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Interactive effects of initial pH and nitrogen status on soil organic carbon priming by glucose and lignocellulose

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Highlights

- **The carbon priming effect was lowest in the moderately acidic soils**
- **Glucose amendment induced greater priming effect than lignocellulose**
- **Nitrogen decreased priming more with lignocellulose than glucose**
- **Reduced priming by nitrogen was greatest in the moderately acidic soils**

Keywords: Liming. Acidification. Priming effects. Substrate decomposition. Microbial biomass C. Microbial biomass N. Microbial metabolic quotient (qCO_2). Soil organic matter

Abstract
Soil pH and the availability of carbon (C) substrate and nutrients to microorganisms are well recognized to influence C priming. However, the mechanisms underpinning such interplay so far remain elusive. Given that liming acid soils, residue retention and fertilization are common agricultural practices, small changes in SOC content by these practices could have a big impact on the global C budget. This study aimed to gain insight into the impact of initial pH and mineral N availability on the priming effect of two C substrates with contrasting biodegradability. Stable ^{13}C-labelled substrates, glucose and lignocellulose, were applied at the rate of 0.5 mg C g^{-1} soil with or without NH_4NO_3 to the same soil matrix with three different initial pH levels; pH 4.1 (strongly acidic), 4.7 (moderately acidic) and 6.6 (slightly acidic). The N treatment was based on a C:N of 10 of the added substrate (0.05 mg N g^{-1} soil) to ensure N was non-limiting. Interestingly, the priming effect was not linearly related to soil pH; greatest at pH 4.1, followed by pH 6.6 and lowest at pH 4.7. The greater net increase in microbial biomass upon C supply in strongly acidic soils compared to the moderately and slightly acidic soils would have enhanced co-metabolic decomposition of native soil organic C (SOC). The cumulative amount of primed SOC during the 30-day incubation period was greater in glucose- (21 μg C g^{-1}) than lignocellulose-amended soils (13 μg C g^{-1}). Nitrogen application reduced the C priming effect of both C substrates at all pH levels. This reduction was more prominent with lignocellulose and in the moderately acidic soils. The results suggest that maintaining optimal soil pH for nutrient availability and N application that exceeds the microbial N requirements in agricultural fields may minimize SOC loss via the priming effect in the short term.

1. Introduction

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

Numerous studies have implicated the quality of C substrates and their availability to decomposers as a major controller of the magnitude and direction of the priming effect (De Graaff et al., 2010; Fontaine et al., 2007; Shahbaz et al., 2017). Availability here refers to biochemical recalcitrance of organic compounds, i.e., susceptibility of substrates to enzymatic degradation followed by the uptake of reaction products by the soil microorganisms (Chen et al., 2014). It has generally been recognized that the added labile C substrate serves as available energy source for microorganisms to produce energetically-expensive extracellular enzymes that degrade native SOC (Hessen et al., 2004). Recalcitrant C substrates, with a higher C:N ratio (>25), also enhanced native SOC decomposition mainly through microbial-N mining processes (Moorhead and Sinsabaugh, 2006). However, reports on the magnitude and direction of the priming effect induced by C substrates with different quality are inconsistent. Many studies have revealed that addition of labile

C substrates such as glucose yielded a higher priming effect than more recalcitrant substrates (Mary et al., 1993; Nottingham et al., 2009) at least in the short term (days). However, others observed the reverse (Di Lonardo et al., 2017; Mondini et al., 2006;) and clear trends have not been drawn so far. These inconsistencies could be due to variation in the amount of added C substrate in relation to the existing SOC pool (Blagodatskaya and Kuzyakov, 2008), the quality of added substrate (C:N) and other background soil physical and chemical properties among the studies.

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

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

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

2. Materials and Methods

2.1. Soil sampling and processing

Surface (0-10 cm) soils were collected from a 35-year lime trial at the Agriculture Reserve, La Trobe University, Victoria, Australia (37°72′ S, 145°05′ E). The soil was classified as Sodosol based on the Australian Soil Classification (Isbell, 2002) and Solonetz in the WRB system (WRB, 2014). It had a silty clay loam texture comprised of 9% sand, 60% silt, and 31% clay, 20.5 mg C g^{-1}, and 1.9 mg N g^{-1}. The soil was managed as a low-input and irregular cropping rotation including cereals, pasture and grain legumes and fallow, and the annual C input was estimated to be less than 1 t C ha^{-1} (Aye et al., 2016). Soil samples were taken from each of the 3-field replicate lime plots that had received 0 and 25 t ha^{-1} lime ($CaCO_3$) 35 years ago, resulting in pH_{CaCl2} of 4.7 and 6.6, respectively. After homogenizing and removing visible plant materials, the soils were air-dried and sieved (≤2 mm).

2.2. Soil pH manipulation

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

2.3. Experimental design and incubation conditions

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

Sufficient amounts (~ 1.2 kg) of each soil were pre-incubated (50% field capacity) at 25 °C for 14 days prior to C and N treatments in order to allow microbial stabilization (Butterly et al., 2010). During the pre-incubation, the containers were opened every 3 days to ensure the maintenance of aerobic conditions. Pure extracts of uniformly ^{13}C-labelled D-glucose (99 atom%, Sigma Aldrich, Missouri, USA) and ^{13}C-labelled lignocellulose (high degree of polymerization derived from maize, 97 atom%, Isolife, Wageningen, The Netherlands) were used as labile and recalcitrant C substrates, respectively (Table 1). Initial characteristics of the soils are presented in Table 1.

Six treatments were imposed on each soil pH level: non-C-amended control with N [C+N] and without N [C-N], glucose with [G+N] and without N [G-N] and lignocellulose with [L+N] and without N [L-N]. Each experimental unit was set up by weighing 30 g (oven-dry equivalent) of pre-incubated soil into a 50-ml PVC core and either ^{13}C-labelled glucose or lignocellulose substrate was added and thoroughly mixed. The same mixing procedure was subjected to the non-C-amended controls to maintain uniform disturbance across all treatments. Inorganic N was added as aqueous NH_4NO_3 (2 ml) in the volume of water required to adjust soil to 60% field capacity and to maximize the activity of aerobic decomposer microorganisms. The non-N treatment (-N) received the same volume of solution as pure Milli-Q water. The liquid was applied evenly to the surface, allowed to settle for 15 min and then mixed with a spatula. Each core was placed in a 1-L air-tight Mason jar

with a screw-cap lid containing a vial with 8 ml of water to reduce drying during the incubation. An open wide-mouth scintillation vial containing 8 ml of 1 M NaOH was also placed in the jar to absorb CO_2 released during the subsequent incubation. Three jars containing only the NaOH traps and water vials were also incubated as blanks. The jars were sealed and incubated at 25 °C for 30 days. The NaOH traps were replaced on days 3, 10, 20, and 30 to analyse $^{12}CO_2$ and $^{13}CO_2$. These sampling times were chosen according to the peaks in CO_2 release from each substrate identified during a preliminary experiment. Before replacing a new trap and resealing, each jar was thoroughly flushed with ambient air to ensure that the air in each jar was consistent. A set of experimental units (54 cores) were destructively harvested at 10 and 30 days after incubation to determine soil pH, microbial biomass C and N, and mineral N ($NH_4^+ + NO_3^-$) contents.

