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

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Abstract
Lime application is the most common method to improve crop production in acid soils and has been shown to change soil organic carbon content. However, the impact of liming history on the priming effect on soil organic C is not well understood. This study examined the effect of liming history on C priming in response to the addition of crop residues of different qualities. Soils with pH ranging from 4.7 to 7.4 were collected from two adjacent field experiments whereby lime was applied at different rates, 6 and 35 years ago. A 90-day incubation study was conducted by applying ^{13}C-labelled wheat (C:N 42) and field-pea (C:N 29) residues at a rate of 5 g kg^{-1} soil. Residue application to soils yielded the positive priming effect in all pH levels with the magnitude of C priming being the greatest at initial soil pH 6.6. In comparison, the optimal pH for residue decomposition (7.3) was higher than that for the priming. The overall priming effect was about 17% greater with field-pea than wheat residue. However, cumulative decomposition of added field-pea residue was 15% lower than that of wheat residue. Furthermore, the C priming was greater in soils from the 35-year-old than the 6-year-old limed plots, indicating that a longer history of liming did not enhance the protection of indigenous C from mineralization. The results suggest that increases in soil pH by liming enhanced native C priming through greater microbial biomass and activity and that the magnitude and dynamics of the priming effect largely depended on residue quality and its consequent nutrient supply to decomposer organisms. The study implies that over-liming would likely have negative impacts on the long-term C sequestration.

Keywords δ^{13}C, Crop residues, Limed soils, Priming effect, Soil pH, Soil organic matter

Introduction

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

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

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

Materials and Methods

Site description

Surface soil (0-10 cm) of a Sodosol (Isbell 2002) or Solonetz (WRB 2014) was taken from two adjacent long-term lime trials (100 m apart) initiated in 2008 (6-year-old) and 1979 (35-year-old) within the Agricultural Reserve of La Trobe University, Victoria, Australia (37°42′58″S 145°02′53.5″E). The experimental site has a temperate climate with an average air temperature of 16 °C and annual precipitation of 666 mm. The soils had native pH of 4.7-4.8, total SOC 15.9-20.4 g kg^{-1}, Total N 1.48-1.73 g kg^{-1}, C:N ratio 10.7-12.3, δ^{13}C -22.3 to -19.8‰ (Table 1), clay 29% and silt 61%, and an electrical conductivity (EC) of 131 μS cm^{-1} (1:5 water). The predominant clay mineral in this soil were illite (70%) with some kaolinite (30%) (Wang et al. 2015b). Prior to the commencement of the lime trials, the entire site was under unimproved pasture. The 6-year-old lime trial had volunteer pasture for five years and was recently cropped to lucerne (*Medicago sativa* L.), while the 35-year-old trial had been under irregular rotation since liming with above-ground residue removal. These different management practices had resulted in differences in C and N content between the corresponding soil pH of each trial (Table 1). Both trials had surface lime applied (10 cm) only once at the commencement of treatment. The 35-year-old trial was laid out in a completely randomised design, with lime rates of 0, 12.5, 25, 50, 75 and 100 t ha^{-1} in three replications. The 6-year-old trial was laid on a randomized block design, with lime rates of 0, 3, 6, 12.5, 25 and 50 t ha^{-1} in three blocks. Details of the lime trials are described in Aye et al. (2016).

Soil sampling

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

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

Wheat (*Triticum aestivum* L.) and field pea (*Pisum sativum* L.) plants were grown under field conditions and repeatedly pulse-labelled with ^{13}C in a growth chamber until the full-maturity stage where the atmosphere was enriched with $^{13}CO_2$ (from $Na_2^{13}CO_3$ at 98 atom%, Sigma Aldrich, Miamisburg, USA) as described by Butterly et al. (2015). Plants were also labelled with ^{15}N by adding $Ca(^{15}NO_3)_2$ fertiliser (20 atom%, Shanghai Research Institute of Chemical Industry, Shanghai, China). After crop senescence, the plants were harvested, dried at 70 °C and the grain was removed. The remaining above-ground residues were ground and sieved (<2 mm) such that the particle size was between 0.5 and 2 mm. The C:N ratio of wheat and field-pea residues were 41.9 and 28.6, respectively (Table 1).

Incubation study

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

Soil measurements

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

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

Both microbial biomass C (MBC) and N (MBN) at each sampling time were determined according to the chloroform fumigation-extraction method described by Brookes et al. (1985) and Vance et al. (1987). Briefly, 8 g of fresh soils were fumigated with ethanol-free chloroform in a desiccator for 24 h at 25 °C. After removal of the fumigant, the soils were extracted for 1 h on an end-over-end shaker with 40 ml of 0.5 M K_2SO_4 solution (1:5, w v^{-1}) and filtered through Whatman 42 (Whatman International, Maidstone, England). Another 8 g of non-fumigated samples were also extracted in the same way as formerly described at the time the fumigation commenced. Extracts were stored frozen at -20 °C before analysis. Total C within extracts was quantified colorimetrically following chromic acid digestion (Heanes 1984). Total N within extracts were determined by the phenol hypochlorite reaction and copperized-Cd reduction with a flow injection analyser (FIA) (QuickChem 8500, Lachat Instruments, USA) following an alkaline persulphate oxidation (Cabrera and Beare 1993). Microbial biomass C and N were computed as the differences between their respective concentrations, in the fumigated and non-fumigated samples. The extraction efficiency was adjusted by a factor of 0.45 for C and 0.54 for N (Brookes et al. 1985; Jenkinson et al. 2004). The extractable C from non-fumigated extracts was assigned as K_2SO_4-extractable C. Inorganic N (NH_4^+-N and NO_3^--N) from non-fumigated and non-oxidised extracts (3 ml) was also determined by FIA as previously mentioned.

