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

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Abstract

This study used two field trials with 5 and 34 years of liming histories, respectively, and aimed to elucidate the long-term effect of liming on soil organic C (SOC) in acid soils. It was hypothesized that long-term liming would increase SOC concentration, macro-aggregate stability and SOC concentration within aggregates. Surface soils (0-10 cm) were sampled and separated into four aggregate-size classes: large macro-aggregates (>2 mm), small macro-aggregates (0.25-2 mm), micro-aggregates (0.053-0.25 mm), and silt and clay fraction (<0.053 mm) by wet sieving and the SOC concentration of each aggregate-size was quantified. Liming decreased SOC in the bulk soil and in aggregates as well as macro-aggregate stability in the low-input and cultivated 34-year-old trial. In contrast, liming did not significantly change the concentration of SOC in the bulk soil or in aggregates but improved macro-aggregate stability in the 5-year-old trial under undisturbed unimproved pastures. Furthermore, the single application of lime to the surface soil increased pH in both topsoil (0-10 cm) and subsoil (10-20 cm), and increased K_2SO_4-extractable C, microbial biomass C (C_{mic}), and basal respiration (CO_2) in both soil layers of both lime trials. Liming increased the percentage of SOC present as microbial biomass C (C_{mic}/C_{org}) and decreased the respiration rate per unit biomass (qCO_2). The study concludes that despite long-term liming decreased total SOC in the low-input systems, it increased labile C pools and the percentage of SOC present as microbial biomass C.

Keywords: carbon protection, lime, microbial biomass, microbial carbon-use efficiency, soil organic matter, soil pH

Introduction

Lime is widely applied to acid soils to counteract soil acidification and to minimize toxic effects of Al^{3+} and Mn^{2+} (Scott et al. 2003). However, there are concerns about the impact of liming on the stability of soil organic C (SOC) and on its contribution to CO_2 emissions (Paradelo et al. 2015). The application of lime to acid soils ameliorates soil acidity, but it can also contribute to soil CO_2 emissions due to chemical dissolution of lime and to changes in biological processes which enhance SOC mineralization in response to increased soil pH (Fuentes et al. 2006; Smolander et al. 1994). Given that soil

acidification and lime utilization are increasing worldwide (Fisher et al. 2003), the effects of liming on soil CO_2 impact the global C cycle cumulatively. Studies have investigated the dynamics of SOC induced by liming (Ignacio Rangel-Castro et al. 2004; Staddon et al. 2003), however, contradictory results have been reported: liming decreased (Bertrand et al. 2007), increased (Johnson et al. 2005) or did not change (Lorenz et al. 2001) SOC concentrations in acid soils. Although there are many studies showing changes of SOC following liming, these studies mainly focused on changes in the quantity of SOC in the short-term while little is known how liming affects changes in net SOC in the long term.

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

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

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

Materials and Methods

Site description

This study utilised two long-term lime trials initiated in 1979 and 2008, respectively, at the La Trobe University farm, Victoria, Australia (37°42'58.00"S 145°02'53.50"E and 83 m above mean sea level). The mean maximum and minimum air temperatures at the trial site are 20 °C and 9.6 °C, respectively, and the mean annual rainfall is 666 mm. The soil was classified as Sodosol (Isbell 2002) with the following characteristics: 10% sand, 61% silt, and 29% clay, electrical conductivity (EC) of 131 µS cm^{-1}, bulk density of 1.3 g cm^{-3}, total organic C 20.5 mg g^{-1}, and total N 1.8 mg g^{-1}. The clay minerals of this soil were predominantly illite (70%) with some kaolinite (30%) (Wang et al. 2015).

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

Soil sampling

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

Soil analysis

Soil moisture content of the samples was determined immediately after sampling by weighing the soil prior and after oven drying at 105 °C for 24 h. Soil pH was measured with a Thermo Orion pH meter (Thermo Orion 720A+, USA) after adding 0.01 M $CaCl_2$ to soil (1:5) and shaking on an end-over-end shaker for 1 h. Soil microbial biomass C (C_{mic}) was determined using field-moist soil immediately after sampling by 24-h fumigation with ethanol-free chloroform followed by 1-h extraction according to Vance et al. (1987). Eight grams of fumigated and non-fumigated soil samples were extracted with 40 mL 0.5 M K_2SO_4 and filtered through Whatman 42 filter papers (nominal pore size 2.5 µm) (Whatman International, Maidstone, England). Three blanks without soil were also filtrated to correct potential C

contamination from the filter papers. Extracts were frozen at -20 °C until extractable C analysis by using chromic acid digestion and spectrophotometer method (Heanes 1984). The C_{mic} was calculated as the difference between extractable C from fumigated and non-fumigated samples, and incomplete extractability was corrected by a conversion factor (K_{EC}) of 0.45 (Jenkinson et al. 2004). The percentage of SOC present as C_{mic} (C_{mic}/C_{org}) was calculated by dividing C_{mic} by SOC multiplied 100 in order to better understand changes in microbial C-use efficiency due to liming. Organic C extracted from non-fumigated soils was denoted as K_2SO_4-extractable C, which is potential C substrates for microbial biomass.