2.4. Soil analysis

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

To quantify the total CO_2 evolved, a 2-ml aliquot from each NaOH trap was precipitated with 8 ml of 0.25 M $BaCl_2$ solution and then titrated with standardized 0.5 N HCl against the phenolphthalein indicator using a digital burette (BRAND Titrette, Wertheim, Germany) according to Zibilski (1994). The ^{13}C abundance ($\delta^{13}C$ Pee Dee Belemnite, PDB) of CO_2 released from respective treatments was measured on $SrCO_3$ precipitates (Harris et al., 1997) using an Elemental Analysis-Isotope Ratio Mass Spectrometer (SerCon Hydra 20-20, Crewe, UK). Concisely, to form $SrCO_3$ precipitates, a 2-ml aliquot from each trap was placed into a 50-ml conical flask, mixed with 2 ml of 1.0 M $SrCl_2$ solution and 15 ml Milli-Q water. A pH probe was then immersed in the solution and 0.5 M HCl was added drop-wise under magnetic stirring until the pH was neutralized. The solution was then transferred to a 50-ml tube and centrifuged at 1579 × *g* for 3 min and supernatants were discarded. The precipitate was subjected to series of resuspension with 40 ml Milli-Q water followed by centrifugation at 2808 × *g* for 6 min, 702 × *g* for 3 min and 274 × *g* for 3 min and discarding the washes. Finally, each precipitate was vortexed with 1 ml Milli-Q water and collected into a glass vial and oven-dried at 60 °C.

Soil microbial biomass C (MBC) and N (MBN) were determined by the chloroform fumigation-extraction method (Vance et al., 1987). Eight grams of moist soil was fumigated with about 50 ml ethanol-free chloroform in a desiccator at 25 °C for 24 h in the dark. Another 8 g of non-fumigated soil was extracted with 40 ml of 0.5 M K_2SO_4 (1:5, w v^{-1}) by shaking on an end-over-end shaker for 1 h followed by filtering through Whatman #42 (Whatman International, Maidstone, England). Fumigated soils were extracted the same as non-fumigated soils following the evacuation of chloroform. Extracts were stored at -20 °C before total organic C analysis (GE Sievers Innovox Laboratory TOC analyser, Boulder, USA). Total N was determined by a flow injection analyser (FIA) (QuickChem 8500, Lachat Instruments, Loveland, USA) after oxidising with alkaline potassium persulphate at 120 °C for 30 min (Cabrera and Beare, 1993). Microbial biomass C and N were expressed as the difference between organic C and total N from fumigated and non-fumigated soil, respectively. Potential incomplete extraction of C and N was corrected by a factor of 0.45 (Jenkinson et al., 2004). Mineral N (NH_4^+-N and NO_3^--N) from non-fumigated and non-oxidised extracts (3 ml) was also determined by FIA through phenol hypochlorite reaction and copperized-Cd reduction. To express all the results on mass-basis of oven-dry soil, gravimetric moisture content of the incubated soil at each sampling date was determined immediately after harvesting by weighing the soil prior and after oven drying at 105 °C for 24 h.

2.5. Calculations and statistical analyses

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

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

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

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

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

All the statistical analyses were performed with the GenStat 17th Edition (VSN International, Hemel Hempstead, UK) after evaluating homogeneity of variance by plotting the residuals vs. the fitted values. No data transformation was necessary. A three-way analysis of variance (ANOVA) with a *post-hoc* least significant difference test (LSD) was carried out for each time point to investigate the main effects of initial pH, C substrate and N and the potential interactions between the dependent variables. With the aim of determining changes in soil pH during incubation within the same initial pH soil and the effect of C substrate and N on soil pH within each observation, the data were also subjected to one- and two-way ANOVA with least significant difference test (LSD) (Table S1).

3. Results

3.1. Soil pH

Changes in soil pH in response to the initial application of C and N substrate varied among different initial soil pH. In the strongly acidic soil (pH 4.1), irrespective of treatments, soil pH increased with incubation succession up to 0.2 units (Table S1). In the moderately acidic soil (pH 4.7), addition of N decreased soil pH (by 0.2 units), but application of lignocellulose or glucose alone (-N) increased the pH up to 0.08 and 0.23 units, respectively (Table S1). Nevertheless, in the slightly acidic soil (pH 6.6), regardless of the treatments, the soil pH decreased by up to 0.36 units during incubation, except for a slight increase in pH (0.07 units) with the lignocellulose only treatment (L-N) and no change with the glucose only (G-N) treatment (Table S1).

3.2. Carbon dioxide release

Initial soil pH had a small but significant effect on total CO_2 release. The CO_2 release during the 30-day incubation from the strongly acidic soil (pH 4.1) was only 7% lower than that of the moderately and slightly acidic soils. There was no significant difference in CO_2 release between the moderately and the slightly acidic soils (pH 4.7 and 6.6; Fig. 1).

Carbon substrate amendment significantly increased total CO_2 release with contrasting dynamic patterns between the two substrates throughout the incubation period (Fig. 1). The CO_2 release from the C-amended soils was on average 52% greater than that of control at the end of the 30-day incubation. Nevertheless, during the first 20 days of incubation, total CO_2 release was greater in glucose-amended soils than lignocellulose-amended soils with the magnitude of this difference declining as the incubation progressed (Fig. 1a, b, c).

Nitrogen also showed a minor but significant effect ($P<0.05$) on CO_2 release with 6% less total CO_2 with N amendment relative to that without N during the 30-day study. This effect of N was only exhibited in the C-amended soils and not in the control soils throughout the incubation (Fig. 1). Nonetheless, there was a significant ($P<0.05$) initial pH × N interaction in which the effect of N on CO_2 release occurred only in the slightly acidic soils (pH 6.6; Fig. 1c).

3.3. Substrate derived CO_2-C

Initial soil pH had a marked effect on substrate-derived CO_2-C which varied among different periods of incubation (Fig. 2). Mineralisation of C substrate, as measured by $^{13}CO_2$ evolution, in the strongly acidic soils (pH 4.1) was about 12% greater than that in the moderately and slightly acidic soils (pH 4.7 and 6.6) during days 0-10 and the reverse trend was true for the rest of the incubation period (days 11-30), in which C substrate mineralisation in the moderately and slightly acidic soils

was on average 36% greater than those of the strongly acidic soils (Fig. 2b, 2d, 2f). Thus, cumulative CO_2-C derived from substrate was about 6% greater in the moderately and slightly acidic soils compared to the strongly acidic soils (Fig. 2b, 2d, 2f, Table 2).