Calculations and statistical analyses

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

$$C_R = C_T \times (\delta_T - \delta_C)/(\delta_R - \delta_C)$$
$$C_{SOC-AME} = C_T - C_R$$
$$PE = C_{SOC-AME} - C_{SOC-CON}$$

where $C_T = C_R + C_{SOC-AME}$, and is the total CO_2 release from residues and amended soil, C_R is CO_2 release from residues, δ_T is δ^{13}C value of C_T, δ_C is δ^{13}C value of CO_2 release from non-amended control soil, δ_R is δ^{13}C value of residues, $C_{SOC-AME}$ is SOC-derived CO_2 from amended soil, $C_{SOC-CON}$ is SOC-derived CO_2 from the control soil, and PE is priming effect of residues. As indigenous SOC contents varied between the lime treatments (Table 1), the magnitude of C priming was then normalised to per gram of SOC for Figure 2 and Table S2. Taking into account the possibilities of inorganic C contribution from the soils which once received 25 and 50 t ha^{-1} lime to the total CO_2 release, inorganic C content of these soils before and after incubation study were determined. The results showed that the potential contribution of inorganic C to the CO_2 release in the soils that received 25 (0.2-0.7% total CO_2 released) and 50 t ha^{-1} lime (0.6-1.0%) during the study was negligible and thus was unlikely to overestimate the priming effect of these limed soils.

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

Results

Soil pH

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

Carbon dioxide (CO_2) release

Increasing initial soil pH significantly ($P<0.001$) increased CO_2-C efflux. The CO_2-C efflux was 9%, 21% and 29% greater at pH 5.7, 6.6 and 7.3, respectively, relative to the low pH (4.8) soils (Fig. 1). The most rapid CO_2-C efflux occurred during the first week, and then the rate declined over time.

The total CO_2-C emission was significantly greater (6%) in soils amended with wheat residue compared to the field-pea-amended soils. The dynamic pattern of CO_2-C efflux during the incubation period varied between the two residues. During the first week, the CO_2-C release was significantly higher (20%) with field-pea residue but thereafter it was on average 21% higher with wheat residue. However, the differences in total CO_2-C evolution between the two lime trials were not significant (Fig. 1 and Table S1).

Primed soil organic C (SOC)

Like total CO_2 release, the cumulative SOC primed per gram indigenous SOC significantly ($P<0.001$) increased with increasing initial pH. However, it reached a maximum at pH 6.6, and then decreased with further increasing pH to 7.3. The greatest C priming was observed at pH 6.6 which was 37% and 20% greater than C priming at low (4.8) and high pH (7.3) (Fig. 2).

There was a highly significant ($P<0.001$) difference in cumulative C priming between the two residue treatments. Total C priming from soils with filed-pea residue was about 18% greater than those with wheat residue over 90 days of incubation (Fig. 2). However, there was a remarkable difference in the dynamic pattern of C priming between the two residues across the incubation time. Particularly, during the first week of the incubation, the C priming with field-pea was about 6-fold greater than with wheat residue, while that was about 34% greater in wheat-amended soils for the rest of the incubation period (Fig. 3). Moreover, the liming history also had a significant ($P<0.001$) effect on C priming. In limed soils (pH 5.5, 6.6 or 7.3) SOC primed was on average about 8% higher in the 35-year-old than 6-year-old limed soils at the end of the study (Fig. 3 and Table S2).

However, the temporal dynamics of SOC primed during the 90-day incubation period was considerably different between the two lime trials. In the first week of incubation, the SOC primed from the 6-year-old limed soils was significantly higher (16%) than its 35-year-old counterpart, whilst the latter was up to 18% greater during days 8-49. Nevertheless, there was no significant difference in those between the two lime trials at the last incubation phase of days 50-90 (Fig. 3 and Table S2).

Residue-derived CO_2-C

The residue-derived CO_2-C was markedly affected by the initial soil pH with a general trend similar to that of total CO_2 release. Overall, the decomposition of wheat was 15% greater than field-pea residue at the end of the 90-day study (Fig. 4). However, the dynamic of decomposition rate with incubation succession varied between the two residues. The decomposition rate of field-pea residue was significantly ($P<0.001$) greater (7%) than that of wheat residue during the first week, but was 6-14% lower thereafter (Fig. 5). Furthermore, the overall residue decomposition rate was 11% faster in the 35-year-old than the 6-year-old limed plots (Fig. 5).

Relationships between initial soil pH and cumulative CO_2 release from SOC and residues

The cumulative SOC primed during the 90-day incubation period increased with increasing initial pH, reaching the maximum at around pH 6.5, and then decreased with further increasing the soil pH. In comparison, the residue-derived CO_2-C increased linearly with initial pH (Fig. 6).

Microbial biomass C and N

Initial soil pH had a great impact on MBC and MBN (Table 2), and its effect generally followed the same pattern as the effect on SOC primed. Both MBC and MBN increased with soil pH up to 6.6 and then decreased at pH 7.3. The MBC and MBN decreased with time except that the MBN in the no-residue controls was the highest at day 30 (Table 2).

The residue type had some effects on MBC and MBN with this effect varying over time. Specifically, MBC and MBN of the two residue treatments did not significantly differ at day 7 but were 23% and 8% greater with wheat than with field-pea residue, respectively, at day 30. In contrast, the MBN with field-pea residue was 14% larger at day 90 (Table 2).

Liming history had only a minimal effect on both MBC and MBN. The 35-year-old limed soils had significantly ($P<0.05$) greater MBC (4-6%) and MBN (4%) during days 0-30, compared to the 6-year-old soils (Table 2). There was a significant ($P<0.05$) Liming history × Residue effect on MBC at all three sampling times and on MBN at days 30 and 90. The interaction on MBC resulted from differences in the MBC between the two lime trials which only occurred with wheat residue but not with field-pea residue. At days 30 and 90, MBN of wheat residue-amended soil was higher in 35-year than that 6-year-old trial, whereas the MBN with field-pea residue was higher in the 6-year-old trial (Table 2).

K_2SO_4-extractable C and inorganic N

Like the effect on total CO_2 release and residue decomposition, increasing the initial pH generally increased the concentration of K_2SO_4-extractable C with the magnitude decreased with incubation

time (Table 3). There was a significant ($P<0.05$) difference in the concentration of K_2SO_4-extractable C between the two residue treatments with 9 and 14% greater with wheat than with field-pea residue at days 7 and 90. The K_2SO_4-extractable C was 9-23% greater ($P<0.001$) in 6-year-old than 35-year-old limed plots at all observations in 90 days (Table 3).