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

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

Soil aggregate size fractionation

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

Density fractionation and sand correction

It is well recognised that SOC exists in soils in forms with different turnover rates due to different degree of physical and chemical protections. The light fraction is labile and tends to be readily decomposable compared with the heavy mineral fraction when incubated. To make an appropriate comparison of SOC concentration in different aggregate-size classes, it is important to quantify sand and undecomposed organic matter (light fraction) which are used to correct SOC content in aggregates (Stevenson and Elliott 1989).

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

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

Mean weight diameter

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

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

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

Statistical analyses

All values are the arithmetic means of three replicates measured on an oven-dried soil basis. One-way analysis of variances (ANOVA) was conducted to investigate the effects of liming on the selected variables of each lime trial by using GENSTAT 16th Edition (VSN International, Hemel Hempstead, UK). To determine the significant differences (P<0.05) among the treatment means, a Tukey's HSD multiple comparison within each depth was performed. Since the samples from three replicates were combined for the analysis of SOC content in the light fractions, no statistical analyses were performed on these data.

Results

Soil pH

After 5 and 34 years since the initial application of lime increased surface soil pH by up to 1.63 and 1.52 units and subsoil pH 0.39 and 1.72 units, respectively. The increase in subsoil pH was about 4 times greater at the 34-year-old site compared to that at the 5-year-old but the pH increase of surface soil was similar. These results highlight the progressive downward movement of surface-applied lime over time (Fig. 1).

Soil organic C and total N

After five years since the application, liming did not affect total soil organic C (SOC), total N, or C/N ratio in surface (0-10 cm) or subsoil (10-20 cm) layers, except a significant increase in C/N in surface soil with the lime rate of 25 t ha^{-1}. In comparison, after 34 years of an initial application, liming decreased total SOC and the C/N ratio and tended to decrease total N in both soil depths, particularly, in surface 0-10 cm (Fig. 2). The average concentration of SOC, total N, K_2SO_4-extractable C and C/N ratio of 34-year-old lime plots were lower than those of 5-year-old in both surface and subsoil layers. Differences in those between the two lime trials were greater in lime-treated plots than untreated control.

Increases in rate of lime application increased K_2SO_4-extractable C in the two depths of both trails. Liming increased the extractable C by up to 127 and 142% in surface and 67 and 93% in subsoil in 5- and 34-year-old limed trials, respectively (Fig. 2).

Aggregate size distribution and SOC contents

There were contrasting patterns of aggregate size distribution between the liming trials. In the 5-year-old lime trial, the higher rates of lime application increased large (>2 mm) and small (2-0.25 mm) macro-aggregates by up to 11% and 44%, but decreased micro-aggregates (0.25-0.053 mm) and silt and clay fractions (<0.053 mm) by up to 13% and 18%, respectively. However, in a 34-year-old lime trial, liming decreased large macro-aggregates by up to 30% and increased small macro-aggregates, micro-aggregates, and silt and clay fractions by up to 54%, 40%, and 72%, respectively, (Fig. 3). As a consequence, the mean weight diameter (MWD) (as a soil structural stability index) increased (P<0.05) with increasing lime rate in 5-year lime plots (from 1.18 ± 0.02 to 1.29 ± 0.02 mm) and decreased (P<0.002) MWD in 34-year-old lime plots (from 1.13 ± 0.01 to 1.01 ± 0.01 mm).

The distribution of all the aggregate-size classes between the two lime trials was similar in the unlimed control. The percentage of large macro-aggregate from the 25 t ha^{-1} treatment was about 28% lower in the 34-year-old than 5-year-old trial (Fig. 3).

The SOC concentration in the sand-free heavy fraction aggregates displayed a similar trend to SOC concentration of the whole soil, i.e., liming decreased the SOC concentration of the aggregates <2 mm in 34-year-old trial but those decreases were only exhibited in small macro-aggregates in 5-year-old one (Fig. 3). Liming did not change the amounts and proportions of SOC in the light fraction, which represented <1.5 % of total SOC, in both lime trials (data not shown).