Mineralisation rates of the two C substrates were very different during the initial phase but the difference diminished as the incubation progressed (Fig. 2b, 2d, 2f). Glucose mineralisation rate was about 890% and 14% higher than its counterpart lignocellulose during the early 0-3 and 4-10 days of incubation, where the glucose mineralisation rate peaked. About 66% of the total amount of glucose mineralized was released within the first 3 days, irrespective of initial soil pH (Fig. 2b, 2d, 2f). Nevertheless, from day 10, mineralisation of lignocellulose was about 7% higher than that of glucose. Lignocellulose mineralisation peaked during days 4-10 and at least 59% of total lignocellulose derived CO_2-C was released in this period (Fig. 2b, 2d, 2f). However, the overall mineralisation of C substrate (% of the total amount added) during the study was similar between the two substrates, i.e. 34% for glucose and 37% for lignocellulose. Nonetheless, the difference in the mineralisation between the two substrates was greater in the moderately acidic soils (12%) than the strongly and slightly acidic soils (6%; Fig. 2d, Table 2).

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

3.4. Carbon priming effect

Initial soil pH had a marked effect on native SOC mineralisation induced by the C addition (priming effect). On average, the cumulative priming effect was greatest at pH 4.1 which was approximately 7- and 3-fold greater than that at pH 4.7 and 6.6, respectively after 30 days (Fig 2a, 2c, 2e, Table 2).

The direction and magnitude of C priming was also significantly influenced by the type of C substrate. The addition of labile C substrate, glucose, yielded greater loss of native SOC compared to that of recalcitrant C, lignocellulose. The differences in magnitude of SOC primed between the two substrates diminished with incubation, for example from 80 μg CO_2 g^{-1} soil at days 0-3 to about 8 μg CO_2 g^{-1} soil at days 21-30 (Figs. 2a, 2c, 2e, 3, Table S2). In addition, the pattern of SOC primed differed between the two C substrates throughout the incubation. In glucose-amended soils, a strong positive priming effect was observed during days 0-3, followed by negative priming (Fig 3a, 3c, 3e, Table S2). On the other hand, in lignocellulose-amended soils, there was negative priming during days 0-3 which turned to positive priming during days 4-10 and followed by negative priming again during days 11-30 (Fig. 3b, 3d, 3f, Table S2). At the end of incubation, cumulative priming with glucose was about 8 μg CO_2 g^{-1} soil (69%) greater than that with lignocellulose (Fig. 2a, 2c, 2e, Table 2).

There was an interaction (P<0.05) between initial pH and C substrate, where the differences in priming effect between the two C substrates was highest at pH 6.6 (Fig. 3e, 3f, Table 2) and lowest at pH 4.7 (Fig. 3c, 3d, Table 2). In addition, the prominent negative priming effect in lignocellulose-amended soils during days 0-3 was observed only in the strongly acidic soils (pH 4.1; Fig. 3b, Table S2).

Mineral N addition showed a significant effect on native SOC mineralisation irrespective of the type of C substrate (Fig. 3, Table 2). The overall reduction of SOC priming in response to N addition in this 30-day incubation study was 8 μg CO_2 g^{-1} soil (69%) (Fig. 3, Table 2).

3.5. *Microbial biomass C and N*

Unlike CO_2 release, initial soil pH exerted a substantial effect on both MBC and MBN. The MBC and MBN concentrations in the slightly acidic soils (pH 6.6) were on average 100% and 14% greater than those of the strongly (pH 4.1) and the moderately (pH 4.7) acidic soils (Table 3).

The addition of C substrates increased the MBC and MBN concentrations by 55% relative to the control soils. The MBC was about 10% greater with glucose than with lignocellulose at 10 days. However, MBC and MBN concentrations with glucose amendment were higher than those with lignocellulose at pH 4.1 and pH 6.6 but the reverse applied at pH 4.7, resulting in significant pH × C substrate interactions at both sampling times (Table 3).

Nitrogen addition showed a significant (P<0.001) suppressive effect on MBC and positive effect on MBN concentrations (Table 3). It decreased MBC by about 10%, but increased MBN by up to 26%. There was a significant (P<0.01) C substrate × N interaction at day 30, whereby the suppressive effects of N on MBC was 15% greater at pH 4.7 and 6.5 in the lignocellulose-amended soils than in the glucose-amended soils (Table 3).

3.6. *Microbial metabolic quotient, qCO_2*

The qCO_2 decreased with increasing initial soil pH in which the qCO_2 of moderately and slightly acidic soils were 43% and 51% lower than that of strongly acidic soils (Table 3). Interestingly, at day 10, the qCO_2 with the glucose amendment was 63% lower than the non-amended control, while that with lignocellulose was about 13% higher than the control. However, by day 30, the qCO_2 of both C-amended soils was lower than the control. Overall, there was a significant pH × C interaction (P<0.001), in which the difference in qCO_2 between the two C substrates was greatest in the strongly acidic soils and lowest in the moderately acidic soils (Table 3).

Nitrogen addition also exhibited a prominent effect on the qCO_2. The average qCO_2 across C types was 30% and 17% higher with N than without N addition at day 10 and 30, respectively (Table 3). The N-induced increase in qCO_2 was greater at pH 4.1 compared to that at pH 4.7 and 6.6, leading to a significant pH × N interaction (P<0.05; Table 3).

3.7. *Inorganic nitrogen (NH_4^+-N and NO_3^--N)*

Contrary to the previously described parameters, inorganic N concentration was negatively correlated with initial soil pH with this trend more prominent for NH_4^+-N (Table 4). The concentration of NH_4^+-N in strongly acidic soils (pH 4.1) was almost 5- and 15-fold greater than those in the moderately acidic (pH 4.7) and slightly acidic (pH 6.6) soils whereas those differences in NO_3^--N were only about 33 and 50%.

Both glucose- and lignocellulose-amended soils had 26% lower inorganic N concentration relative to the control soils, particularly during days 0-10 (Table 4). At the end of the study, inorganic N in lignocellulose-amended soils was 28% less than the control. In addition, there was a significant (P<0.001) interactive effect of Initial pH × C substrate on inorganic N in which the differences in inorganic N concentrations between the two substrates was greatest in the moderately acidic soils (Table 4).

