 2004; Yagi et al. 2003). In addition, solid-state ¹³C nuclear magnetic resonance (NMR) spectroscopy has become a popular and important tool for direct chemical characterization of C in whole soil or in each C fraction (Baldock et al. 2013b; Courtier-Murias et al. 2014; Simpson et al. 2012).

Recently, the potential of agricultural soils to sequester C has received significant attention, considering its potential contribution to mitigate emissions of greenhouse gases and to improve soil sustainability. The application of lime, a common agricultural practice for ameliorating soil acidity, has also been shown to affect SOC preservation and decomposition. For example, liming can enhance soil C loss by increasing C solubility, microbial activity and thus the rates of C decomposition (Bezdicsek et al. 2003; Fuentes et al. 2006). In contrast, other studies have found that liming enhanced SOC stabilization through redistribution of C from labile to more humified soil pools, and/or the complexation of SOC with Ca²⁺ thereby enhancing its resistance to decomposition (Manna et al. 2007; Morries et al. 2007). Also, lime-induced increases in root and shoot growth and thus organic residue inputs to soil could

contribute to observed increases in SOC storage (Briedis et al. 2012; Bronick and Lal 2005).
The final change in total SOC content following liming depends on the balance between SOC
gains and losses. However, it is still not clear under what conditions a particular mechanism
dominates.

Liming could also influence the chemical composition of SOC in addition to altering
the overall soil C balance. Soil pH is known to impact the selective preservation of some
macromolecules (e.g. lipids) during SOC decomposition (Bull et al. 2000; Guggenberger et al.
1995; van Bergen et al. 1998). Moreover, liming has been reported to reduce the C:N ratio of
soil organic matter (Fornara et al. 2011; Tonon et al. 2010), indicating an alteration to the
chemical composition of soil organic matter. It is not clear how lime affects different pools of
SOC that are lost or accumulated. For example, liming can increase or decrease the allocation
of SOC to the humus C fraction irrespective of changes in SOC content (Fornara et al. 2011;
Yagi et al. 2003). To date, studies examining the long-term impact of liming have focused on
assessing the change in total SOC storage or physically separated C fractions. Little
information is available on lime-induced change in the chemical composition of SOC, e.g.
amount and proportional distribution of each type of SOC. Knowledge of the chemical
structure of SOC is essential as it could reflect SOC stability and provide an indication of
long-term C sequestration mechanisms.

This paper aimed to quantify the long-term effects of liming on SOC content, the
allocation of SOC to component fractions and the chemical composition of SOC using
diffuse-reflectance mid-infrared (MIR) spectroscopy and solid-state ^{13}C nuclear magnetic
resonance (NMR) spectroscopy. Soils with various liming histories (5-35 years) and soil
textures (clay content 5-36%) were sampled from four long-term field trials. We hypothesised
that (1) liming would decrease soil C storage by increasing C loss through enhanced microbial
decomposition; (2) SOC in limed soils would be dominated by C in a more decomposed state
than unlimed soils; and (3) alkyl C would be preferentially accumulated in the limed soils due
to enhanced SOC decomposition and its high chemical recalcitrance.

Materials and methods

Liming trial sites and soil sampling details

Four long-term liming trials were used in the study. Two sites were located on the La Trobe
University farm (37°42'S, 145°02'E), Victoria, Australia. The soil was similar between the
two sites (100 m apart) and was classified as a Sodosol (Isbell 2002) with 36% clay content.
The first trial was established in 1979, and consisted of a completely randomized design with
1 m × 2 m plots replicated three times and was mainly sown with legumes including lentils
and medic pasture. The second site was established in 2008 and consisted of a randomized
complete block design with three replicates, and the site had been under unmanaged pasture
before the liming trial. Three lime treatments were selected from each site; 0, 12.5 and
25 t ha⁻¹. For both sites, the organic matter inputs had been minimal.

The third trial (Wongan Hills site) was established in 1984 on a paddock, 15 km east
of Wongan Hills, Western Australia (30°54'S, 116°51'E). The soil was classified as an acidic
Tenosol (Isbell 2002) characterized by low clay content (6%), low organic matter (7 g kg⁻¹),
with gravels (~5%) below 10 cm. The trial consisted of 12 plots of 1.8 m × 22 m (Tang et al.
2003). Soils were sampled in May 2013 from four lime treatments; no lime (no lime), lime
applied at 1.5 t ha⁻¹ in 1999 (lime99), lime applied at 6.2 t ha⁻¹ in 1984 (lime84), and lime
applied at 6.2 t ha⁻¹ in 1984 and 1.5 t ha⁻¹ in 1999 (lime84+99), with 3 replicates. The annual
crop rotation on the trial and surrounding paddock was wheat and barley. Crop residues
(straw) were returned to the field after each harvest.

The fourth trial (Kellerberrin site) was located in a paddock 17 km north of
Kellerberrin, Western Australia (31°29'S, 117°47'E). The soil was classified as an acidic

Tenosol (Isbell 2002) and had clay content of 6.8% at 0-10 cm, 10.4 % at 10-30 cm and 8.4% below 30 cm. The trial consisted of a completely randomised design with 15 m × 100 m plots replicated three times. Soil samples were taken in May 2013 from three liming treatments; lime applied in 1991 at 0, 2.5 and 5 t/ha with a further 1 t/ha applied to all treatments in 2000. The trial was cropped to wheat, barley, lupins or canola annually, with crop residue returned to the field after harvest.

At the Wongan Hills and Kellerberrin sites, soils were sampled from each plot by taking 5 cores (4 cm diameter) at depth intervals of 0-10, 10-20, 20-30, 30-40 and 40-50 cm. At the La Trobe University sites, only the top 0-10 cm was sampled. All soils were air-dried and passed through a 2-mm sieve.

Basic soil physiochemical analyses

Soil pH was measured in 0.01 M CaCl₂ (1:5 soil solution ratio, 1 hour end-over-end shaking, centrifuging at 700 g for 10 min). Soil pH buffer capacity (pHBC) was determined using the method described by Wang et al. (2015). Soil texture was measured using a laser particle size analyser (Malvern Mastersizer 2000, Worcester, UK). Dissolved organic C (DOC) was determined for all soils collected from Kellerberrin and Wongan Hills sites. Briefly, 20 g of air-dried soil and 20 ml of 0.5 M K₂SO₄ were shaken end-over-end for 10 min, centrifuged at 2800 g for 10 min and filtered through a 0.45-µm nylon membrane. The concentration of DOC in the extracts was determined colorimetrically after wet digestion with K₂Cr₂O₇ (49 g L⁻¹) and concentrated H₂SO₄ (1:2) (Heanes 1984). The aromaticity of DOC was quantified by measuring the specific UV absorbance at 280 nm (Weishaar et al. 2003).

Soil organic C (SOC) was determined by dry combustion using a CHNS Analyser (PerkinElmer EA2400, Shelton, USA). Prior to SOC analysis, soils were ball-milled (MM400, Retsch GmbH, Haan, Germany) and tested for the presence of CaCO₃ using 4 M HCl. Only one soil collected from the plot limed at 25 t/ha lime of the second trial showed visible effervescence and was pretreated with H₂SO₃ to remove inorganic C (Schmidt et al. 2012).

SOC characterisation using MIR and NMR analyses

MIR spectroscopic analyses were carried out using the ball-milled soils on a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc., MA, USA) equipped with a Pike AutoDiff-Automated diffuse reflectance accessory (Pike Technologies, WI, USA). Samples (approximately 100 mg) were packed into stainless steel cups and loaded into the 60 position Pike AutoDiff sample wheel. MIR spectra were acquired over 8000-400 cm⁻¹ with a resolution of 8 cm⁻¹ using a KBr beam-splitter and a DTGS detector. The background signal intensity was quantified by collecting 240 scans on a silicon carbide disk prior to analysing each set of 60 soil samples and used to correct the signal obtained for the soil samples. A total of 60 scans were acquired and averaged to produce MIR reflectance spectra for each individual sample and the Omnic software (Version 8.0) was used to convert the acquired reflectance spectra into absorbance spectra (log transform of the inverse of reflectance). All chemometric analyses (spectral transformation and predictions using partial least squares regression, PLSR, prediction algorithms) were completed using Unscrambler 10.3 (CAMO Software, Oslo, Norway). All acquired MIR spectra were truncated to a spectral range of 6000-1030 cm⁻¹ and a baseline offset transformation was applied. The square root of the gravimetric contents of particulate organic C (POC) (SOC associated with 50-2000 µm particles excluding poly-aromatic C), humic organic C (HOC) (SOC associated with particles ≤50 µm excluding poly-aromatic C) and resistant organic C (ROC) (poly-aromatic C associated with particles ≤2 mm) were predicted from the transformed MIR spectra using the

MIR/PLSR algorithms developed by Baldock et al. (2013a) using measured POC, HOC and ROC contents derived using an automated size fractionation protocol (Baldock et al. 2013b).

