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 topsoil and subsoil acidities 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 humic 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 were 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.

Keywords 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 behavior 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 (Balock 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 (Bezdicek 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 Ca\(^{2+}\) thereby enhancing its resistance to decomposition (Manna et al. 2007; Morris 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 (Formara 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 (Formara 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 hypothesized 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 1×2-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 gravel (~5 %) below 10 cm. The trial consisted of 12 plots of 1.8×22 m (Tang et al. 2003). Soils were sampled in May 2013 from the following 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 three replicates. The annual crop rotation on the trial and surrounding paddock were 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 randomized design with 15×100-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\(^{-1}\) with a further 1 t ha\(^{-1}\) 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 five 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 h end-over-end shaking, centrifuging at 700g 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 analyzer (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 2800g 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).

SOC was determined by dry combustion using a CHNS Analyzer (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 was limed at 25 t ha⁻¹ lime of the second trial showed visible effervescence and was pretreated with H₂SO₄ to remove inorganic C (Schmidt et al. 2012).

SOC characterization 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 8000–4000 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 analyzing 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, Olso, 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 ¹³C NMR analysis were prepared by forming single composite samples for each treatment at each site by combining a mass of soil normalized 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 h 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 backward 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 lyophilized and ground samples were treated with 2 % hydrofluoric acid (HF) to further concentrate SOC and remove paramagnetic materials (Skjemstad et al. 1994).

Solid-state ¹³C 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 hexamethylenzene at 17.36 ppm, and a 50-Hz Lorentzian line broadening was applied to all spectra. Three separate ¹³C NMR experiments were performed. An inversion recovery pulse sequence using eight inversion recovery times varying from 0.001 to 3.0 s and a recycle delay (d₁) of 5 s was applied to each sample. T₁H 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 T₁H values) to avoid saturation in the subsequent cross-polarisation (CP) ¹³C 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 spinlock experiment using an array of spinlock 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 T₁H 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 to 85 % for other three sites.

All spectral processing including the calculations of $T_1H$ and $T_1ρH$ and integration of spectral regions were 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 sidebands 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 analyzed 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 total organic carbon (TOC), with the content of each type of C lower in the limed than unlimed soils, 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 ($≤0.5\%$), 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 gravel 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 mg kg$^{-1}$) (Godsey et al. 2007; Conyers et al. 2003). Given the soil pHBC of 6.3 mmol,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.

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

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**Fig. 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 three replicates.

**Fig. 2** Dissolved organic C (mg kg$^{-1}$) (a, c) and its specific UV absorbance (L mg$^{-1}$ C m$^{-1}$) (b, d) in soil profiles of long-term lime trials at Wongan Hills (a, b) and Kellerberrin (c, d), 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 three replicates.
Therefore, liming could increase or decrease DOC concentration. Enhanced solubility and leaching of DOC due to deprotonation or desorption have also been observed after liming (Andersson et al. 1994, 2000). Erich and Trusty (1997) found that DOC in the limed plots was more susceptible to microbial attack 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). 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 sub-surface 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 (Karltn 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 lower aromaticity of DOC in the limed than unlimed soils 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 soils 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

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**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

| Soil depth (cm) | Lime treatments (t ha$^{-1}$) | SOC (mg g$^{-1}$) | C/N ratio | LSD ($P=0.05$) |
|----------------|-------------------------------|------------------|-----------|----------------|
|                | No lime | Lime99 | Lime84 | Lime84+99 |                |
| 0–10           | 7.7     | 6.9    | 5.7    | 7.3      | 0.38           |
| 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.           |

Lime treatments include no lime (0), liming at 2.5, and liming at 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)

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

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**Table 2** 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 Kellerberrin

| Soil depth (cm) | Lime treatments (t ha$^{-1}$) | SOC (mg g$^{-1}$) | C/N ratio | LSD ($P=0.05$) |
|----------------|-------------------------------|------------------|-----------|----------------|
|                | 0                              | 2.5              | 5         |                |
| 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.           |

Lime treatments include no lime (0), liming at 2.5, and liming at 5 t ha$^{-1}$ in 1991

n.s. not significant at $P=0.05$
C to microbial mineralization 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⁻¹ at the Wongan Hills site. Liming has been shown to decrease (Chan and Heenan 1999; Marschner and Wilczynski 1991), increase (Briedis et al. 2012; Formara 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, 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 depend 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 counterbalance the C losses from enhanced decomposition. On the other hand, the TOC content of Wongan Hills site 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 the 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.

Table 3  Soil pH buffer capacity (pHBC) and MIR-predicted particulate organic C (POC), humic 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, and La Trobe University farm (1979 and 2008)

| Site                      | Lime treatment (t ha⁻¹) | MIR-predicted | Soil pHBC (mmolkg⁻¹ pH⁻¹) |
|---------------------------|-------------------------|---------------|---------------------------|
|                           | POC (mg g⁻¹)            | HOC           | ROC (mg g⁻¹)              | TOC (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                       |
|                           | 6.2–0 (lime84)          | 1.2           | 2.6                       | 3.1                       | 5.3                       | 6.0                       |
|                           | 6.2–1.5 (lime84+99)     | 1.8           | 4.1                       | 4.2                       | 8.0                       | 6.0                       |
|                           | LSD (P=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 (P=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 (P=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 (P=0.05)            | 0.8           | 1.0                       | n.s.                      | 2.4                       | n.s.                      |

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 liming at 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 n.s. not significant at P=0.05
It has been proposed that liming can promote SOC storage via increased aggregate stability and the formation of Ca\(^{2+}\) 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 Heenan 1999; 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 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 liming at 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. 

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 levels of POC and HOC pools should be governed by the following two main processes: C migration from plant residue to POC and then onto 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 the 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 Heenan 1999; 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 solubilization 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.

Fig. 5 Alkyl/O-alkyl C ratio in the 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).
at the Wongan Hills site. We speculate that liming, at high rates, will favor the formation of new complexes between functional groups of the humus and Ca\(^{2+}\), 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 unplotted 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 soils.

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 the following 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 aboveground 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 \(^{13}\)C 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 maximize 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 mineralization 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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The text contains references to various studies and experiments related to soil fertility, organic matter, and liming effects. It mentions the establishment of a site by Dr. Nick Uren and the importance of scientific research in understanding soil properties and ecological processes. The references include studies on microbial activity, soil fertility, and carbon dynamics in limed and organic plots. The text is a part of a scientific article discussing the role of lime and organic matter in soil fertility and crop productivity.
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