4. Discussion

The study provides new insights into the priming effect induced by C substrates as affected by initial pH and soil N status. We demonstrated that the priming effect was greatest in strongly acidic (pH 4.1) soil, followed by slightly acidic (pH 6.6) soil and lowest in moderately acidic (pH 4.7) soil in a 30-day incubation period. The findings from this study support the first hypothesis that labile C substrate (glucose) yielded a greater priming effect compared to the recalcitrant C substrate (lignocellulose) and verify previous observations (Mary et al., 1993; Nottingham et al., 2009). Although 63-66% of the substrates remained in the soil at the end of the study (324-335 μg C g^{-1} soil), this was less than the total CO_2-C derived from SOC (361-379 μg C g^{-1} soil), indicating a

negative C balance. For the first time, we showed that addition of mineral N reduced both substrate mineralisation and the priming effect regardless of C substrate quality (availability) and initial soil pH. This finding did not fully support our second hypothesis that N increased substrate mineralisation but decreased the priming effect. Furthermore, the greatest net reduction in C priming due to N addition occurred in the moderately acidic (pH 4.7) soil which was contrary to our third hypothesis.

4.1. Soil pH effect on priming

This study showed that the priming effect in response to exogenous C addition was largely controlled by initial soil pH, consistent with previous work (Aye et al., 2017; Luo et al., 2011; Perelo and Munch, 2005). The influence of initial soil pH on the priming effect could be attributed to variation in abundance and community composition of decomposer microorganisms among the soils with different pH (Fierer and Jackson, 2006) as the priming effect is expected to be mediated by decomposer organisms (Rousk et al., 2009). In addition, the variation in the proportion of added substrate C relative to the indigenous MBC among different pH soils might also have played a significant role in priming (Blagodatskaya and Kuzyakov, 2008). The different MBC concentrations across the initial pH range (Table 3) meant that the amount of C added (0.5 mg g^{-1} soil) was about 6 times higher than indigenous MBC at pH 4.1, while that was only 2.5 and 2 times higher than the MBC at pH 4.7 and 6.6 (Table 3). Therefore, more C would have been available to microorganisms in the former soils at a given addition rate of C substrate, and thereby facilitating a stronger priming effect through microbial activation theory (Chen et al., 2014). The addition of C substrate might also trigger microorganisms to switch from a dormant to an active stage (Wang et al., 2016).

The net increase in MBC in response to C-substrate amendment at pH 4.1 was larger (69%) relative to those at pH 4.7 (29%) and 6.6 (48%; Table 3). Such a surge in microbial biomass and activity in this strongly acidic soil could have been responsible for the greatest mineralisation of native SOC (Fig. 2) through co-metabolism and microbial N-mining mechanisms (Kuzyakov et al., 2000). However, it is noteworthy that not all the extra C mineralisation due to substrate C addition should be assumed as accelerated non-biomass SOC mineralisation because it can be contributed from CO_2 released by rapid microbial respiration upon receiving labile C substrates (Wu et al., 1993), which is known as apparent priming effect (Blagodatskaya and Kuzyakov, 2008).

The manipulation of soil pH to obtain the strongly acidic (pH 4.1) soil would have altered C availability and likely changed the functional capacity of the microbial community. Microbial biomass was reduced by half (~110 μg C g^{-1} soil) when the pH was reduced from 4.7 to 4.1. It is likely that some of the dead microbial biomass contributed to the primed C after glucose or lignocellulose was added and that this labile C pool might not be present in soils with a more static soil pH. Utilization of such microbial necromass as a primer (Shahbaz et al., 2017) in this strongly acidic soil could also have been part of the reason for the largest priming effect. The heterogeneous nature of the necromass would have been able to serve as labile C source for living microorganisms and enhanced SOC priming. This may indicate that pH effects on priming occur via its effect on C availability since soil microbes had the capacity to utilise this C in the strongly acidic soil once the other substrates were added.

The effect of initial soil pH on the priming effect differed between the two C substrates. Even though the magnitude of the priming effect in glucose-amended soils was consistently higher in the strongly acidic soils throughout the study, the pattern in lignocellulose-amended soils was more dynamic (Fig. 3). A negative priming effect occurred in lignocellulose-amended-strongly acidic soils during the early incubation stage (days 0-3) which coincided with faster mineralisation of this substrate (Fig. 2). This could be ascribed to a greater abundance of microbes adapted in strongly acidic soils which were more efficient in degrading and utilizing the high-polymer lignocellulose in a short time (Fontaine et al., 2011). A greater proportion of active microbes, which respond rapidly to substrate addition (e.g., by producing enzymes), is considered to be more important than microbial biomass in priming effect (Blagodakskaya and Kyuzayakov, 2013). Consequently,

microbes would have utilised the abundant labile C that was added rather than mining from soil organic matter, in turn caused the negative priming in accordance with the preferential substrate utilization mechanism (Cheng, 1999).

The priming effect was greater in the strongly acidic soil (pH 4.1) than the two higher pH soils (pH 4.7 and 6.6) even though lignocellulose mineralisation was lower in the former soil after day 3 (Fig. 2). The low mineralisation of lignocellulose in the strongly acidic soils would not be able to supply labile C to rapidly growing microbial biomass in this particular phase, resulting in greater priming effects through mining C and N from soil organic matter to meet nutritional demand of microbes (Shahbaz et al., 2017). Therefore, the direction of priming effect would have been mediated by microbial adjustment between C demand and supply from added substrates. This reflects the inconsistencies of priming effect demonstrated by the studies under different soil environments along with substrate amendments of various quantities and/or qualities. This greater priming effect could also be ascribed to the greater net increase in microbial biomass during the initial stage following C-substrate amendment in this strongly acidic soil (Table 3). Greater biomass would have prolonged their activity by using the labile C substrates from the early stages of incubation as an energy source according to the 'microbial activation' theory (De Nobili et al., 2001) in the strongly acidic soil, which in turn decomposed a larger amount of native SOC than the two higher pH soils either indirectly (glucose) or through the co-metabolism (lignocellulose) phenomenon (Kuzyakov and Bol, 2006).

Nevertheless, if the priming effect was compared only between the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils, the priming effect was greater in the latter soils (Fig. 3, Table 2). This could also be attributed to a greater net increase in microbial biomass upon substrate supply in the slightly acidic soils compared to the moderately acidic soils (Table 3), which is consistent with our previous study (Aye et al., 2017). Similar results have also been reported (see Blagodatskaya and Kuzyakov 2008) despite most of these studies being conducted in different soil matrixes with very narrow range of pH. Greater priming effect in soils with pH 6.1 than those with pH 5.9 was ascribed to greater increase in soil-derived C in microbial biomass (Perelo and Munch, 2005). Substrate-induced immobilization of SOC would have been greater in slightly acidic soils (pH 6.6) compared to the moderately acidic soils (pH 4.7) due to more favorable conditions for microbial activity in the former soils. As such, greater CO_2 evolution from rapid microbial turnover would also have contributed to primed C.