Soils for solid-state ^{13}C NMR analysis were prepared by forming single composite samples for each treatment at each site by combining a mass of soil normalised to measured bulk densities from each field replicate for the 0-10 cm depth layer. To remove the sand particles, all composite soils were dispersed (20 g soil in 80 ml of a 50 g L⁻¹ sodium hexametaphosphate solution shaken for 16 hours on a flatbed orbital shaker) and passed through a 50- μm sieve. The coarse organic materials retained on the 50- μm sieve were separated from sand by backwards pushing the organic material on the sieve with a stream of water into a separate container. The collected coarse organic materials were combined with the collected <50 μm suspension, and then freeze-dried (Baldock et al. 2013b). The final lyophilised and ground samples were treated with 2% hydrofluoric acid (HF) to further concentrate SOC and remove paramagnetic materials (Skjemstad et al. 1994).

Solid-state ^{13}C NMR analyses were completed on a Bruker 200 Avance spectrometer equipped with a 4.7 T wide-bore superconducting magnet operating at a resonance frequency of 50.33 MHz. Weighed samples (150-600 mg) were packed into 7-mm diameter zirconia rotors with Kel-F end caps and spun at 5 kHz. Chemical shift values were calibrated to the methyl resonance of hexamethylbenzene at 17.36 ppm and a 50 Hz Lorentzian line broadening was applied to all spectra.

Three separate ^{13}C NMR experiments were performed. An inversion recovery pulse sequence using 8 inversion recovery times varying from 0.001 to 3.0 s and a recycle delay (d_1) of 5 s was applied to each sample. $T_1\rho\text{H}$ values were calculated for each sample from the inversion recovery data and indicated that a recycle delay of 1 s was adequate (>5 times calculated $T_1\rho\text{H}$ values) to avoid saturation in the subsequent cross polarisation (CP) ^{13}C NMR analyses. The CP analyses used a 3.2 μs , 195 w, 90° pulse and a contact time of 1 ms. Between 10,000 and 20,000 scans were collected for each CP analysis. The number of scans was increased as the amount of C contained in the rotor declined across the various samples. A variable spin lock experiment using an array of spin lock times (1, 2, 6, 10, 15 and 20 ms), a contact time of 1 ms and a recycle delay of 1 s was performed to calculate sample specific $T_{1\rho}\text{H}$ values and allow the CP NMR observability of organic C in the samples to be quantified as described by Baldock and Smernik (2002). The observability of C was close to 100% (99-108%) for samples collected from Wongan Hills site, and ranged from 75-85% for other three sites.

All spectral processing including the calculation of $T_1\rho\text{H}$ and $T_{1\rho}\text{H}$ and integration of spectral regions was completed using the Bruker TopSpin 3.2 software. After phasing and baseline corrections were applied, the absolute NMR signal intensities acquired for each sample were divided by the number of scans collected and corrected for empty rotor background signals. The resultant spectra were integrated using the chemical shift limits and calculations defined by Baldock et al. (2013b) and used to quantify the allocations of soil C to alkyl, N-alkyl/methoxyl, O-alkyl, di-O-alkyl, aryl, O-aryl, carbonyl/amide and ketone forms. In this integration process any signal intensity present in spinning side bands was mathematically added to the parent resonances from which they were derived.

Statistical analyses

The effects of liming on soil pH, DOC, SOC, C:N ratio, MIR-predicted C fractions and soil pHBC at each depth were analysed using a one-way analysis of variance (ANOVA). Significant ($P=0.05$) differences between means were identified using the least significant difference (LSD) test. For NMR analysis, duplicates were performed for one treatment at each site to assess the experimental error.

Results

The magnitude of the increase in soil pH reflected both liming rates and soil pHBC. Applying lime at 12.5 and 25 t ha⁻¹ increased the soil pH by 1.0 and 1.6 pH units, respectively, at the University sites (data not shown). At the Kellerberrin site, liming at 5 t ha⁻¹ increased the soil pH by nearly 1 pH unit to a depth of 30 cm, when compared with the unlimed control (Fig. 1). At the Wongan Hills site, marked increases in soil pH (1.5-1.8 pH units) were detected even below 30 cm, when lime was applied at 6.2 or 6.2+1.5 t ha⁻¹. Liming at lower rates increased soil pH by less than 0.5 pH units at both sites (Fig. 1).

Liming significantly decreased DOC concentration at 0-30 cm (Fig. 2a, c). However, when compared with the unlimed control, DOC concentration increased at 40-50 cm at the Wongan Hills site following liming at 6.2 or 6.2+1.5 t ha⁻¹. Similarly, liming increased the aromaticity of DOC, except at the depth below 30 cm at the Wongan Hills site, where higher specific UV absorbance of DOC was observed in the plots limed at 6.2 or 6.2+1.5 t ha⁻¹ than the control (Fig. 2b, d).

In general, SOC content at 0-10 cm decreased by increasing lime rates at all sites (Tables 1 and 2); however SOC remained unchanged when lime was applied at 6.2+1.5 t ha⁻¹ at the Wongan Hills site. Changes in C/N ratio, MIR-predicted POC and HOC fractions showed a similar trend to total SOC at all sites (Tables 1, 2 and 3). For example, liming decreased soil C/N ratio in most cases, but not when lime was applied at 6.2+1.5 t ha⁻¹ at the Wongan Hills site (Tables 1 and 2). At the University farm, MIR-predicted POC and HOC decreased consistently with SOC in response to liming. Lime application had no significant effect on SOC below 10-cm soil layers and on the MIR-predicted ROC fraction at all depths (data not shown for below 10 cm). The pHBC of soils was also decreased by liming, except where residual lime was detected in the University farm limed at 25 t ha⁻¹ in 2008.

As revealed by the NMR spectra, O-alkyl C (24.6-27.7%) and alkyl C (22-25.4%) were the dominated C types in the topsoils at all sites (Figs. 3 and 4). Change in each C type was affected by the change in TOC, with the content of each type of C lower in the limed than unlimed soil, except at the Wongan Hills site where lime applied at 6.2+1.5 t ha⁻¹ did not affect the C content. Only alkyl C showed an apparent difference in relative intensity distribution in the spectra of the limed and unlimed soils (Fig. 4). The relative alkyl C content and alkyl/O-alkyl C ratio were lower in the limed plots, compared with the unlimed plots established at the University farm in 1979 (Figs. 4 and 5). There was also an apparent trend of decreased proportion of alkyl C and alkyl/O-alkyl ratio with liming at the other three sites. The experiment error ($\leq 0.5\%$), as revealed by the duplicate measurements, was lower than the magnitude of change in the relative content of alkyl C (1.7-2%).

Discussion

Effect of surface liming

The apparent pH increase due to liming to a depth of 30 cm at Kellerberrin and 50 cm at Wongan Hills sites suggested that surface application of lime was effective in ameliorating subsoil acidity in soils with low clay content in the long term. High liming rates, low soil pHBC and the presence of gravels in the subsurface layers could account for the significant downward movement of lime at the Wongan Hills site. Little movement of surface-applied lime was frequently detected on soils with relatively higher soil pHBC as indicated by their higher clay (24-32%) and organic C content ($> 20 \text{ mg kg}^{-1}$) (Godsey et al. 2007; Conyers et al. 2003). Given the soil pHBC of 6.3 mmol_c kg⁻¹ pH⁻¹ at the Wongan Hills site, applying lime at 6.2 and 6.2+1.5 t ha⁻¹ equated to twice the amount of lime required to increase the soil pH to a depth of 50 cm by 1 and 1.4 pH units, respectively. Blevins et al. (1978) also observed a vertical movement of lime to a depth of 30 cm when lime was applied at a rate of three times the lime requirement. Thus, liming at relatively high rates on sandy soils with low pHBC

allows vertical movement and amelioration of subsoil acidity. Moreover, when compared with soil pH data obtained in 2003 (Tang et al. 2003), re-acidification of whole soil profile (0.1-0.9 pH units) had occurred during last 10 years of continuous cropping, indicating that re-liming every 5-10 years would be essential to prevent pH decrease due to re-acidification.