The treatment effects on inorganic N ($NH_4^+ + NO_3^-$) did not follow the same pattern as those on the extractable C (Table 3). The significant increase in inorganic N ($NH_4^+ + NO_3^-$) concentration with increasing initial pH exhibited at days 30 and 90 which reached the maximum at pH 6.6. It was the greatest in the non-amended control and smallest in the wheat-amended soils across the incubation period. The significant effect ($P<0.001$) of liming history on the concentration of inorganic N displayed only at day 30 and it was 13% greater in the 35-year-old than the 6-year-old limed plots (Table 3). There were Residue × Liming history and Initial pH × Liming history interactions (Table 3). The Residue × Liming history interaction was inconsistent with incubation time. In comparison, the old limed soils had greater concentrations of inorganic N at initial pH of 4.8 and 5.7 while the opposite was true at a higher initial pH, leading to the Initial pH × Liming history interaction.

Discussion

Effect of initial pH

The initial pH of the soils greatly influenced the magnitude of priming effects induced by incorporation of crop residues. Importantly, this study revealed that the optimum pH for the priming occurred around pH 6.5. In comparison, residue decomposition increased linearly with initial pH up to the maximum of pH 7.4 (Fig. 6). The lower optimum pH observed for the priming effect than for residue decomposition is probably a function of the dominant decomposer organisms and associated metabolic capacity including the concentration of extracellular oxidative enzymes at the time of C substrate addition as suggested by Rousk et al. (2016) and their location within the soil. Ivarson (1977) observed that although total bacterial biomass increased with increasing pH up to 7.5, only fungal community composition was different at pH 5.5 and 7.5. Even though the direct effect of soil pH on fungal abundance was weaker than that on the bacteria community, indirect effects due to competitive interactions between bacteria and fungi at different pH values would have changed fungal community composition (Rousk 2010). Such changes in microbial composition could be partly responsible for this lower priming effect in the high initial pH (>7) relative to the moderately high pH (6.6) soils of the present study. Differences in competition for energy and nutrients between fresh organic matter-degrading and SOC-degrading microorganisms would also have contributed to this difference in priming among different pH levels as postulated by Fontaine et al. (2003). Moreover, variation in maintenance energy requirements of microbes among different soil pH would also have influenced microbial C-use efficiency, which plays a prominent role in C priming (Cotrufo 2013). However, relative importance of these mechanisms along with interdependence between bacteria and fungi in priming remains uncertain.

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

However, lesser magnitude of C priming in slightly alkaline (7.3) than slightly acid (6.6) soil in this study was not in agreement with other studies. This could possibly be due to a negative impact

of residual lime (0.1-0.2% inorganic C) remaining in the high pH soils (received 50 t ha^{-1} lime) on microbial growth and metabolic activity. There could be some free $CaCO_3$ (and high concentrations of HCO_3^-, Powlson and Jenkinson 1976), which might have negative effects on survival of some microorganisms (Bashan and Vazquez 2000; Farhangi et al. 2013). Yaganza (2009) suggested that osmotic stress causing an increased maintenance metabolism would have been partly responsible for the strong inhibition of bacteria growth by HCO_3^-. Garau et al. (2007) also observed a reduction in microbial population and β-glucosidase enzyme activity as soil pH$_{(H_2O)}$ increased from 4.2 to 7.1 following liming. This reduction was proposed to be due to specific mineral deficiencies caused by carbonate. The lower (9%) microbial biomass in these slightly alkaline soils than slightly acid soils supports this hypothesis (Table 2). Similar to our findings, Aciego Pietri and Brookes (2008) also revealed that increases in microbial biomass with increasing soil pH reached maximum at around pH 6.7 and declined at higher pH in Chromic Luvisol soils with a liming history of more than 100 years.

Moreover, the 8% and 18% higher specific respiration rates (qCO$_2$) of our strongly acid (4.8) and slightly alkaline (7.3) than slightly acid (6.6) soils, respectively (data not given), also indicate that microorganisms from both extremes in pH utilized more energy for maintenance (Anderson and Domsch 1993). The greater requirement of maintenance energy for microbes in these pH soils reflects its negative impact on the ratio of microbial growth to C uptake, i.e., microbial C-use efficiency (Manzoni et al. 2012). Lower microbial biomass and C-use efficiency in these pH soils might have been associated with a lower activity of extracellular enzymes (Dorodnikov et al. 2009) and consequently decreased the C priming compared to the slightly acid soils. The results highlight the important role of changes in pH following liming in microbial turnover of SOC.

Effect of residue type

The quality of plant residues added to the soils determined the magnitude of the priming effect and its dynamic with incubation time. The six-fold higher initial priming with field-pea (C:N 29) than with wheat (C:N 42) residue during days 0-7 (Fig. 3) could be ascribed to easier degradability of the field-pea residue, as indicated by Hobbie (2005). The results are consistent with previous findings that the application of easily degradable residues with low C:N induced a greater priming effect than the application of high C:N residues during early stages of incubation (Conde et al. 2005; Thiessen et al. 2013; Wang et al. 2015a). The greater CO$_2$ release and decomposition of field-pea relative to wheat residue during the first week of incubation indicate the greater microbial activity with the former residue (Figs. 1 and 4). However, during this first week of incubation, even though the priming effect was considerably greater with field-pea relative to wheat residue, the residue type had little effect on microbial biomass C (Table 2). This indicates that microbial activity was more strongly influenced by residue C:N ratio than microbial biomass C as suggested by Nguyen and Marschner (2015).

The rapid decomposition of field-pea residue would have supplied a greater amount of C substrate to microorganisms than wheat residue in the first week of incubation. This energy-rich C source would have facilitated the synthesis of SOC-degrading enzymes (Fontaine et al. 2011; 2003) which are capable of breaking down the added residues and decomposing native SOC, enhancing the priming effect according to the co-metabolism theory (Kuzyakov et al. 2000). Greater increase in microbial activity due to readily degradable immature wheat and vetch residues incorporation compared to the mature wheat residue at the beginning of incubation study was also observed (Moreno-Cornejo et al. 2015). Dilly et al. (2007) demonstrated that an increased production of enzymes after C amendment effectively degraded C polymers and caused a positive priming effect. During this process, microbes would also have obtained other essential nutrients, particularly N. However, the priming effect associated with field-pea residue declined sharply after the initial phase

of incubation (days 0-7) in this present study. Such a diminishing priming effect could be partly due to an exhaustion of labile C substrate, and reductions in microbial activity, indicated by the 66% and 33% reduction in residue-derived CO_2-C, and in microbial abundance from the first to the second sampling time (Fig. 5 and Table 2). Besides, this decrease in C priming could also be coupled with depletion of labile nutrients which are required for the microbes to produce enzymes for SOC decomposition (Koyama 2013). This assumption was in agreement with Joshi et al. (1993), who demonstrated that microbial abundance increased rapidly as litter decomposition progressed, and then decreased towards the end of the process. Increases in microbial abundance following incorporation of this field-pea residue would likely increase microbial assimilation of substrate C which then enhanced the contribution of microbial metabolites to SOC stabilization according to the new paradigm of C stabilization proposed by Cotrufo et al. (2013). Flessa (2000) also observed that litter with high N contributed a higher leaching of C to the mineral soil.