4.2. Effect of C substrate on SOC priming

Overall, the application of both glucose and lignocellulose substrates induced a positive priming effect in all pH soils during this 30-day incubation study. Generally, C priming was largest through the first 3 days with glucose and 10 days with lignocellulose, which was also the period of highest substrate mineralisation. After these peaks, the priming effect of both substrates diminished and negative values remained until the end of the study (Fig. 3, Table S2). Consequently, the priming effect of both C substrates in this current study was much lower than those reported in previous studies using comparable substrates and observation periods (Bastida et al., 2013; Fontaine et al., 2004b). The amount of C added here is considered to be relevant to agricultural systems whereby only a small proportion of fixed C by plants is released into the soil (see Qiao et al. 2017). Nevertheless, the low cumulative SOC primed in this present study could be partially ascribed to the amount of C substrate added which was 2.4-5.9 times the MBC. Chowdhury et al. (2014) showed that priming effects were greater at lower rates of malic acid (100 mg C kg^{-1} soil) addition than higher rates (1000 mg C kg^{-1} soil). However, Blagodatskaya and Kuzyakov (2008) reported that the addition of substrate induced linear increases in C priming up to rates equal to 15% of MBC but when high amounts of substrate (>50% of MBC) were applied, the priming effect tended to be close to zero or even negative. Here, C substrate addition (500 mg kg^{-1}) was equivalent to 598% (initial pH 4.1) and 240% (initial pH 6.6) of MBC and potentially outside the range expected to induce a positive impact on C priming. Preferential substrate utilization was assumed to be the

major process involved in this phenomenon with the switch of substrate conditions for decomposer organisms from energetically-expensive soil organic matter to easily accessible added substrates (Cheng and Kuzyakov, 2005; Kuzyakov, 2002). The increase in C-use efficiency (decrease qCO_2) in C substrate-amended soils compared to the control at the end of the study (Table 4) also indicates that a less stressful environment for microbes in the former soils, which might have led to this negative priming effect (Odum 1985). In addition, the overall small magnitude of net primed C observed in this study have resulted from the balance between positive and negative priming (Fig. 3, Table S2) and the fact that the soils used in the study were from a cropping system with low C inputs (<1 t C ha^{-1} yr^{-1}) (Aye et al., 2016) and as a result the C present in this soil is likely to be more recalcitrant than soils used in other studies.

On the whole, the greater priming effect in the soils amended with glucose relative to those with lignocellulose might be partly explained by the greater net increase in MBC in the glucose-amended soils than in the lignocellulose-amended soils (Fig. 2, Table 3). Similar results have been revealed earlier (Mary et al., 1993; Nottingham et al., 2009). Even though the duration of positive priming induced by glucose lasted only 3 days, the cumulative native SOC loss was greater in glucose-amended soils than in lignocellulose-amended soils (Fig. 2). The labile glucose amendment provided sufficient labile C for microbial growth as indicated by greater net increases in MBC (Table 3) and this 'activation' of the soil microbes could have facilitated the production of extracellular enzymes able to degrade native SOC. Although indirectly, the effect of glucose addition may have facilitated SOC mineralisation during the first 3 days of incubation similar to the co-metabolism mechanism (Kuzyakov and Bol, 2004), whereby the additional enzymes produced degrade SOC. The abundance and/or activity of extracellular enzymes are positively correlated with microbial population as long as fresh substrate is not limiting (Kshattriya et al., 1992; Joshi et al., 1993). However, considerable amount of CO_2 released from rapid microbial turnover during this early incubation stage (3 days) would also have been recorded as primed C.

In the case of lignocellulose, there was a lag phase of about 3 days to reach maximum substrate mineralisation and priming compared to glucose (Figs 2, 3). Such a delay in the mineralisation of both substrates and native SOC might be accredited to poor initial adaptation of the microbial community to decompose polymeric lignocellulose substrate, particularly in the moderately and slightly acidic soils (Torres et al., 2014). Furthermore, mineralisation of lignin-rich recalcitrant organic compounds requires a depolymerization step to produce soluble components for microbial absorption and metabolism (Fontaine et al., 2003), while glucose can be directly assimilated by microbes (Jagadamma, 2014). However, accelerated priming in the lignocellulose-amended soils after this lag phase could be attributed to the similar degradability of added substrate and SOC which were decomposed by the same enzymes (Van der Wal and De Boer, 2007).

Such differences in mineralisation between the two substrates could be a reason for the different lag phases of negative priming, 3 days with glucose and 10 days with lignocellulose (Fig. 3). Preferential utilization of labile C source rather than more recalcitrant indigenous SOC resulted in the negative priming effect. With glucose amendment, such preferential substrate utilization occurred earlier than that with lignocellulose since glucose is readily assimilated by microbes, while lignocellulose is more recalcitrant and requires a period of depolymerization prior to the products being preferentially utilized as labile C.

4.3. Effect of N on SOC priming

The lower amount of SOC primed in soils amended with N than without, reflects that N addition alleviated N limitation and consequently reduced microbial N-mining. This result is in line with many other studies (Blagodatskaya et al., 2007; Foereid et al., 2004; Fontaine et al., 2004a; He et al., 2016; Henriksen and Breland, 1999). Reduced heterotrophic respiration occurs in the presence of inorganic N but not organic N (Chen et al., 2018), which may subsequently influence C priming. The greater inorganic N concentrations in N-amended soils (Table 4) indicate that N availability was not limited, decreasing the need for microbes to acquire N from soil organic matter.

The reduction in inorganic N due to microbial N immobilisation following C substrate amendment, especially within 10 days after incubation was more prominent with lignocellulose than glucose (Table 4), suggesting that more N was needed by microbes while degrading the high polymeric substrate (Vahdat et al., 2011). In another study, Szili-Kovács et al. (2007) observed that the application of sucrose and sawdust in soils reduced the concentrations of inorganic N by 6 and 20%, respectively. Differences in N demand between the two C substrates might have modified stoichiometry of soil N, which in turn affects the growth and activity of decomposers and the SOC mineralisation processes as suggested by Allison et al. (2008) and Bowden et al. (2004). Kirkby et al. (2014) also demonstrated that augmenting the residues with supplementary nutrients decreased SOC mineralisation and suggested that the application of additional nutrients beyond that required for crop production is needed to minimise extra SOC losses. The addition of N to the soil amended with maize residue also reduced SOC priming by 9.5% (Qiu 2016). Reductions in C mineralisation in three grassland soils after N addition have been associated with reduced microbial biomass rather than changes in oxidative enzyme activity and C-use efficiency (Riggs and Hobbie 2016). However, the decreased activity of SOC-degrading enzymes due to mineral N addition (Sinsabaugh et al., 2005) could have also contributed to the decreased priming. Nevertheless, many other studies reported alternative results that the application of mineral N enhanced the C priming effect (Conde et al., 2005; De Graff et al., 2006). Such inconsistency among the studies could possibly be due to different biotic and abiotic characteristics of soils, variation in the amount and quality of added substrate C and N relative to stoichiometry of indigenous microorganisms. If the soil is rich in available C but low in N availability, the priming may increase due to microbial N mining. On the other hand, high amounts of available N may enhance microbial activity which in turn would increase the priming effect. Therefore, the effect of N on priming effect would mainly depend on adjustment of microbial activity and composition in accordance with N availability in the soil (Qiu et al., 2016). Moreover, soils are composed of a complex mixture of organic molecules that vary in N content and the energy required by microbes to break them down. As such, the addition of inorganic N is likely to increase the mineralisation of some SOC fractions but decrease that of others (Neff et al., 2002). For example, turnover of protected C (mineral associated C) was more inhibited by N addition than that of less protected SOC pools (within micro- and macroaggregates) and the intensity of C mineralisation inhibition increased with N level (Tan et al., 2017).