Dissolved organic C (DOC)

The DOC concentration of limed soils decreased proportionally to the amount of added lime. Lower amounts of DOC in the limed soil could be due to enhanced decomposition or leaching associated with higher soil pH. It is well recognized that soil pH can modify the quantity of DOC through affecting its production, decomposition, solubility and adsorption by soil minerals. Liming could increase the production and decomposition rate of DOC by stimulating microbial activity (Andersson et al. 1994; 2000). Erich and Trusty (1997) found that DOC in the limed plots was more susceptible to microbial attacks as Al-organic complexes might have been replaced by Ca-organic complexes. Furthermore, enhanced solubility and leaching of DOC due to deprotonation or desorption have also been observed after liming (Andersson et al. 1994; 2000). Therefore, liming could increase or decrease DOC concentration in soil, depending on which processes dominate. In this study, lower DOC contents of limed soils suggest that DOC loss via enhanced microbial mineralisation or leaching was greater than DOC production via decomposition or processes that increased DOC availability such as deprotonation or desorption. However, lime-induced increases in DOC have been frequently reported in some short-term field and laboratory studies (Andersson and Nilsson 2001; Erich and Trusty 1997; Garbuio et al. 2011). Possibly, DOC did increase immediately following liming, but this increase was temporary and could not be sustained against enhanced microbial degradation or leaching in these lightly textured soils over long periods.

Leaching of DOC down the soil profiles at the Wongan Hills and Kellerberrin sites is likely given their coarse texture. Many studies detected rapid adsorption of DOC by soil minerals (Kaiser and Guggenberger 2000; Kawahigashi et al. 2006), which could lead to low rates of DOC transport to deeper layers. However, vertical movement of DOC had been detected in sandy soils with limited sorption sites (Ahmad et al. 2013; Li and Shuman 1997). The higher DOC, together with the relatively higher clay content, at 20-30 cm at the Kellerberrin site could indicate that DOC was leached down from the surface layer and adsorbed by clay minerals at subsurface layers. At Wongan Hills site, increased DOC at a depth of 40-50 cm due to liming at 6.2 or 6.2 + 1.5 t ha⁻¹ could provide evidence of DOC migration to deep layers.

In addition to lower DOC content, DOC within the limed soil was more aromatic and more chemically recalcitrant than in the unlimed soil. The DOC could be derived from both recent litter and humus, but most studies suggested that DOC consisted mainly of highly altered humified material (Karlton et al. 2005; Sanderman et al. 2008). The aromatic signal in DOC was found to be dominated by lignin-derived compounds which were highly chemically recalcitrant (Nakanishi et al. 2012; Sanderman et al. 2008). Other studies also revealed a higher proportion of labile hydrophilic material (e.g. carbohydrates) in DOC of the unlimed soils, compared with a higher proportion of aromatic hydrophobic material in the limed soils (Andersson and Nilsson 2001; Andersson et al. 2000). The higher aromaticity of DOC in the limed than unlimed soil could be attributed to two possible reasons: 1) DOC was more decomposed in the limed soil due to enhanced microbial activity at higher soil pH (Andersson et al. 2000), and 2) aromatic C compounds containing less polar hydrophobic acids became soluble as pH increased (Guggenberger et al. 1994). The lower aromaticity of DOC at a depth of 30-50 cm at the Wongan Hills site in the limed than unlimed soil possibly reflected the labile or mobile nature of DOC leached. It has been well documented that only structurally

simple molecules, such as carbohydrates held by minerals through weaker bonds, could be more easily leached down to the soil profile than aromatic compounds bound via ligand exchange (Corvasce et al. 2006; Kawahigashi et al. 2006). The susceptibility of this labile C to microbial mineralisation would be relatively low due to reduced microbial biomass and activity at deeper soil layers. Overall, the quality of DOC could be greatly affected by liming, and DOC in the limed soil showed higher stability against either microbial degradation or leaching than in the unlimed soil.

Total SOC and MIR-predicted SOC fractions

There was a substantial loss of SOC from limed soils, except when lime was applied at 6.2+1.5 t/ha at the Wongan Hills site. Liming has been shown to decrease (Chan and Heenan 1999; Marschner and Wilczynsk 1991), increase (Briedis et al. 2012; Fornara et al. 2011), or not impact SOC content (Garbuio et al. 2011; Kemmitt et al. 2006). Liming-induced decreases in SOC are mainly attributed to enhanced C mineralization following increased C solubility, or microbial activity, or both (Ahmad et al. 2013; Bezdicek et al. 2003; Fuentes et al. 2006; Kemmitt et al. 2006). However, greater C inputs as crop residues following liming would be expected to increase SOC (Bronick and Lal 2005; Briedis et al. 2012). The rate and direction of changes in SOC following liming depends on the balance between SOC gains and losses. In our study, lower SOC content in the limed than unlimed plots would suggest that additional C inputs, if they occurred, were too low to counter-balance the C losses from enhanced decomposition. On the other hand, TOC content of Wongan Hills sites receiving the highest lime rates (6.2+1.5 t ha⁻¹) was not different from that of the unlimed plots, indicating that lime-induced increase in the C input matched the enhanced decomposition of SOC. Tang et al. (2003) showed that, 18 years after liming at Wongan Hills site, shoot biomass at maturity increased by 60% due to liming at 6.2+1.5 t ha⁻¹, compared with 48% and 46% following liming at the 1.5 and 6.2 t ha⁻¹, respectively. This suggests that less than 50% increase in shoot biomass, or possibly in C input as plant residues, could not counteract the C losses from limed soils. The relative high shoot biomass production following liming at 6.2+1.2 t ha⁻¹ could be related to the great alleviation of soil acidity at both topsoil and subsoil layers (Fig. 1). The rates of liming were supposed to have a profound effect on regulating the balance and size of total SOC in the limed plots through affecting their total C input.

It has been proposed that liming can promote C storage via increased aggregate stability and the formation of Ca²⁺ complexes with SOC and clay (Baldock et al. 1994; Bronick and Lal 2005). These processes could reduce the decomposition of both soil organic matter and added plant residues (Chan and Heena 1998; Muneer and Oades 1989). However, the loss of C following liming at all sites except at Wongan Hills where lime was applied at 1.5+6.2 t ha⁻¹ does not support this. Also, the low clay content (5-7%) at Kellerberrin and Wongan Hills would render the amount of SOC that could potentially be protected through the formation of Ca²⁺ bridges between clay and SOC very low. Baldock et al. (1994) and Briedis et al. (2012) found that increases in aggregate stability and SOC storage become more significant when lime was incorporated with straw, compared with lime alone. Therefore, the direct or indirect stabilizing effects of Ca²⁺ ions may contribute little to long-term C storage in plots where C inputs and/or clay contents were low.

Decrease in TOC following liming occurred mainly in the POC and HOC pools. The ROC, mainly in the form of char, was the most chemically protected and biologically recalcitrant pool (Skjemstad et al. 1996), and was not affected by liming. Similar to TOC, the level of POC and HOC pools should be governed by two main processes: C migration from plant residue to POC, and then on to HOC with continued decomposition of POC. Following liming, more C from the POC pool could have been processed and incorporated into HOC as a consequence of fast C turnover (Baldock et al. 1994; Marschner and Wilczynski 1991;

Tonon et al. 2010). This could explain the decreased C:N ratio and decreased proportion of POC fraction by liming in this present study. Previous findings on changes in HOC after liming are contradictory, with both decreases (Marschner and Wilczynski 1991; Yagi et al. 2003) and increases reported (Chan and Heena 1998; Fornara et al. 2011). The net decrease in HOC in this study indicates that the loss of HOC outweighed the newly incorporated C from POC. In contrast, little C loss from HOC when limed at 6.2+1.5 t ha⁻¹ at the Wongan Hills site might be attributed to a greater C flux from POC to HOC associated with high C input or enhanced abiotic C sequestration through Ca-humus complex. Although HOC is considered to be relatively chemically recalcitrant, our results suggest that liming could accelerate its decomposition and decrease its contribution to long-term C storage. Lime-induced solubilisation or desorption of humic substances from mineral surface were possibly responsible for the decreased stability of HOC pool (Garbuio et al. 2011). Nevertheless, liming at high rates may result in an increase in total C input as plant residue or enhanced humus stabilization which may offset the C losses from the HOC pool due to enhanced microbial decomposition.