The study showed considerably lower priming effect of wheat than field-pea residue during the first week of incubation (Fig. 3). This result indicated the existence of a lag phase in the priming effect of wheat residue which might be attributed to its low N content (9.7 g N kg^{-1}) compared to field-pea residue (14.3 g N kg^{-1}). A N shortage rather than C shortage in wheat residue-amended soils would have limited microbial activity at this early incubation stage (Merckx et al. 1987; Knapp et al. 1983). Jenkinson et al. (1985) also suggested that when soils contain plenty of fresh organic materials of wide C:N, microbial N demand cannot be met from inorganic N reserves. Besides, Henriksen and Breland (1999) also demonstrated that a residue N concentration below 12 g kg^{-1} markedly reduced its mineralization and the growth of soil microorganisms. Moreover, N limitation is also known to negatively impact on the production of extracellular enzymes necessary to degrade polymers contained within high C:N wheat residue (Jingguo and Bakken 1997). Generally, decomposer organisms have lower C:N ratios than most residues (Berg and McClaugherty 2003) and they immobilize inorganic N during the early phase of decomposition (Manzoni et al. 2008).

Furthermore, the approximate 3-fold lower concentration of inorganic N in wheat than field-pea residue-amended soils was also likely an additional cause of this one-week lag phase with wheat residue. The slower microbial activity in soils with wheat residue at this stage coincided with less CO_2 release and slower residue decomposition compared with field-pea residue (Figs. 1, 2, 4, Table 3). Notwithstanding, after this one-week delay, the priming effect with wheat residue caught up with that of field-pea residue and even surpassed from 3 weeks onwards (Fig. 3). This could be partially attributed to slow and progressive increases in labile C substrates and inorganic N for microorganisms from gradual decomposition of the wheat residue (Fig. 4 and Table 3). Such progressive decomposition of high C:N (>45) residues was also reported during days 45 onwards in a previous incubation study (Kriaučiūnienė et al. 2012). Increased availability of C and N substrates with progressive decomposition would have led to both co-metabolism and N-mining phenomena to occur simultaneously during days 8-21 when the priming effect reached the maximum. Microbial N-mining occurs whereby microbes acquire N from SOC to balance available C substrate in N-limited environments (Fontaine et al. 2003). These two mechanisms are not mutually exclusive despite the fact that one mechanism could dominate at any given period depending on C substrate quality and nutrient availability (Cheng and Kuzyakov 2005). However, the low indigenous soil N (1.48-1.73 g kg^{-1}) (Table 1) and high N immobilization (Table 3) indicated that the predominant mechanism for this surged priming effect was mainly microbial N-mining while acknowledging the contribution of other proposed mechanisms (Kuzyakov et al. 2000). This also corresponds well with Craine et al. (2007) who suggested that the priming effect caused by N-mining should be greatest when labile C is available and N is shortage.

A further driving mechanism behind this considerable increase in the priming effect with wheat residue after one week-lag phase could be accredited to changes in microbial community composition. Shift from fast-growing r-strategists to slow-growing K-strategists that are more

competitive in decomposing substrates with low N can occur when N is limited (Blagodatsky et al. 2010). Substantially faster residue decomposition and a greater priming effect with wheat residue after day 7 (Figs. 3 and 4) might have partially resulted from increased N-mining by these K-strategists. This intensive C priming coincided with increased microbial respiration, residue decomposition and microbial biomass (Figs. 1, 3, 4, Table 3).

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

Effect of liming history

The greater priming effect in 35-year-old than 6-year-old limed soils in this study might be partially ascribed to the effect of duration since liming on microbial biomass and community composition. The larger microbial biomass in the soils from 35-year-old limed plots would have utilized more C from decomposition of added residues as an energy source, leading to the greater priming effect through the mechanisms of co-metabolism and microbial N-mining relative to the 6-year-old limed soils (Figs. 2, 4, Table 3). The consistently lower K_2SO_4-extractable C in the 35-year than the 6-year-old trial in the first 30 days despite faster decomposition of residues in the former indicates the greater microbial demand and utilization of labile C substrate in the former than the latter trial (Fig. 4 and Table 3). Greater decomposition of wheat residue in soils from the older than newer limed plots suggests that microorganisms were more efficient in utilizing the higher C:N wheat residue. Substantially greater microbial biomass C upon wheat residue addition in older than the newer limed soils could also be attributed to lower soil fertility (Table 1). An et al. (2015) demonstrated that the contribution of straw C to microbial biomass C was more effective in a low-fertility soil than a high-fertility soil. This was also in line with the finding of Moreno-Cornejo et al. (2015) that soils with lower indigenous SOC content had a higher microbial turnover rate and greater priming effect than soils with higher SOC. The combination of greater microbial biomass and activity and utilization of labile C substrate from intensive decomposition of residue would have led to greater N-mining and hence the priming effect, in soils from the older limed plots.

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

Conclusion

This study used soils from two long-term lime trials to investigate the role of soil pH in the priming effect following addition of crop residues. It showed that increased pH resulting from long-term liming of acid soils favoured the decomposition of native SOC (priming effect). The magnitude of the priming effect depended largely on the quality of the added residues. Although faster during the initial period, cumulative decomposition of field-pea residue (low C:N) during the 90-day study was lower than that of wheat residue (high C:N). It appears that the incorporation of residues of low C:N ratio would favour soil C storage and improve soil fertility through increasing microbial C-use efficiency in the long term.