The more pronounced reduction in the priming effect with added N in soils amended with lignocellulose than glucose could be ascribed to the lower soil pH and MBC in the former treatments (Fig. 3, Tables 3, S1). In other studies, decreasing soil pH decreased the priming effect (Aye et al., 2017), and the optimum pH for the priming effect was between 6 and 8 (Aye et al., 2017; Blagodatskaya and Kuzyakov, 2008). Such an effect on SOC decomposition had resulted from the effect of soil pH on microbial growth and activity (Andersson et al., 2000; Briedis et al., 2012; Foereid et al., 2004). It was postulated that N application might exert a direct negative effect on microbial biomass through increased solute concentrations and/or indirectly by decreasing the soil pH via nitrification of added N. This interactive effect of C substrate and N could also be ascribed to the different chemical structures of the two substrates. Lignocellulose amendment would have caused N-limited environment for soil decomposers, since microbes require both C and N (Thangarajan et al., 2013). The application of N would have satisfied N demand, resulting in reduced priming caused by N mining. Therefore, the results confirmed that N was less important in priming in soils amended with glucose compared to those with lignocellulose. This indicated that the application of sufficient amounts (optimal but not excessive) of N would provide not only better crop yield, but may also minimise indigenous SOC loss upon crop residue returning to the soil. However, such a speculation needs to be verified in long-term field trials.

5. Conclusions

To our knowledge, this is the first study to investigate the impact of pure C substrates and N on priming at a range of initial pH levels within the same soil matrix. The effect of initial soil pH on

priming was non-linear such that priming was greatest in the strongly acidic (pH 4.1) soils, followed by the slightly acidic (pH 6.6) soils and lowest in the moderately acidic (pH 4.7) soils. This greatest priming effect in the strongly acidic soil could be ascribed to the greatest net increase in MBC resulted from the largest proportion of added C substrate to indigenous MBC pool. The effect of N addition on priming depended on substrate quality, with N addition decreasing SOC priming, and this effect being greater with lignocellulose than glucose. Such a decreased priming effect could be attributed to reduction of microbial-N mining and/or reduction in microbial activity as a result of decreased pH following N application. Albeit small in magnitude compared with total C mineralisation, the study highlighted that N could reduce C priming and this effect was greatest in the slightly acidic soil. Hence, targeted N and pH management may have significant impacts on SOC in the longer term. Further studies with examination of shifts in microbial community composition and functional activity upon C and N amendment at different initial pH levels are needed.

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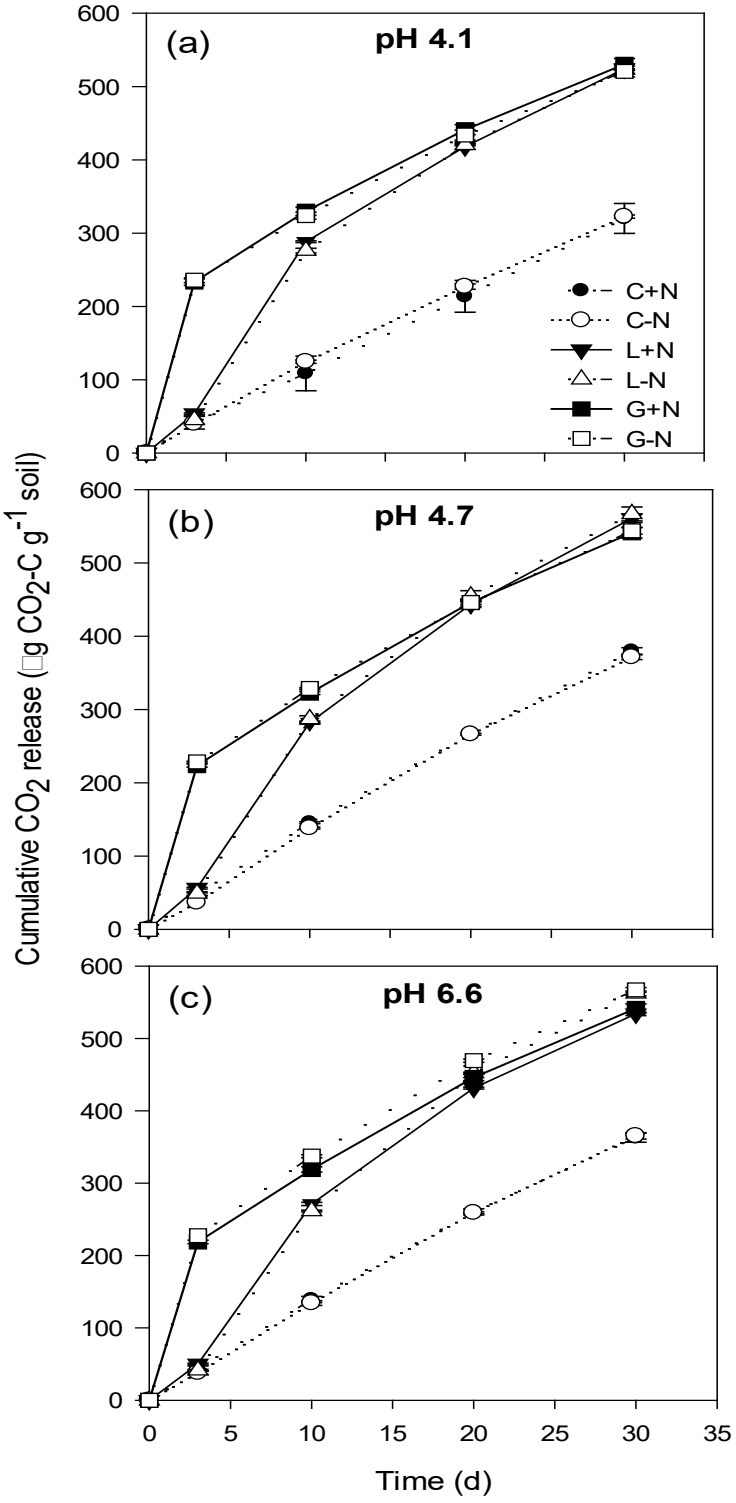

Fig. 1 Cumulative CO_2 release from control with (C+N) and without N (C-N), glucose with (G+N) and without N (G-N), and lignocellulose with (L+N) and without N (L-N) in the strongly acidic (pH 4.1) (*a*), the moderately acidic (pH 4.7) (*b*) and the slightly acidic (pH 6.6) (*c*) soils over 30 days. Error bars represent the standard error of the means (n=3). Where error bars not visible, symbols are larger than error bars.