The humus fraction enriched with carboxyl functional groups was expected to be closely related to change in soil pHBC. Nevertheless, while soil pHBC decreased consistently with liming, HOC was not affected by the lime applied at 6.2+1.5 t ha⁻¹ at the Wongan Hills site. We speculate that liming, at high rates, will favour the formation of new complexes between functional groups of the humus and Ca²⁺, in addition to the replacement of the Al with Ca in the organic complexes. Subsequently, the availability of functional groups for soil pH buffering might be lowered. After liming, soil pHBC probably depended more on the availability rather than the quantity of functional groups indicated by HOC content or carbonyl groups. This can also partly account for the low correlation between soil pHBC and carbonyl groups.

Soil organic C composition

A lower proportion of alkyl C or alkyl/O-alkyl C ratio was observed in the limed than unlimed plots. This suggests enhanced decomposition of alkyl C in the limed soils, or the selective accumulation of alkyl C in the unlimed acid soil. However, without the baseline chemical data prior to liming, the exact process cannot be identified. A greater chemical recalcitrance of alkyl C suggests that it should be selectively preserved during SOC decomposition (Baldock et al. 1992). Thus, if the enhanced SOC decomposition following liming acted as the main mechanism, a greater decrease in the O-alkyl than alkyl C would be expected. A consistent increase in alkyl/O-alkyl ratio with increasing the degree of decomposition of fresh organic materials has been frequently observed (Baldock et al. 1992, 1997). This indicates that the alkyl/O-alkyl C ratio may not be a good indicator of the extent of C decomposition between the limed and unlimed soil.

On the other hand, alkyl C which is sourced from lipids, cutin, and suberin polyesters (Winkler et al. 2005) can be selectively preserved in acidic soils. For example, numerous studies reported that lipids or lipid-derived fatty acids (van Bergen et al. 1998; Nierop et al. 2003, 2005), or cutin- and suberin-derived moieties (Nierop 1998; Nierop and Buurman 1999) accumulated during long-term SOC formation under acidic conditions. The selective preservation of cutin and suberin was attributed to the low cutinase or suberinase activity at low soil pH (Nierop et al. 2003). Also, lime-induced changes in microbial community composition, particularly a shift from fungi to bacteria (Bååth and Anderson 2003), could also be responsible for the different C compounds accumulated in soils differing in pH. Several studies have shown that, in the long-term, lignin can be effectively degraded by fungi in acid soils, whereas lipids and suberin-derived moieties accumulated and remained relatively unaltered (Stevenson 1994; Nierop and Verstraten 2003).

To conclude, the relative proportion of alkyl C was possibly controlled by two contrasting processes: selective preservation of alkyl C due to the accumulation of lipid or polyester in the unlimed soil, and selective preservation of alkyl C during the decomposition of plant residues in both limed and unlimed soils. Litter decomposition in the limed soil at the two cropping sites was expected to contribute more to the increase in alkyl C than at the trials at the University farm with little C input. This would have narrowed the difference in the proportion of alkyl C between limed and unlimed soils where the accumulation of lipid or polyester dominated but the decomposition of litter was limited by low pH. The duration of the liming treatments at the University farm limed in 2008 might not have been long enough for greater changes in SOC composition to be detected. Rosenberg et al. (2003) also found that liming decreased the proportion of alkyl C and the alkyl/O-alkyl C ratio in a mature Norway spruce forest. They attributed the decrease of alkyl-C in the limed soil to the greater root biomass production, which was lower in alkyl C than above-ground litter. This was less likely to occur at our University site with low C input. Other studies by Lorenz et al. (2001) and Tonon et al. (2010) revealed that the ¹³C NMR spectra of limed and unlimed soils were very similar and concluded that pH did not significantly affect the chemical and structural composition of SOC. Among all studies, soil microbial communities were expected to differ among soils with different environmental physico-chemical properties (Delmont et al. 2014), which could possibly account for the observed discrepancies. Our study suggests that differences in the quantity and quality of C inputs associated with liming rates or land management practices might also have a great impact on the final SOC composition.

Conclusion

Total SOC at 0-10 cm decreased or remained unchanged following long-term liming, depending on the rates of lime application. Decreases in SOC due to liming occurred mainly in the labile and moderately labile C pools, and only in the surface soil layer. To maximise C input as crop residues and to prevent the loss of C from limed soil, lime should be applied periodically at relatively high rates or more frequently at lower rates. Liming practices should also be accompanied by retention of crop residues, considering enhanced mineralisation associated with high soil pH. On the other hand, liming possibly had a marked effect on regulating the decomposition and preservation of certain C compounds. The apparent accumulation of alkyl C in the unlimed soil could indicate the potential ability of acid soils to store SOC. Less changes in the chemical composition of SOC of the two cropping sites in Western Australia was supposed to be partly masked by the continuous replenishment of C via residue return, although further research is required to confirm this speculation.

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References

- Ahmad W, Singh B, Dijkstra FA, Dalal RC (2013) Inorganic and organic carbon dynamics in a limed acid soil are mediated by plants. *Soil Biol Biochem* 57:549-555
- Andersson S, Nilsson SI (2001) Influence of pH and temperature on microbial activity, substrate availability of soil-solution bacteria and leaching of dissolved organic C in a mor humus. *Soil Biol Biochem* 33:1181-1191

Andersson S, Nilsson SI, Saetre P (2000) Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. *Soil Biol Biochem* 32:1-10

Andersson S, Valeur I, Nilsson I (1994) Influence of lime on soil respiration, leaching of DOC, and C/S relationships in the mor humus of a haplic podsol. *Environm Int* 20:81-88

Bååth E, Anderson TH (2003) Comparison of soil fungal/ bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biol Biochem* 35:955-963

Baldock JA, Aoyama JM, Oades JM, Susanto Grant CD (1994) Structural amelioration of a South Australian Red brown earth using calcium and organic amendments. *Aust J Soil Res* 32:571-594.

Baldock JA, Hawke B, Sanderman J, Macdonald LM (2013a) Predicting contents of carbon and its component fractions in Australian soils from diffuse reflectance mid-infrared spectra. *Soil Res* 51:577-595

Baldock JA, Oades JM, Nelson PN, Skene TM, Golchin A, Clarke P (1997) Assessing the extent of decomposition of natural organic materials using solid-stage ¹³C NMR spectroscopy. *Aust J Soil Res* 35:1061-1083

Baldock JA, Oades JM, Waters AG, Peng X, Vassallo AM, Wilson MA (1992) Aspects of the chemical structure of soil organic materials as revealed by solid-state ¹³C NMR spectroscopy. *Biogeochemistry* 16:1-42

Baldock JA, Sanderman J, Macdonald L, Puccini A, Hawke B, Szarvas S, McGowan J (2013b) Quantifying the allocation of soil organic carbon to biologically significant fractions. *Soil Res* 51:561-576

Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. *Org Geochem* 33:1093-1109

Bezdicsek DF, Beaver T, Granatstein D (2003) Subsoil ridge tillage and lime effects on soil microbial activity, soil pH, erosion, and wheat and pea yield in the Pacific Northwest, USA. *Soil Till Res* 74:55-63

Blevins RL, Murdock LW, Thomas GW (1978) Effect of lime application on no tillage and conventionally-tilled corn. *Agron J* 70:322-326

Briedis C, Sá JCM, Caires EF, Navarro JF, Inagaki TM, Boer A, Neto CQ, Ferreira AO, Canalli LB, Santos JB (2012) Soil organic matter pools and carbon-protection mechanisms in aggregate classes influenced by surface liming in a no-till system. *Geoderma* 170:80-88

Bronick CJ, Lal R (2005) Soil structure and management: a review. *Geoderma* 124:3-22