Microbial biomass and respiration

Irrespective of liming history, lime application markedly increased microbial biomass C (C_{mic}), and the percentage of SOC present as microbial biomass C (C_{mic}/C_{org}) in both surface and subsoil. Liming

increased C_{mic} and C_{mic}/C_{org} up to 80% in surface 10 cm in both 5 and 34-year-old limed plots compared to those unlimed. However, the increase in subsoil varied widely between the two lime trials. In the 5-year-old trial, liming increased subsoil C_{mic} and C_{mic}/C_{org} by up to 155% and 209% while those in the 34-year-old were increased only 26% and 50%, respectively (Fig. 4). Microbial biomass C of surface 10 cm of any treatments did not differ between the two lime trials. However, C_{mic} of subsoil from 5-year-old plots was nearly double that from 34-year-old plots. Difference in C_{mic} between the two lime trials only exhibited in limed plots but not in the control.

Likewise, liming increased cumulative soil respiration (CO_2) by up to 13% in surface and 9% in subsoil in both lime trials (Fig. 4). Notwithstanding, the magnitude of increases in cumulative soil respiration due to liming was considerably lower than that of increase in microbial biomass C, resulting in decreased microbial metabolic quotient (qCO_2), by up to 38 and 34% in surface and 50 and 15% in subsurface in 5 and 34-year-old limed plots, respectively (Fig. 4). The cumulative CO_2 release, qCO_2 and the percentage of SOC present as C_{mic} (C_{mic}/C_{org}) from the surface soil were similar between the two lime trials. However, those of subsoil were greater in the 5-year-old lime plots.

The increased cumulative soil respiration by liming is consistent with increased respiration rate (Fig. 5). The respiration rate was the highest at day 2 during the incubation, and decreased with time but the effect of liming tended to be persistent over the 31-d period at the two depths of both trials.

Discussions

Effect of long-term liming on SOC of whole soil

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

The decreased SOC content in the surface soil 34 years after liming in this present study is in agreement with the results of studies in Australia and Brazil involving continuous cropping systems. Chan and Heenan (1999) reported an 11% decrease in SOC content at 0-10 cm depth 3 years after 1.5 t ha^{-1} of lime was applied to an acidic Ferrosol under continuous wheat with stubble burning.

Similarly, the surface incorporation of 18 t ha^{-1} of dolomitic limestone in an acidic Ferrosol with continuous corn cultivation raised soil pH (1:1 water) from 4.7 to 6.5 in Southern Brazil but decreased SOC by 42% in the surface 0-17 cm layer over a 7-year period (Ernani et al. 2004).

In other studies, applications of lime to cropping soils did not significantly change the SOC content. Kemmitt et al. (2006) reported no increase in SOC despite large increases in crop production (up to 250%) over 37 years in southern England, while Hati et al. (2008) reported similar results over 30 years in India. The long-term SOC maintenance was explained by the annual rates of organic matter input matching the CO_2 outputs. Briedis et al. (2012a) reported a 15% increase in SOC content at 0-27 cm depth 15 years after lime application to the surface of an acidic Red Latosols. This was ascribed to an increase (26 %) in annual C inputs from crop residues in limed soils relative to the control soils. It appears that increased C input as a result of liming treatment under no-till system would have been able to offset SOC mineralization and thereby increased SOC accumulation.

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

Microbial biomass C and carbon-use efficiency

The substantial increases (up to 80%) in microbial biomass C and respiration in limed soils in both lime trials reflect the persistence of the initial liming effect on soil pH, and on the size and activity of the microbial biomass. These results are similar to those reported in previous studies (Kemmitt et al. 2006; Rousk et al. 2010b). The increases have been attributed to proliferation of indigenous acid-intolerant bacteria (Rousk et al. 2010a) as bacteria exhibit a narrow optimal pH range for growth (Wheeler et al. 1991). However, the effect of liming on basal respiration was small relative to that on microbial biomass C (Fig. 4). This appears to be due to a higher efficiency of microbial C reutilization in limed soils which have led to decreased microbial specific respiration (qCO_2). The results were in agreement with findings of Webster et al. (2000). In addition, the subsoil respiration from the two lime trails was similar although C_{mic} in the limed plots was halved in the 34-year-old trial. The reason for such a difference in C_{mic} between the lime trials is unknown. One possible explanation is that the soils from the 34-year-old trail were drier than those from the 5-year-old trial at the time of field sampling.