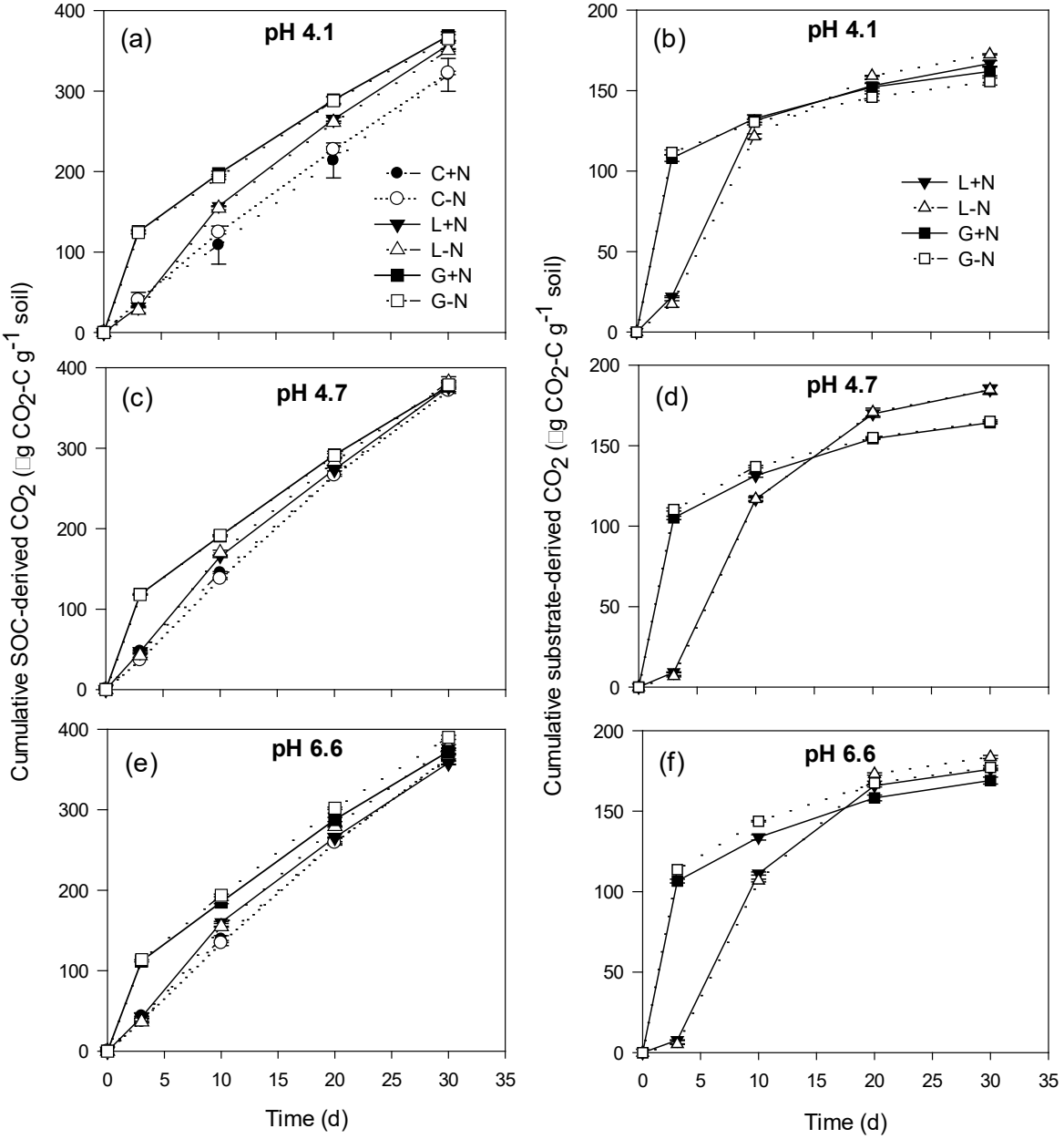

Fig. 2 The cumulative SOC-derived CO_2 (*a, c, e*) and substrate-derived CO_2 (*b, d, f*) from control with (C+N) and without N (C-N), glucose with (G+N) and without N (G-N), and lignocellulose with (L+N) and without N (L-N) in the strongly acidic (pH 4.1) (*a, b*), the moderately acidic (pH 4.7) (*c, d*) and the slightly acidic (pH 6.6) (*e, f*) soils over 30 days. Error bars represent the standard error of the means (n=3). Where error bars not visible, symbols are larger than error bars.