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

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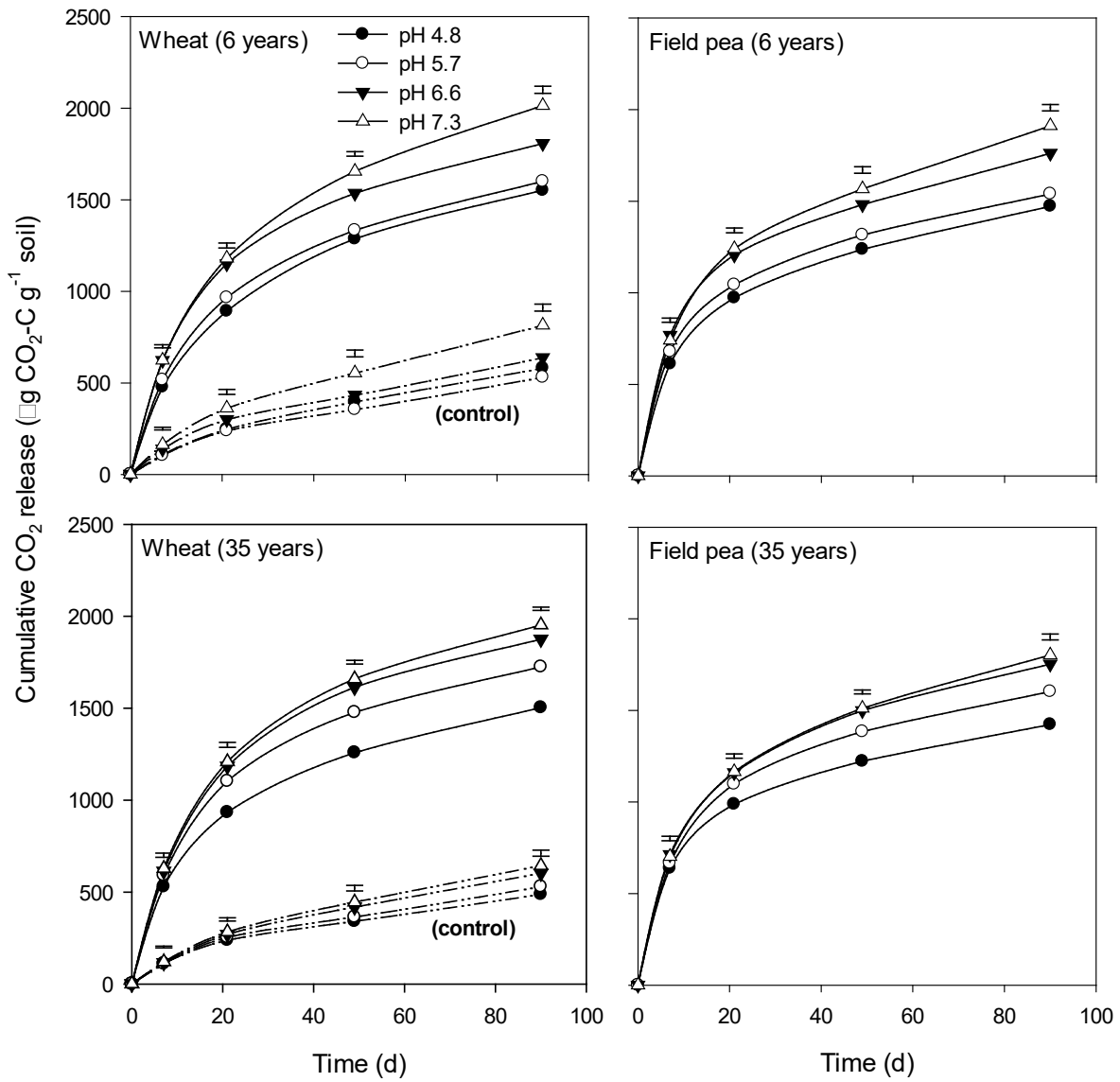
Figure 1. Cumulative total CO_2 release from soils incubated without residue (control) or with wheat (left) or field-pea (right) residue. The soils differing in initial pH were from two lime trials with either 6 (top) or 35 years (bottom) since liming. Vertical bars indicate the least significant difference (P=0.05) between means for each sampling time.

Figure 2. Cumulative amount of **primed C per gram of indigenous SOC** over 90 days in response to the addition of wheat (left) or field-pea (right) residue to soils differing in initial pH. The soils were from two lime trials with either 6 (top) or 35 years (bottom) since liming. Vertical bars indicate the least significant difference (*P*=0.05) between means for each sampling time.