Bull ID, Nott CJ, van Bergen PF, Poulton PR, Evershed RP (2000) Organic geochemical studies of soils from the Rothamsted Classical Experiments – VI. The occurrence and source of organic acids in an experimental grassland soil. *Soil Biol Biochem* 32:1367-1376

522 Chan KY, Heenan DP (1999) Lime-induced loss of soil organic carbon and effect on
aggregate stability. *Soil Sci Soc Am J* 63:1841-1844

524 Conyers MK, Heenan DP, McGhie WJ, Poile GP (2003) Amelioration of acidity with time by
limestone under contrasting tillage. *Soil Till Res* 42:85-94

526 Corvasce M, Zsolnay A, D'Orazio V, Lopez R, Miano TM (2006) Characterization of water
extractable organic matter in a deep soil profile. *Chemosphere* 62:1583-1590

528 Courtier-Murias D, Farooq H, Longstaffe JG, Kelleher BP, Hart KM, Simpson MJ, Simpson
AJ (2014) Cross polarization-single pulse/magic angle spinning (CPSP/MAS): A robust
530 technique for routine soil analysis by solid-state NMR. *Geoderma* 226:405-414

532 Delmont TO, Francioli D, Jacquesson S, Laoudi S, Nesme MJ, Ceccherini MT, Nannipieri P,
Simonet P, Vogel TM (2014) Microbial community development and unseen diversity
534 recovery in inoculated sterile soil. *Biol Fertil Soils* 50:1069-1076

536 Erich MS, Trusty GM (1997) Chemical characterization of dissolved organic matter released
by limed and unlimed forest soil horizons. *Can J Soil Sci* 77:405-413

538 Fornara DA, Steinbeiss S, McNamara NP, Gleixner G, Oakley S, Poulton PR, Macdonald AJ,
540 Bardgett RD (2011) Increases in soil organic carbon sequestration can reduce the global
warming potential of long-term liming to permanent grassland. *Global Change Biol* 17:1925-
542 1934

544 Fuentes JP, Bezdicsek DF, Flury M, Albrecht S, Smith JL (2006) Microbial activity affected
by lime in a long-term no-till soil. *Soil Till Res* 88:123-131.

546 Garbuio FJ, Jones DL, Alleoni LRF, Murphy DV, Caires EF (2011) Carbon and nitrogen
548 dynamics in an Oxisol as affected by liming and crop residues under no-till. *Soil Sci Soc Am J* 75:1723-1730

550 Godsey, CB, Pierzynski GM, Mengel DB, Lamond RE (2007) Management of soil acidity in
552 notill production systems through surface application of lime. *Agron J* 99:764-772.

554 Guggenberger G, Glaser B, Zech W (1994) Heavy metal binding by hydrophobic and
hydrophilic dissolved organic carbon fractions in a Spodosol A and B horizon. *Water Air Soil*
556 *Poll* 72:111-127

558 Guggenberger G, Zech W, Haumaier L, Christensen BT (1995) Land-use effects on the
composition of organic matter in particle-size separates of soils: II. CPMAS and solution ¹³C
560 NMR analysis. *Eur J Soil Sci* 46:147-158

562 Heanes DL (1984) Determination of total organic-C in soils by an improved chromic acid
digestion and spectrophotometric procedure. *Commun Soil Sci Plant Anal* 15:1191-1213.

564 Isbell RF (2002) *The Australian Soil Classification*. CSIRO Publishing, Collingwood

566 Kaiser K, Guggenberger G (2000) The role of DOM sorption to mineral surfaces in the
preservation of organic matter in soils. *Org Geochem* 31:711-725

568 Karlton E, Harrison AF, Alriksson A, Bryant C, Garnett MH, Olsson MT (2005) Old organic
carbon in soil solution DOC after afforestation - evidence from C-14 analysis. *Geoderma*
570 127:188-195

572 Kawahigashi M, Kaiser K, Rodionov A, Guggenberger G (2006) Sorption of dissolved
organic matter by mineral soils of the Siberian forest tundra. *Global Change Biol* 12:1868-
1877

574 Kemmitt SJ, Wright D, Goulding KWT, Jones DL (2006) pH regulation of carbon and
nitrogen dynamics in two agricultural soils. *Soil Biol Biochem* 38:898-911

576 Krull ES, Baldock JA, Skjemstad JO (2003) Importance of mechanisms and processes of the
stabilization of soil organic matter for modelling carbon turnover. *Funct Plant Biol* 30:207-
222

580 Li Z, Shuman LM (1997) Estimation of retardation factor of dissolved organic carbon in
sandy soils using batch experiments. *Geoderma* 78:197-206

582 Lorenz K, Preston CM, Feger KH (2001) Long term effects of lime on microbial biomass and
activity and soil organic matter quality (^{13}C CPMAS NMR) in organic horizons of Norway
584 spruce forests in Southern Germany. *J Plant Nutri Soil Sci* 164:555-560

586 Maana MC, Swarup A, Wanjari RH (2007) Long-term fertilization, manure and liming effects
on soil organic matter and crop yields. *Soil Till Res* 94:397-409

588 Marschner B, Wilczynski A (1991) The effect of liming on quantity and chemical
composition of soil organic matter in a pine forest in Berlin, Germany. *Plant Soil* 137:229-236

590 Morris SJ, Bohm S, Haile-Mariam S, Paul EA (2007) Evaluation of carbon accrual in
592 afforested agricultural soils. *Global Change Biol* 13:1145-1156

594 Muneer M, Oades JM (1989) The role of Ca-organic interactions in soil aggregate stability. II.
Field studies with ^{14}C -labelled straw, CaCO_3 and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$. *Aust J Soil Res* 27:401-409

596 Nakanishi T, Atarashi-Andoh M, Koarashi J, Saito-Kokubu Y, Hirai K (2012) Carbon
isotopes of water-extractable organic carbon in a depth profile of forest soil imply a dynamic
598 relationship with soil carbon. *Eur J Soil Sci* 63:495-500

600 Nierop, KGJ (1998) Origin of aliphatic compounds in a forest soil. *Org Geochem* 29:1009-
602 1016

604 Nierop KGJ, Buurman P (1999) Insoluble organic matter fractions in incipient podzol B
horizon: preservation of aliphatic biopolymers from roots. *Humic Subst Environ* 1:29-37

606 Nierop KGJ, Naafs DFW, Van Bergen PF (2005) Origin, occurrence and fate of extractable
608 lipids in Dutch coastal dune soils along a pH gradient. *Org Geochem* 36:555-566

610 Nierop KGJ, Naafs DFW, Verstraten JM (2003) Occurrence and distribution of ester-bound
lipids in Dutch coastal dune soils along a pH gradient. *Org Geochem* 34:719-729

612 Nierop KGJ, Verstraten JM (2003) Organic matter formation in sandy subsurface horizons of
614 Dutch coastal dunes in relation to soil acidification. *Org Geochem* 34:499-513

616 Rosenberg W, Nierop KGJ, Knicker H, Jager PA, Kreutzer K, Weiss T (2003) Liming effects
on the chemical composition of the organic surface layer of a mature Norway spruce stand
618 (*Picea abies* [L.] Karst.). *Soil Biol Biochem* 35:155-165