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

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

The increase in C_{mic}/C_{org} in limed soils indicates that liming increased the conversion of a relatively large portion of SOC into the microbial biomass (Anderson and Domsch 1989). It has been proposed that the higher the amount of substrate C incorporated into microbial biomass, the lower the loss of microbial-derived organic C (Six et al. 2006). Therefore, in the 5-year-old lime trial, liming might increase labile C content through SOC incorporating into microbial biomass even though it did not change total SOC content. It is likely to overestimate the amount of liming-induced SOC losses unless C assimilated by microbial community is taken into account. However, exact microbial community composition and shifting of fungal:bacteria ratios were not examined in this study. Further studies on the short- and long-term effects of liming on microbial community composition and activities are important to understand the mechanisms underpinning the SOC dynamics in such limed soils.

Aggregate stability

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

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

In the 5-year-old trial, liming increased the stability of large and small macro-aggregates while maintaining SOC content. One plausible reason for this structural improvement was that the soil in this 5-year-old liming experiment was growing a continuous grass pasture with minimal disturbance, whereas the continually cropped 34-year-old trial was subjected to frequent soil disturbance. The

results were in line with Briedis et al. (2012b) and Fornara et al. (2011) who reported that liming increased structural stability under no-till permanent pasture system. In another study, macro-aggregates were increased when the grasses were grown and the soils that were not disturbed (Oades 1984). Furthermore, at the same SOC level, limed soils tended to have higher aggregate stability (Chan and Heenan 1999) than those without. Therefore, liming effects on large macro-aggregate stability might be strongly influenced by degree of soil disturbance, the level of addition of plant biomass shoots and roots to the soil and initial SOC content of the limed soils (Westerhof et al. 1999).

Aggregate-associated SOC

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

In spite of decreasing SOC concentration in the smaller aggregates (<2 mm), long-term liming increased the proportion of those in water-stable micro-aggregates and hence increased the total amount of SOC that is associated with these aggregates (Fig. 3). It seems that long-term liming increased the proportion of SOC which is better protected within micro-aggregates. The C associated with micro-aggregates has been known to be more protected from microbial decomposition and has longer residence time than those associated with macro-aggregates (Six et al. 2002). It may thus be expected that long-term liming increases the amount of better-protected C even though it decreases total SOC content. A further study is needed to explore chemical composition of the SOC in each aggregate-size class to better understand the effect of long-term liming on SOC stabilization.

Amelioration of surface liming on subsoil acidity

The initial surface application of lime at a relatively high rate gradually ameliorated subsurface acidity (Fig. 1), suggesting a gradual downward movement of alkalinity from lime and persistent of this positive effect in a long-term. Increases in soil pH due to liming that extend beyond the point of placement are in agreement with what shown by Wang et al. (2016). The fact that liming increased subsoil pH to lesser extent in the 5-year-old trial than in the 34-year-old trial in this present study, indicates that lime/alkalinity had moved slowly and progressively downward with time, as suggested (Conyers and Scott 1989). Furthermore, it also suggests that surface liming at low application rates may not be effective in ameliorating subsoil acidity. For example, application of 3 t ha^{-1} lime did not increase subsoil pH over 5 years (Fig. 3) which could be explained as there was no excess alkalinity to move downward after neutralizing the surface acidity (Conyers and Scott 1989).

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

system due to liming. Such an increase in aggregate stability would enhance SOC protection within aggregates in subsoil.

Conclusions

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

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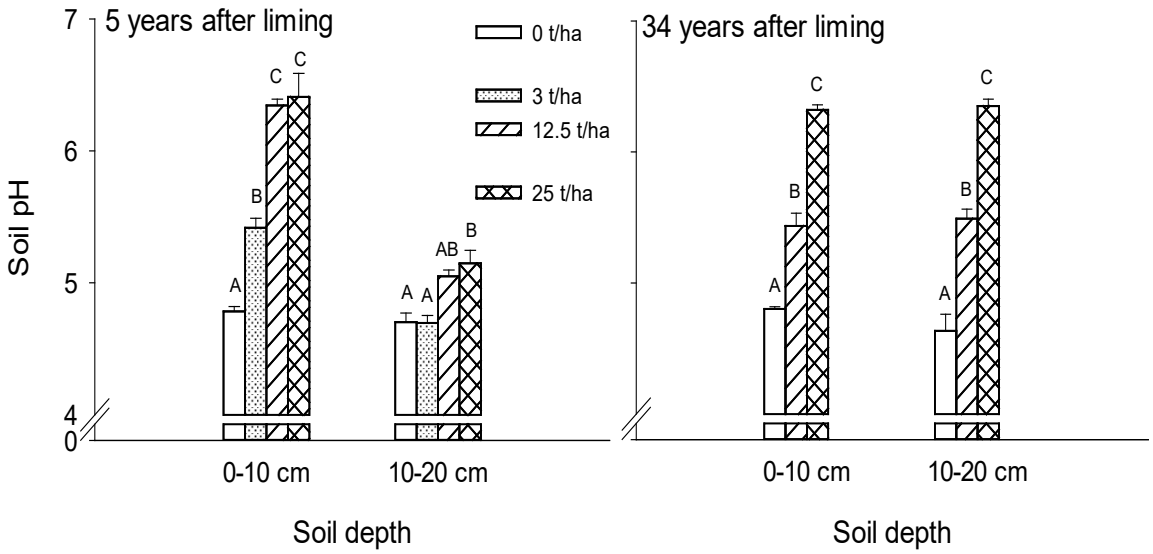