Figure 3. The main effects of initial pH, residue type and liming history on the rate of soil organic C mineralization (SOC primed), in response to the addition of wheat or field-pea residue to soils differing in initial pH. The soils were from lime trials with either 6 or 35 years since liming. Vertical bars indicate the least significant difference (*P*=0.05) between means for each sampling time.
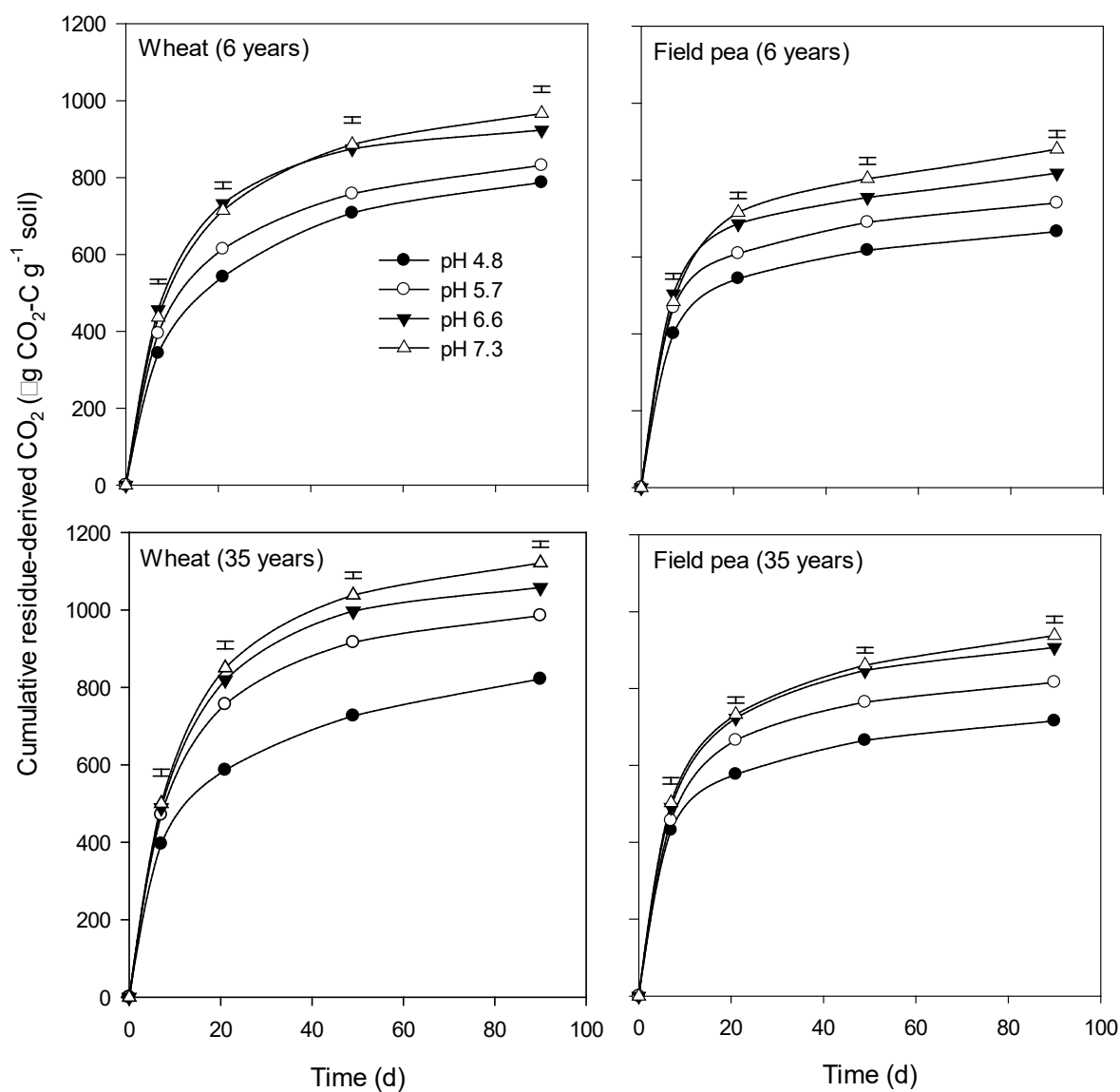
Figure 4. Cumulative residue-derived CO₂ release of soils differing in initial pH incubated with wheat (left) or field-pea (right) residue. The soils were from two lime trials with either 6 (top) or 35 years (bottom) since liming. Vertical bars indicate the least significant difference (*P*=0.05) between means for each sampling time.
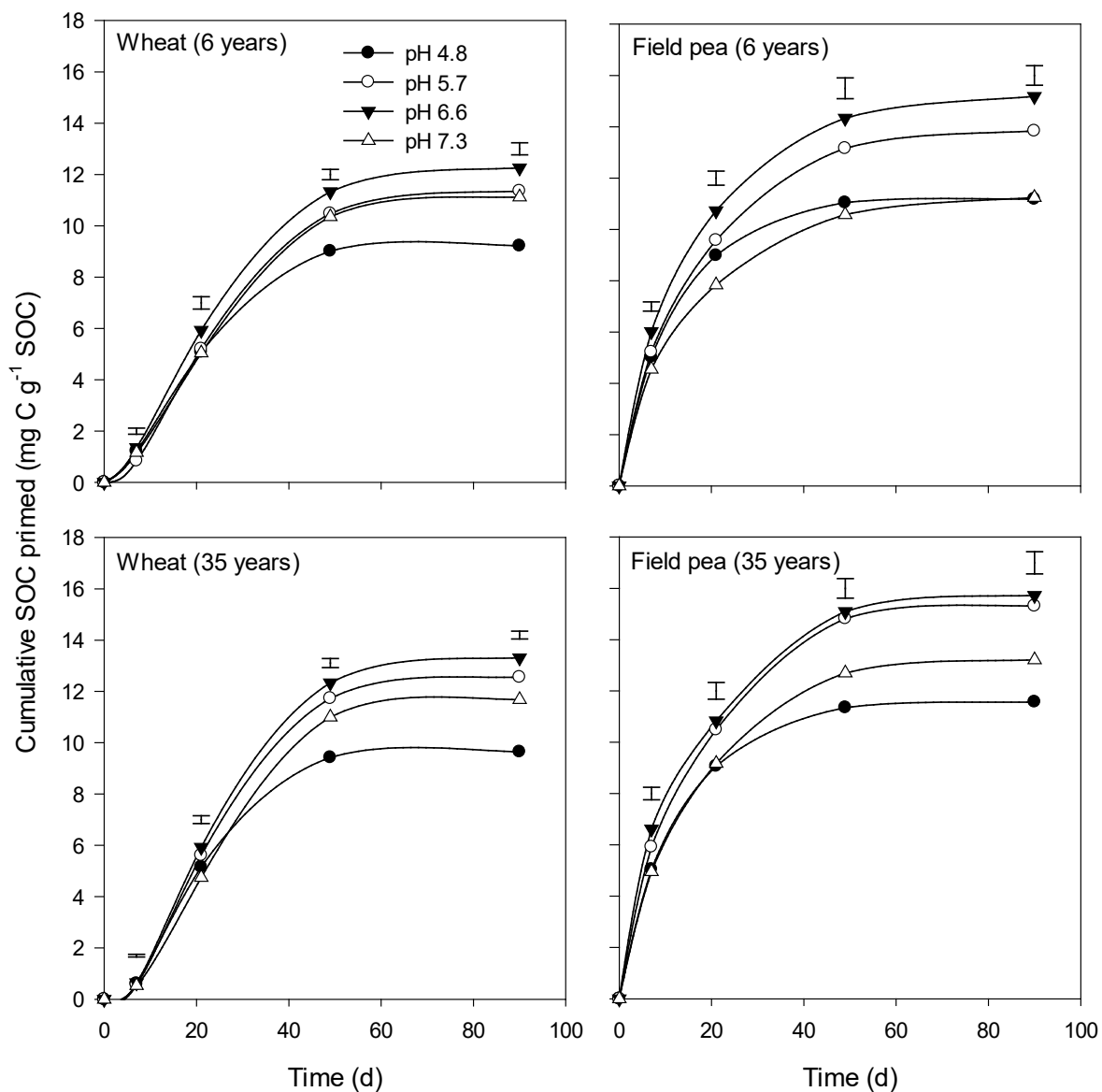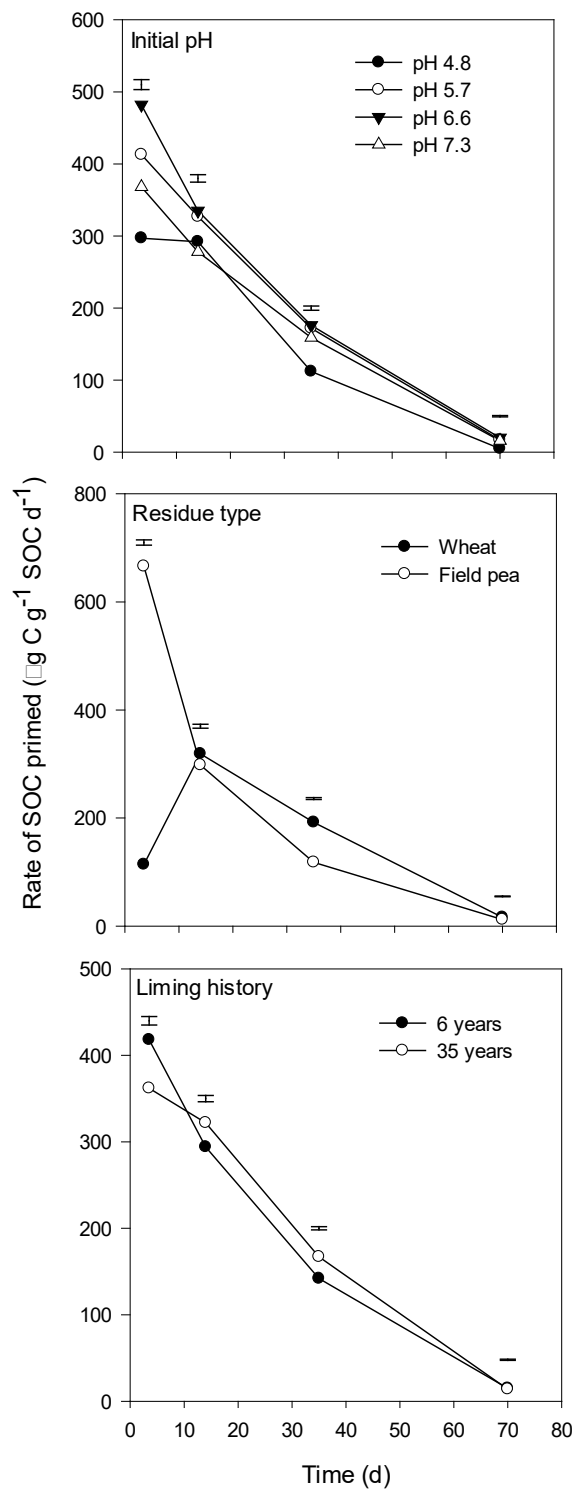
Figure 5. The main effects of initial pH, residue type and liming history on the rate of residue-derived CO_2 release in response to the addition of wheat or field-pea residue to soils differing in initial pH. The soils were from two lime trials with either 6 or 35 years since liming. Vertical bars indicate the least significant difference (P=0.05) between means for each sampling time.