620 Sanderman J, Baldock JA, Amundson R (2008) Dissolved organic carbon chemistry and
 dynamics in contrasting forest and grassland soils, *Biogeochemistry* 89:181–198
 622
 624 Schmidt A, Smernik RJ, McBeath TM (2012) Measuring organic carbon in calcarosols:
 understanding the pitfalls and complications. *Soil Res* 50:397-405.
 626 Simpson AJ, Simpson MJ, Soong R (2012) Nuclear Magnetic Resonance Spectroscopy and
 Its Key Role in Environmental Research. *Environ Sci Technol* 46:11488-11496
 628
 630 Six J, Guggenberger G, Paustian K, Haumaier L, Elliott ET, Zech W (2001) Sources and
 composition of soil organic matter fractions between and within soil aggregates. *Eur J Soil
 Sci* 52:607-618
 632
 634 Skjemstad JO, Clarke P, Taylor JA, Oades JM, McClure SG (1996). The chemistry and nature
 of protected carbon in soil. *Aust J Soil Res* 34:251-271
 636 Skjemstad JO, Clarke P, Taylor JA, Oades JM, Newman RH (1994) The removal of magnetic
 materials from surface soils. A solid-state ^{13}C CP/MAS NMR study. *Aust J Soil Res* 32:1215-
 638 1229
 640 Skjemstad JO, Spouncer LR, Cowie B, Swift RS (2004) Calibration of the Rothamsted
 organic carbon turnover model (RothC ver. 26.3), using measurable soil organic carbon pools.
 642 *Aust J Soil Res* 42:79-88
 644 Stevenson FJ (1994) *Humus Chemistry*. New York: John Wiley and Sons, pp1-211; 259-302.
 646 Tang C, Rengel Z, Diatloff E, Gazey C (2003) Responses of wheat and barley to liming on a
 sandy soil with subsoil acidity. *Field Crop Res* 80:235-244.
 648
 650 Tonon G, Sohi S, Francioso O, Ferrari E, Montecchio D, Gioacchini P, Ciavatta C, Panzacchi
 P, Powlson D (2010) Effect of soil pH on the chemical composition of organic matter in
 physically separated soil fractions in two broadleaf woodland sites at Rothamsted, UK. *Eur J
 Soil Sci* 61:970-979.
 652
 654 van Bergen PF, Bull ID, Poulton PR, Evershed RP (1998) Organic geochemical studies of
 soils from the Rothamsted Classical Experiment- I. Total lipid extracts, solved insoluble
 656 residues and humic acids from Broadbalk Wilderness. *Org Geochem*. 26:117-135
 658 Wang X, Tang C, Mahony S, Baldock JA, Butterly CR (2015) Factors affecting the
 measurement of soil pH buffer capacity: approaches to optimize the methods. *Eur J Soil Sci*
 660 66:53-64
 662 Weishaar JL, Aiken, GR, Bergamashi BA, Fram MS, Fujii Ri, Mopper K (2003) Evaluation
 of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of
 664 dissolved organic carbon, *Environ Sci Technol* 37: 4702-4708
 666 Winkler A, Haumaier L, Zech W (2005) Insoluble alkyl carbon components in soils derive
 mainly from cutin and suberin. *Org Geochem* 36:519-529
 668

Yagi R, Ferreira M.E, Cruz MCP, Barbosa JC (2003) Organic matter fractions and soil
670 fertility under the influence of liming, vermicompost and cattle manure. Sci Agric 60:549-557

672

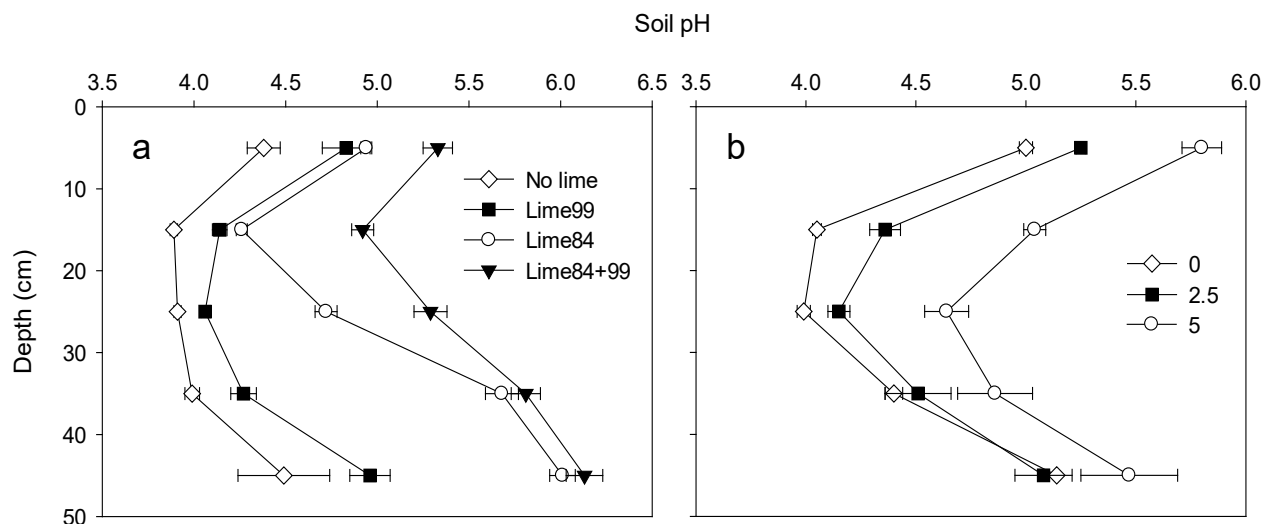
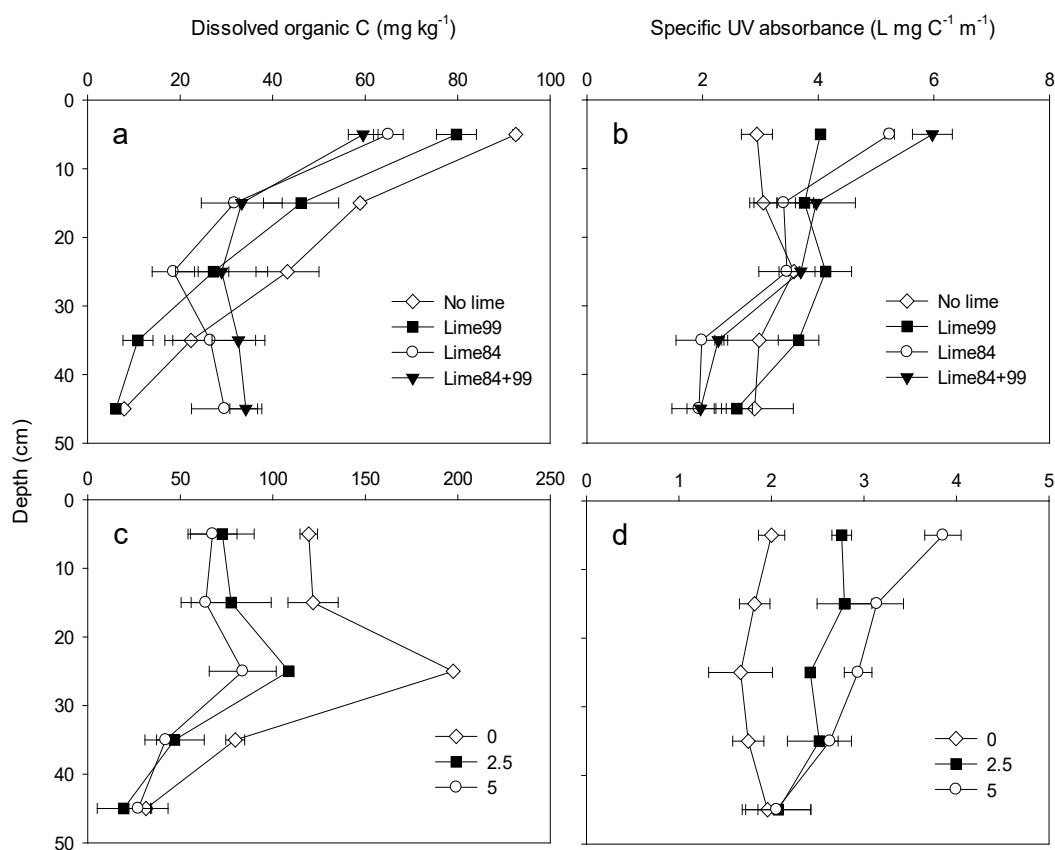


Figure 1. Soil pH profiles for long-term lime trials at Wongan Hills (a) and Kellerberrin (b), Western Australia. Lime treatments at Wongan Hills include: no lime (no lime), liming at 1.5 t ha⁻¹ in 1999 (lime99), liming at 6.2 t ha⁻¹ in 1984 (lime84), and liming at 6.2 t ha⁻¹ in 1984 and 1.5 t ha⁻¹ in 1999 (lime84+99). Lime treatments at Kellerberrin include: no lime (0), liming at 2.5 t ha⁻¹ (2.5) and liming at 5 t ha⁻¹ (5). Horizontal bars indicate the standard error of 3 replicates.