Figure 1. Effect of lime application rate on soil pH of surface (0-10 cm) and subsoil (10-20 cm). Lime was applied once 5 and 34 years ago. Different letters indicate a significant difference between treatment means of lime application rates within a depth at each lime site (Tukey's test, $P < 0.05$). Error bars indicate standard error (n = 3).

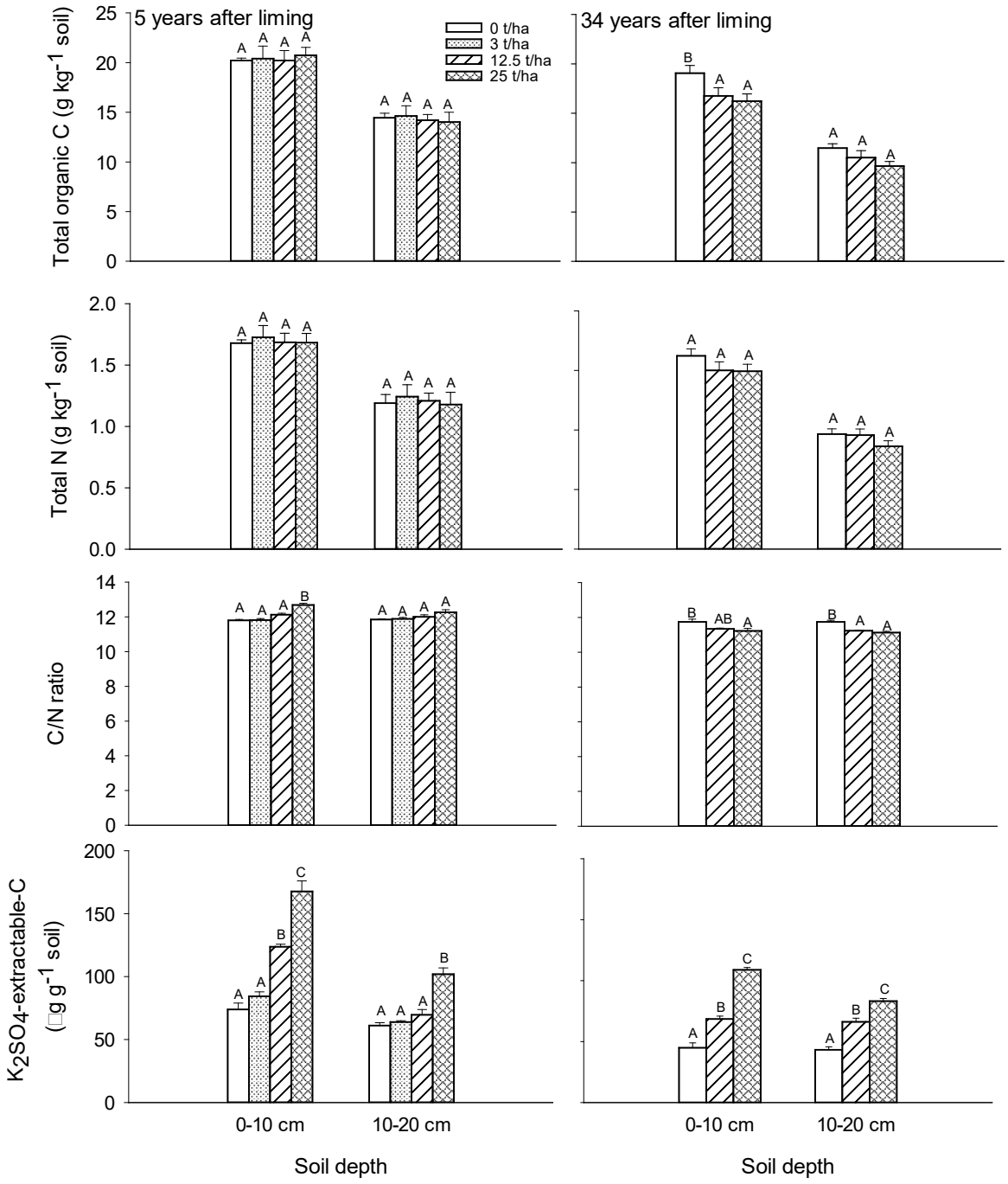

Figure 2. Effect of lime application on total soil organic C and total N, C to N ratio (C/N ratio), and K_2SO_4-extractable C of surface (0-10 cm) and subsoil (10-20 cm). Lime was applied once 5 and 34 years ago. Different