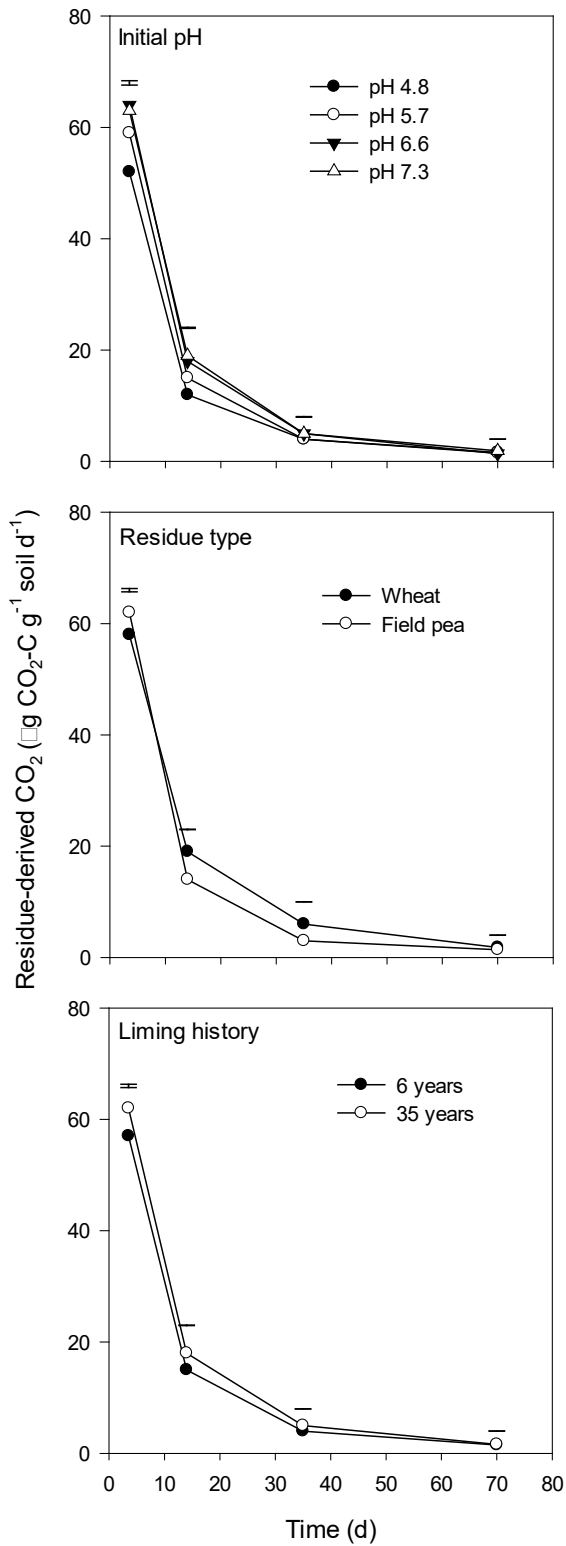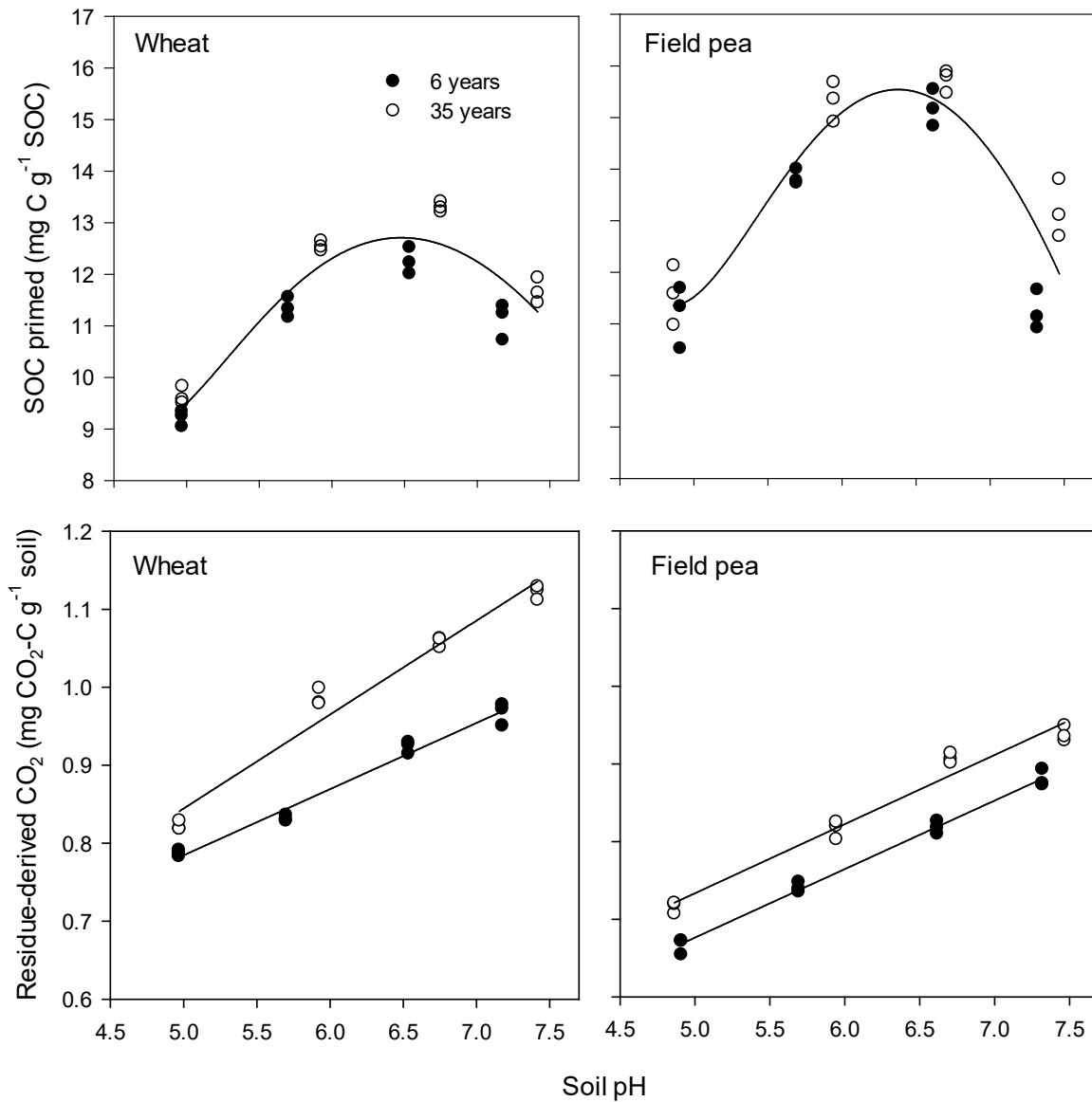**Figure 6.** Relationships between initial soil pH and the amounts of soil organic C (SOC) primed in response to the addition of wheat or field-pea residue to soils from two lime trials with either 6 or 35 years since liming (top) and residue decomposed (bottom).