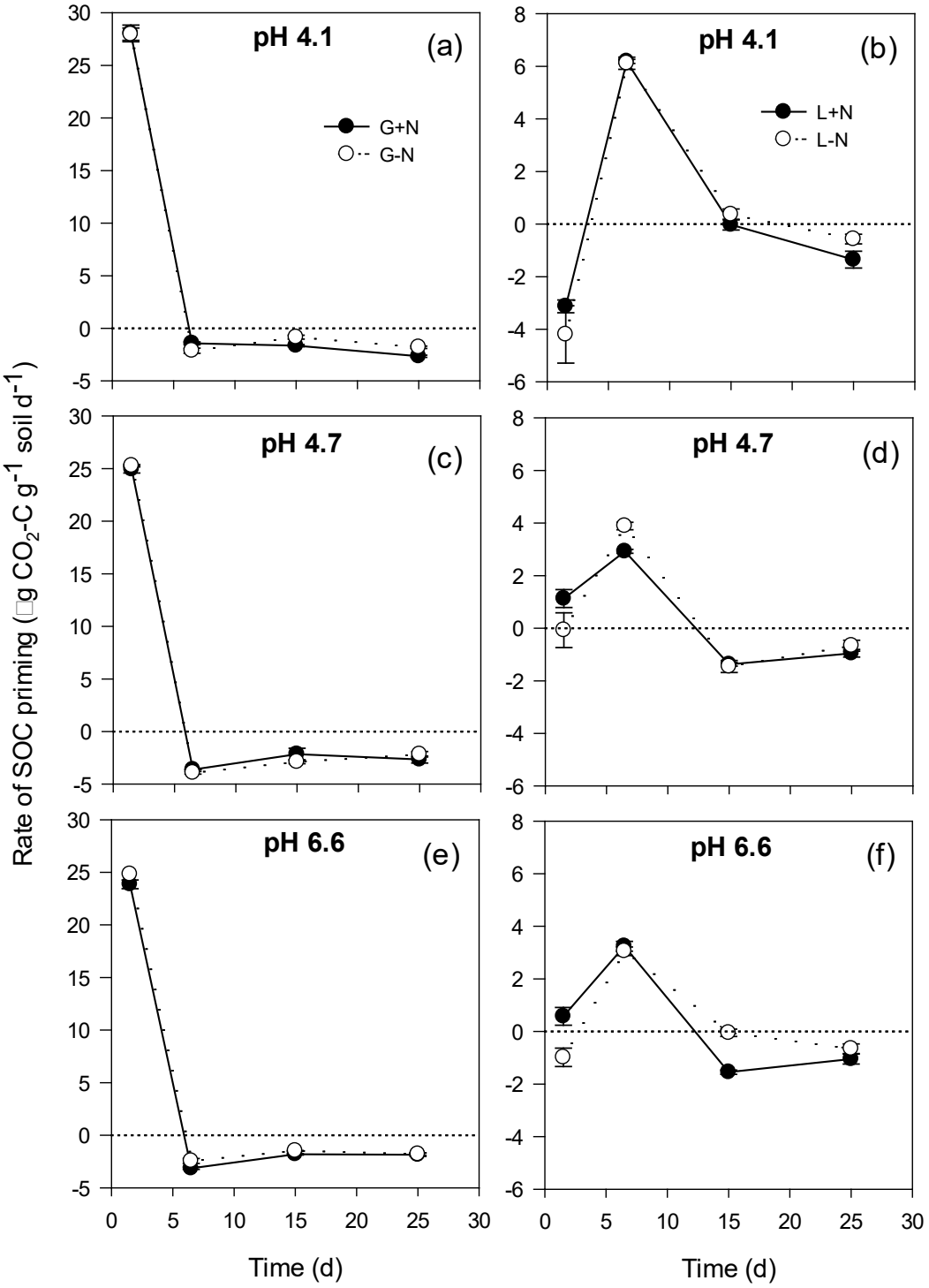

Fig. 3. The rate of SOC priming from glucose with (G+N) and without N (G-N) (*a, c, e*), and lignocellulose with (L+N) and without N (L-N) (*b, d, f*) in the strongly acidic (pH 4.1) (*a, b*), the moderately acidic (pH 4.7) (*c, d*) and the slightly acidic (pH 6.6) (*e, f*) soils over 30 days. Error bars represent the standard error of the means (n=3). Where error bars not visible, symbols are larger than error bars.

Table 1. Physicochemical characteristics of soils and substrates used in the experiment

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

Table 2. Basal soil-derived C, primed-C and substrate-derived C in the strongly acidic (pH 4.1), the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils during the 30-day incubation of glucose and lignocellulose and with (+N) or without N (-N).

Treatments	Basal soil-derived C ($\mu g\ CO_2$-C g^{-1} soil)	Primed-C ($\mu g\ CO_2$-C g^{-1} soil)	Substrate-derived C ($\mu g\ CO_2$-C g^{-1} soil)
pH 4.1			
Glucose + N	369	34.3	162
Glucose - N	365	47.6	156
Lignocellulose + N	357	24.1	167
Lignocellulose - N	351	30.5	173
pH 4.7			
Glucose + N	377	3.2	164
Glucose - N	378	7.0	165
Lignocellulose + N	376	3.1	185
Lignocellulose - N	382	6.0	185
pH 6.6			
Glucose + N	372	12.9	169
Glucose - N	390	18.9	177
Lignocellulose + N	358	2.6	176
Lignocellulose - N	379	8.9	184
LSD (P=0.05) for any two means	*13*	*3.25*	*5*
Significance level			
Initial pH	***	***	***
C substrate	**	***	***
N	*	***	*
Initial pH × C substrate	*	***	***
Initial pH × N	**	***	**
C substrate × N	NS	NS	NS
Initial pH × C substrate × N	NS	NS	*

Not significant (NS) at P=0.05; *, ** and *** indicate significant at the P<0.05, P<0.01 and P<0.001, respectively.

Table 3. Microbial biomass C and N, and microbial metabolic quotient (qCO_2) in the strongly acidic (pH 4.1), the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils at 10 and 30 days (control), or incubated with glucose and lignocellulose and with (+N) or without N (-N).

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

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

Table 4. Concentrations of NH_4^+-N, NO_3^--N and total inorganic nitrogen (NH_4^+-N + NO_3^-- N) in the strongly acidic (pH 4.1), the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils (control), or incubated with glucose and lignocellulose and with (+N) or without N (-N).

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

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

Table S1. Soil pH of the strongly acidic (pH 4.1), the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils at 10 and 30 days in the control, glucose and lignocellulose with (+N) or without N (-N) treatments.

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

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

Table S2. Cumulative primed-C in the strongly acidic (pH 4.1), the moderately acidic (pH 4.7) and the slightly acidic (pH 6.6) soils at 3, 10, 20 and 30 days after glucose and lignocellulose amendment with (+N) or without N (-N).

Treatments	Primed C ($\mu g\ CO_2$-C g^{-1} soil)			
	3 days	10 days	20 days	30 days
pH 4.1				
Glucose + N	84.1	73.2	58.7	34.3
Glucose - N	83.9	74.8	64.4	47.6
Lignocellulose + N	-10.4	32.9	35.2	24.1
Lignocellulose - N	-10.9	31.9	36.8	30.5
pH 4.7				
Glucose + N	74.6	48.1	25.0	3.2
Glucose - N	75.8	49.6	26.8	7.0
Lignocellulose + N	1.7	22.2	9.4	3.1
Lignocellulose - N	1.5	26.2	13.5	6.0
pH 6.6				
Glucose + N	71.6	48.7	31.1	12.9
Glucose - N	74.5	53.2	36.8	18.9
Lignocellulose + N	2.3	25.0	12.8	2.6
Lignocellulose - N	1.4	24.6	17.3	8.9
LSD (P=0.05) for any two means	*2.63*	*2.94*	*3.29*	*3.25*
Significance level				
Initial pH	*	***	***	***
C substrate	***	***	***	***
N	NS	**	***	***
Initial pH × C substrate	***	***	***	***
Initial pH × N	NS	NS	NS	***
C substrate × N	NS	NS	NS	NS
Initial pH × C substrate × N	NS	*	NS	NS

Not significant (NS) at P=0.05; *, ** and *** indicate significant at the P<0.05, P<0.01 and P<0.001, respectively.