letters indicate a significant difference between treatment means of lime application rates within a depth at each lime site (Tukey's test, $P < 0.05$). Error bars indicate standard error (n = 3).

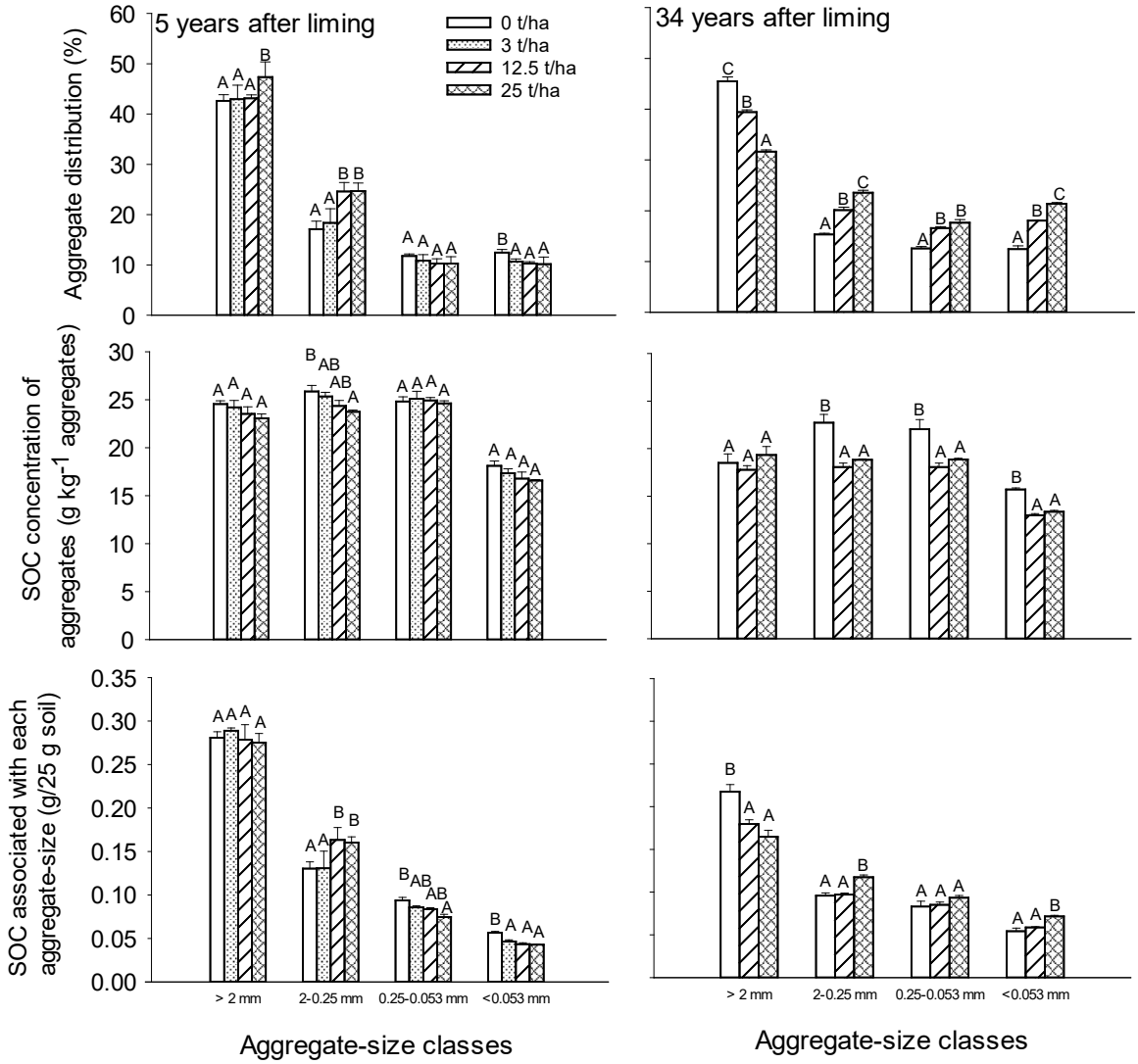

Figure 3. Effect of lime application on distribution of aggregate-size classes as large macro-aggregates (>2 mm), small macro-aggregates (0.25-2 mm), micro-aggregates (0.053-0.25 mm), and silt and clay fraction (<0.053 mm), concentration of soil organic C (SOC) in sand-free heavy fraction of each aggregate-size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