Table 1. Initial chemical characteristics of the soils and labelled wheat and field-pea residues. The soils were from two lime trials with 6 (New) or 35 years (Old) since liming. Values are means (n=3) with the standard error in parentheses.

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

Table 2. The main effects of initial pH, residue and liming history, and the effect of the residue × liming history interaction, on the C and N in the microbial biomass during the 90-day incubation.

Factor	Treatments	Microbial biomass C (µg g^{-1} soil)			Microbial biomass N (µg g^{-1} soil)		
		7 days	30 days	90 days	7 days	30 days	90 days
Initial pH	4.8	282	217	175	22.4	20.7	19.8
	5.7	311	240	188	26.9	23.3	21.1
	6. 6	350	272	209	31.3	26.4	23.6
	7.3	326	272	189	30.3	25.6	22.9
	LSD (_P_=0.05)	_16_	_14_	_14_	_1.1_	_0.8_	_1.8_
		***	***	***	***	***	***
Residue	Control	217	201	172	18.5	19.8	19.3
	Wheat	366	303	198	32.0	27.3	21.5
	Field pea	368	246	201	32.6	24.9	24.6
	LSD (_P_=0.05)	_14_	_12_	_12_	_0.92_	_0.69_	_1.54_
		***	***	***	***	***	***
Liming history	New	311	243	191	27.1	23.6	21.9
	Old	324	258	190	28.3	24.5	21.7
	LSD (_P_=0.05)	_11_	_10_		_0.8_	_0.6_	
		*	**	NS	**	**	NS
Residue × Liming history	Control - New	217	205	176		20.0	19.8
	Control - Old	217	197	169		19.6	18.9
	Wheat - New	348	276	188		25.5	20.0
	Wheat - Old	385	331	208		29.1	23.0
	Field pea - New	367	248	208		25.1	26.0
	Field pea - Old	369	245	193		24.8	23.2
	LSD (_P_=0.05)	_20_	_17_	_17_		_1.0_	_2.2_
		*	***	*		***	**

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

Table 3. The main effects of initial pH, residue and liming history, and the interaction effects of residue × liming history and initial pH × liming history, on the concentrations of K_2SO_4-extractable C and inorganic N ($NH_4^+ + NO_3^-$) in soil, during the 90-day incubation.

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

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

Table S1. The main effects of initial pH, residues and liming history on the cumulative CO_2 release, during the 90-day incubation.

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

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

Table S2. The main effects of initial pH, residue and liming history on the cumulative SOC primed during the 90-day incubation.

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

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