682 **Figure 2.** Dissolved organic C (mg kg^{-1}) (a, c) and its specific UV absorbance (L mg^{-1}
684 C m^{-1}) (b, d) in soil profiles of long-term lime trials at Wongan Hills (a, b) and
686 Kellerberrin (c, d), Western Australia. Lime treatments at Wongan Hills include: no
688 lime (no lime), liming at 1.5 t ha^{-1} in 1999 (lime99), liming at 6.2 t ha^{-1} in 1984
(lime84), and liming at 6.2 t ha^{-1} in 1984 and 1.5 t ha^{-1} in 1999 (lime84+99). Lime
treatments at Kellerberrin include: no lime (0), liming at 2.5 t ha^{-1} (2.5) and liming at
 5 t ha^{-1} (5). Horizontal bars indicate \pm the standard error of 3 replicates.

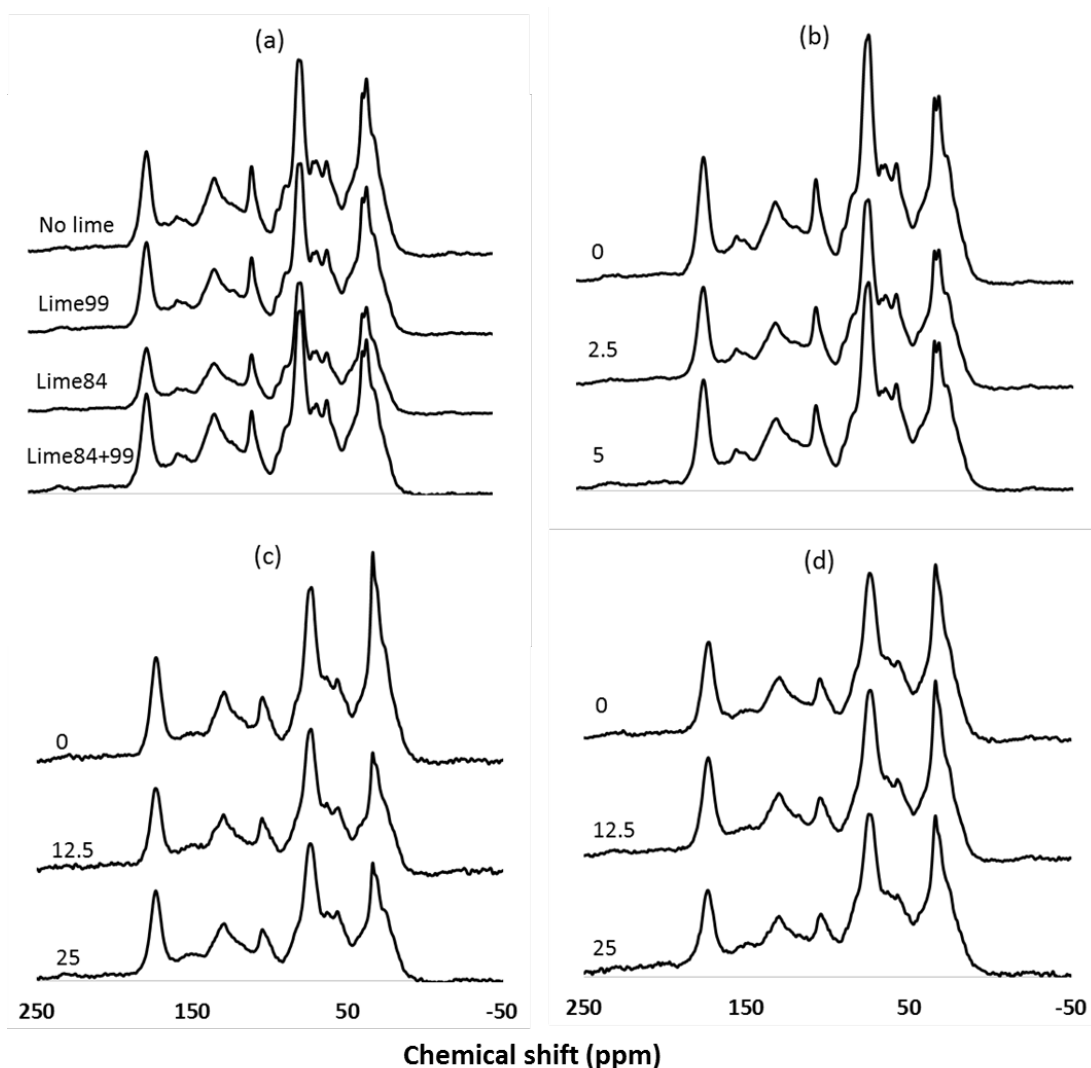


Figure 3. Solid state ^{13}C CP/MAS NMR spectra acquired from HF-treated soils collected from four long-term lime trials (0-10 cm) at Wongan Hills (a), Kellerberrin (b), La Trobe University farm limed in 1979 (c) and 2008 (d). Lime treatments at Wongan Hills include: no lime (no lime), liming at 1.5 t ha^{-1} in 1999 (lime99), liming at 6.2 t ha^{-1} in 1984 (lime84) and liming at 6.2 t ha^{-1} in 1984 and 1.5 t ha^{-1} in 1999 (lime84+99). Lime treatments at Kellerberrin include: no lime (0), liming at 2.5 and 5 t ha^{-1} . Lime treatments at the university farm include: no lime (0), liming at 12.5 and 25 t ha^{-1} in 1979 and liming at 12.5 and 25 t ha^{-1} in 2008.

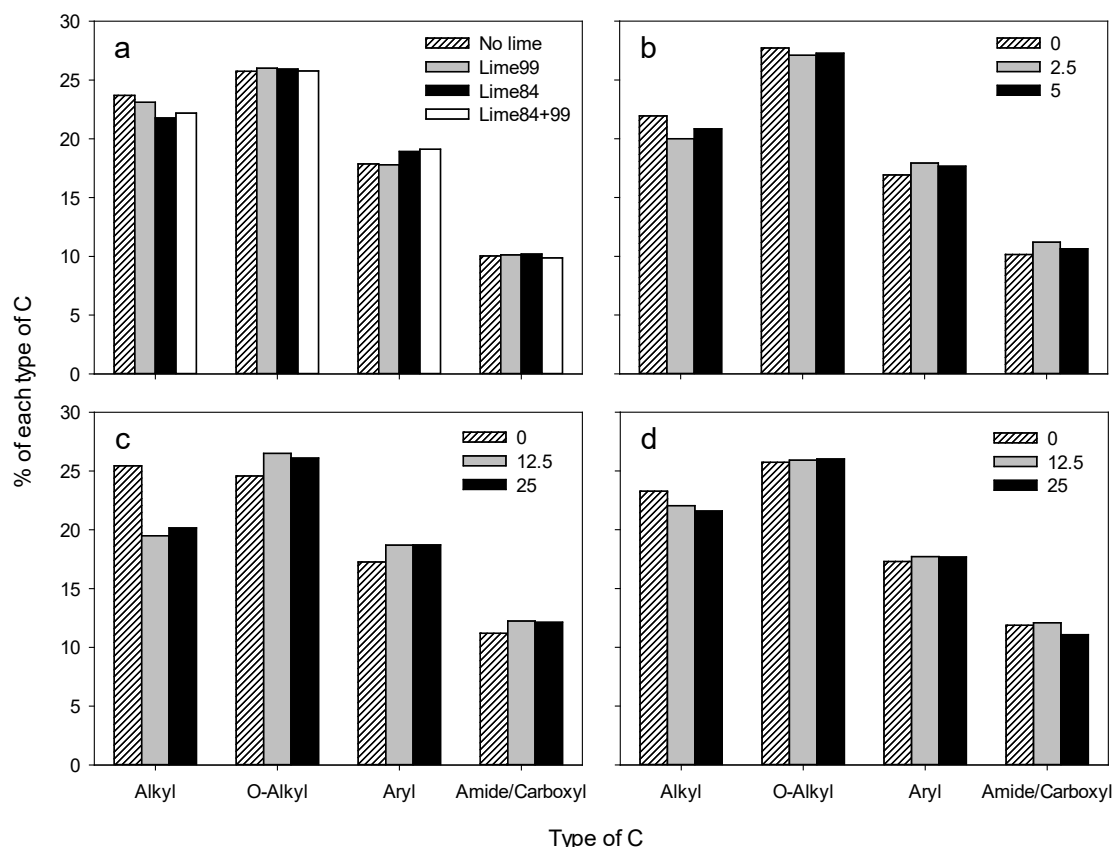


Figure 4. Distribution of each type of C in HF-treated soils collected from long-term lime trials (0-10 cm) at Wongan Hills (a), Kellerberrin (b), and La Trobe University farm limed in 1979 (c) and 2008 (d). Lime treatments at Wongan Hills include: no lime (no lime), liming at 1.5 t ha⁻¹ in 1999 (lime99), liming at 6.2 t ha⁻¹ in 1984 (lime84), and liming at 6.2 t ha⁻¹ in 1984 and 1.5 t ha⁻¹ in 1999 (lime84+99). Lime treatments at Kellerberrin include: no lime (0), liming at 2.5 and 5 t ha⁻¹. Lime treatments at the University farm include: no lime (0), liming at 12.5 and 25 t ha⁻¹ in 1979 and liming at 12.5 and 25 t ha⁻¹ in 2008.