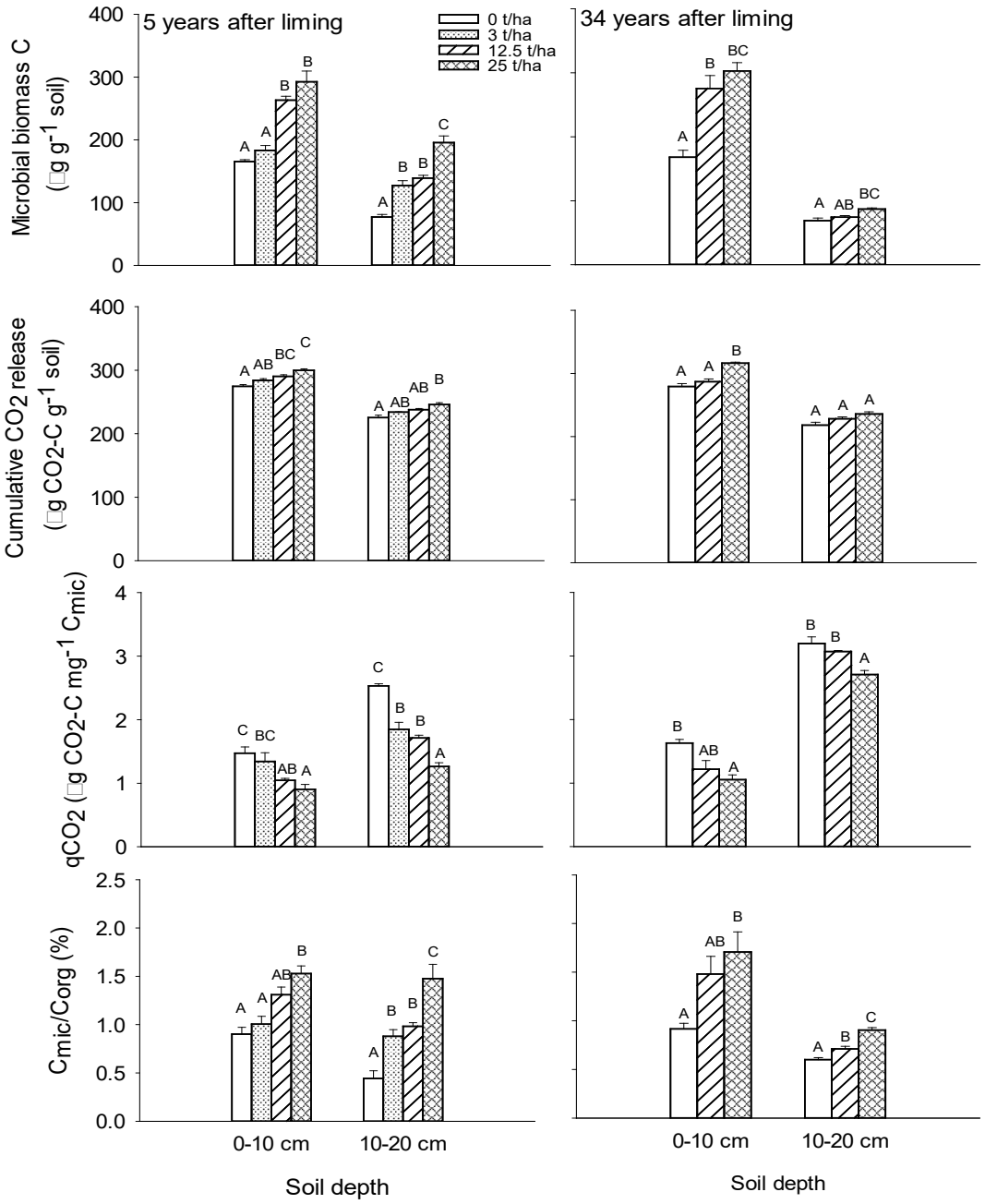

Figure 4. Effect of lime application on soil microbial biomass C (C_{mic}), cumulative soil respiration (CO_2) during 30-day incubation period, microbial metabolic quotient (qCO_2), and the percentage of SOC present as C_{mic} (C_{mic}/C_{org}) of topsoil (0-10 cm) and subsoil (10-20 cm). Lime was applied once 5 and 34 years ago. Different letters indicate a significant difference between treatment means of lime application rates within a depth at each lime site (Tukey's test, $P < 0.05$). Error bars indicate standard error (n = 3).

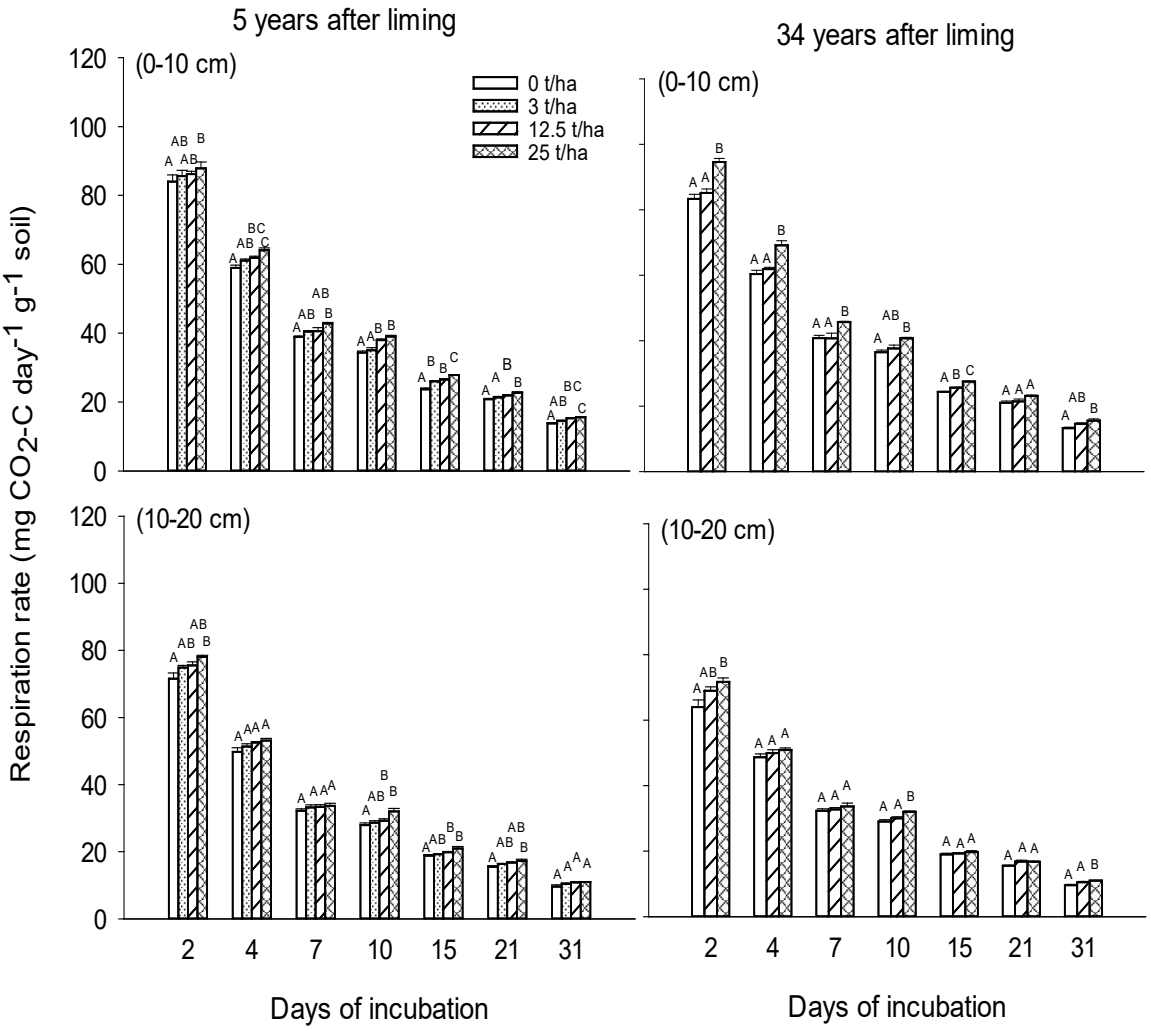

Figure 5. Effect of lime application on soil basal respiration of rewetted air-dried soils incubating under 25 °C of surface (0-10 cm) and subsoil (10-20 cm). Lime was applied once 5 and 34 years ago. Different letters indicate a significant difference between treatment means of lime application rates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