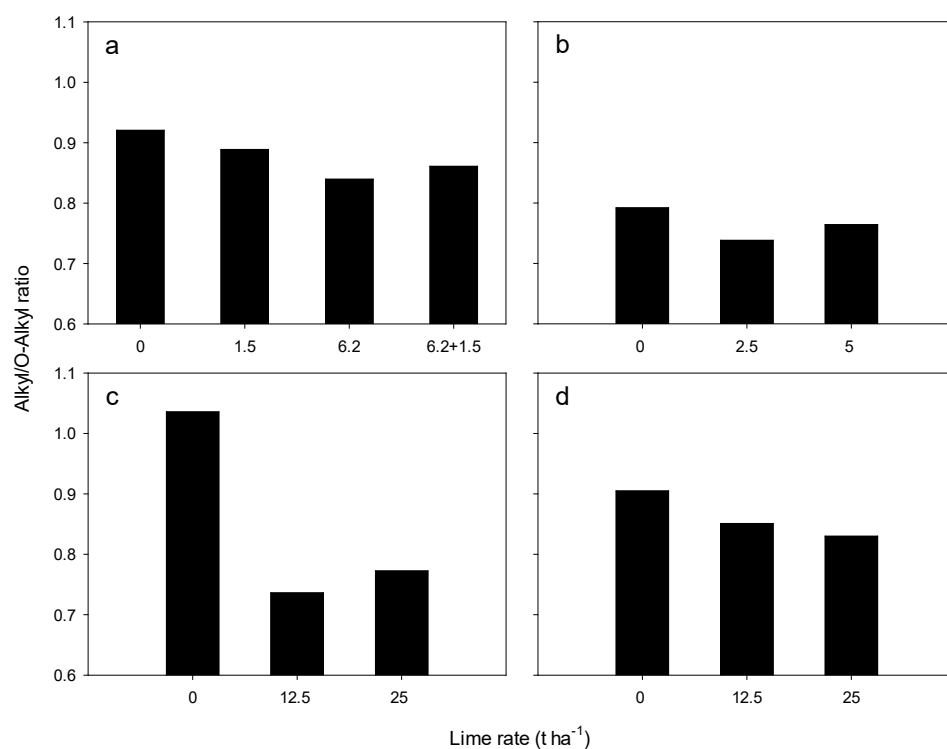


Figure 5. Alkyl/O-Alkyl C ratio in the soils collected from long-term lime trials (0-10 cm) at Wongan Hills (a), Kellerberrin (b), La Trobe University farm limed in 1979 (c) and 2008 (d).

Table 1. Soil organic C (SOC) (mg g^{-1}) and C/N ratio at depths of 0-10, 10-20, 20-30, 30-40 and 40-50 cm at Wongan Hills. Lime treatments include: no lime (no lime), liming at 1.5 t ha^{-1} in 1999 (lime99), liming at 6.2 t ha^{-1} in 1984 (lime84), and liming at 6.2 t ha^{-1} in 1984 and 1.5 t ha^{-1} in 1999 (lime84+99).

Soil depth (cm)	Lime treatments (t ha ⁻¹)				LSD (<i>P</i> = 0.05)
	No lime	Lime99	Lime84	Lime84+99	
<i>SOC (mg g⁻¹)</i>					
0-10	7.7	6.9	5.7	7.3	0.86
10-20	2.3	2.2	2.1	2.5	n.s.
20-30	1.0	1.1	1.0	1.0	n.s.
30-40	0.7	0.6	0.8	0.8	n.s.
40-50	0.6	0.5	0.6	0.7	n.s.
<i>C/N ratio</i>					
0-10	11.2	10.3	10.2	11.1	0.83
10-20	10.0	8.4	7.6	9.7	n.s.
20-30	6.7	7.3	5.6	7.0	n.s.
30-40	4.7	4.2	6.3	6.3	n.s.
40-50	4.2	3.5	5.0	5.3	n.s.

n.s., not significant at $P = 0.05$.

Table 2. Soil organic C (mg g^{-1}) (SOC) and C/N ratio at depths of 0-10, 10-20, 20-30, 30-40 and 40-50 cm at Kellerberrin. Lime treatments include: no lime (0), liming at 2.5 and 5 t ha^{-1} in 1991.

Soil depth (cm)	Lime treatments (t ha^{-1})			LSD ($P = 0.05$)
	0	2.5	5	
<i>SOC (mg g^{-1})</i>				
0-10	11.9	11.0	10.4	1.2
10-20	3.5	3.1	3.3	n.s.
20-30	2.1	1.8	2.1	0.2
30-40	1.6	1.3	1.4	n.s.
40-50	1.4	1.2	1.2	n.s.
<i>C/N ratio</i>				
0-10	13.1	11.3	10.7	1.3
10-20	8.5	9.7	9.3	n.s.
20-30	7.3	6.0	7.0	0.7
30-40	5.3	5.5	6.0	n.s.
40-50	6.5	5.5	6.0	n.s.

n.s., not significant at $P = 0.05$.

Table 3. Soil pH buffer capacity (pHBC) and MIR-predicted particular organic C (POC), humus organic C (HOC), resistant organic C (ROC) and total organic C (TOC) in the soils collected at 0-10 cm from four long-term lime trials at Wongan Hills, Kellerberrin, La Trobe University farm (1979, 2008). Lime treatments at Wongan Hills include: no lime (no lime), liming at 1.5 t ha⁻¹ in 1999 (lime99), liming at 6.2 t ha⁻¹ in 1984 (lime84) and liming at 6.2 t ha⁻¹ in 1984 and 1.5 t ha⁻¹ in 1999 (lime84+99). Lime treatments at Kellerberrin include: no lime (0), liming at 2.5 and 5 t ha⁻¹. Lime treatments at the university farm include: no lime (0), liming at 12.5 and 25 t ha⁻¹ in 1979, liming at 12.5 and 25 t ha⁻¹ in 2008.

Site	Lime treatment (t ha ⁻¹)	MIR-predicted POC	HOC	ROC	TOC	Soil pHBC (mmol _c kg ⁻¹ pH ⁻¹)
		(mg g ⁻¹)				
Wongan Hills (1983)	0-0 (no lime)	2.2	4.1	3.7	8.1	7.0
	0-1.5 (lime99)	1.7	3.5	3.7	7.2	6.3
	2.5-0 (lime84)	1.2	2.6	3.1	5.3	6.0
	2.5-1.5 (lime84+99)	1.8	4.1	4.2	8.0	6.0
	LSD (<i>P</i> = 0.05)	0.4	0.7	0.5	1.9	0.7
Kellerberrin (1991)	0	3.0	7.3	4.0	13.2	9.3
	2.5	1.8	5.6	3.7	9.7	7.9
	5	2.5	6.4	3.9	10.8	8.0
	LSD (<i>P</i> = 0.05)	0.7	0.9	n.s.	2.0	0.5
La Trobe University farm						
(1979)	0	4.3	9.4	6.7	19.7	18.2
	12.5	3.2	8.0	6.2	16.3	12.1
	25	3.4	8.4	6.6	17.1	12.5
	LSD (<i>P</i> = 0.05)	0.7	1.1	n.s.	2.3	1.1
(2008)	0	4.7	11.6	6.4	24.2	18.0
	12.5	3.7	9.8	6.4	20.6	17.7
	25	3.8	10.3	6.4	21.3	20.2
	LSD (<i>P</i> = 0.05)	0.8	1.0	n.s.	2.4	n.s.

n.s., not significant at *P* = 0.05.