Soil Biology and Biochemistry
Volume 65, October 2013, Pages 217-226
http://dx.doi.org/10.1016/j.soilbio.2013.05.019

Soil organic carbon contributes to alkalinity priming induced by added organic substrates

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ABSTRACT
Organic substrate input stimulates mineralisation of native soil organic matter, resulting in CO_2 priming. Our previous studies showed that such CO_2 priming enhanced alkalinity release but the mechanisms behind this are unknown. This study used ^{14}C-labelled compounds to investigate the effect of added organic compounds on decomposition of soil organic matter and how this related to the enhanced release of alkalinity. ^{14}C-labelled glucose and malic acid were added at a rate of 1 mg C g^{-1} to topsoil and subsoil of the Kandosol (pH 5.4-5.8, C 8.9-12.4 mg g^{-1}), the Podosol (pH 4.4-4.5, C 1.5-2.9 mg g^{-1}) and the Tenosol (pH 4.7-6.1, C 1.9-10.9 mg g^{-1}), and incubated for 15 d. 21-27% of the added C was mineralised to CO_2 in the Podosol while 56-74% was mineralised in other two soils with malic acid being mineralised more than glucose. The CO_2 priming, as a result of added C, was substantial, and ranged 110-325 μg g^{-1} for Podosol and 766-1178 μg g^{-1} for the other two soils with the priming being greater in topsoil than subsoil. The addition of both organic compounds resulted in alkalinity priming in the Kandosol and the Tenosol but not in the Podosol; the alkalinity was greater with malic acid than glucose and greater in topsoil than subsoil. The effect of glucose on alkalinity release occurred mainly via NO_3 immobilization while the effect of malic acid via ammonification, NO_3 immobilization and decarboxylation/decomposition of native soil organic matter. This study confirmed that alkalinity priming occurred with concurrent CO_2 priming as a result of C compound addition. This alkalinity priming depended on added C source, initial soil pH and soil organic matter content.

Key words: alkalinity priming, carbon cycle, CO_2 priming, decomposition; indigenous soil organic matter, initial soil pH, nitrogen cycle, ^{14}C-glucose, ^{14}C-malic acid.

1. Introduction
The return of crop residues and addition of organic materials to soil are an important farming practice to sustain soil productivity, and have various impacts on soil pH change which in turn influences plant growth. The addition of organic materials to soil has been shown to increase, decrease or not affect soil pH, depending on biochemical composition of organic material added and soil properties (e.g. Ritchie and Dolling 1985; Pocknee and Sumner 1997; Tang and Yu 1999; Xu and Coventry 2003; Xu et al., 2006; Butterly et al., 2013). For example, different plant residues contain various amounts of carbohydrates, nitrogen compounds, lipids and lignin as the principal organic compounds (Kögel-Knabner, 2002). Plant species also differ in release of low molecular-weight compounds into the rhizosphere (Strobel, 2001). Laboratory incubation studies have shown that the addition of simple sugars such as glucose to soils has little effect on pH change (Yan et al., 1996; Kemmitt et al., 2006; Rukshana et al., 2011). In comparison, the addition of nitrogen compounds to soil has a significant effect on soil pH due to ammonification and nitrification processes (Yan et al., 1996; Rukshana et al., 2012). The addition of carboxylates or carboxylic acids such as malic acid and citric acid changes soil pH through H^+ association/dissociation reactions in soil matrix and subsequent decomposition (Rukshana et al., 2011).

It has been well studied that the addition of organic substrates enhances mineralisation of native soil organic matter with extra CO_2 release, resulting in a priming effect (Hamer and Marschner, 2005; Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010). Our previous study showed that the addition of simple organic substrates to topsoils enhanced CO_2 release which was associated with release of additional alkalinity (termed “alkalinity priming”) in the soil (Rukshana et al. 2012). The degree of alkalinity priming appeared to be influenced by the type of organic substrate, initial soil pH

and/or soil organic matter content. For example, the alkalinity priming occurred in Tenosol (initial pH 6.2) but not in Podosol (pH 4.5). It was greater when malic and citric acid were added than when glucose was added to the soil although the addition of glucose, malic acid and citric acid resulted in the largest and similar cumulative soil respiration among seven organic substrates tested (Rukshana et al. 2012). However, the nature of such alkalinity priming and how this is associated with carbon priming effect is unknown.

The experiment described in this paper aimed to quantify alkalinity generated after the addition of model compounds and the proportion that was derived from either added C or from soil organic matter. We determined the fate of ^{14}C-labelled glucose and malic acid into soil C pools (mineralised to CO_2; immobilised into microbial biomass C; remaining as extractable organic C) in topsoil and subsoil of Kandosol, Podosol and Tenosol which differed in total organic C content and initial pH. We hypothesized that 1) alkalinity priming would result from the stimulated decomposition of soil organic matter, 2) alkalinity priming would be greater in topsoils than subsoils due to higher C content and 3) the extent of alkalinity priming would be greater in soils with higher than lower pH.

2. Materials and methods

2.1. Soil types

Soils were collected from 3 different agro-ecological zones in Australia; Wagga-Wagga, New South Wales (35°02'S, 147°20'E); Frankston, Victoria (38°14'S, 145°22'E) and Shepparton, Victoria (36°28'S, 145°36'E). These soils are classified as Kandosol, Podosol and Tenosol (Isbell, 2002) or Luvisol, Podzol and Cambisol (FAO/ISRIC/ISSS, 2006), respectively. The Kandosol and Tenosol were collected from cropping sites while the Podosol collected from a site under native vegetation. Topsoil and subsoil samples were collected at each site. However, the sampling depth was primarily determined by contrasting pH, total C and pH buffer capacity of soil layers rather than a single depth. Soils were sieved (<2 mm), thoroughly mixed and air-dried for subsequent analysis and the incubation study. Selected chemical and physical properties of soils are outlined in Table 1.

2.2. Model C compounds

Two model C compounds commonly found within decomposing plant materials or the rhizosphere were used in this study to examine their effects on alkalinity priming. Glucose is a simple carbohydrate with neutral OH and CHO chemical functional groups. Malic acid is an organic acid containing two acidic carboxyl (R-COOH) functional groups (pKa_1=3.4, pKa_2=5.13). Stock solutions of glucose (41.66 g l^{-1}; Ajax Finechem) and malic acid (46.52 g l^{-1}; Sigma-Aldrich) were prepared using CO_2 free Milli-Q water. Each stock solution was then divided into two parts, and one set was spiked with an equivalent radioisotope. Stock solutions (100 ml) of glucose and malic acid were spiked with 30 µl of ^{14}C-glucose (5.55 MBq ml^{-1}; D-Glucose-UL-^{14}C; Sigma-Aldrich) and 22.5 µl ^{14}C-malic acid (7.4 MBq ml^{-1}; Malic Acid, L-[U-^{14}C]; PerkinElmer), respectively, to give an approximate activity of 167 kBq. Stock solutions with (labelled) and without (non-labelled) radioisotope were both used in this study.

2.3. Soil incubation

Each soil was rewetted to 40% of field capacity, thoroughly mixed and 50 g (dry weight equivalent) of each soil was packed into individual plastic vials (4.2 cm ID × 5.5 cm high) to a bulk density of 1.4 g cm^{-3}. Soil vials were placed in the air-tight containers containing water reservoirs to maintain headspace humidity and were pre-incubated in the dark at 25 °C for 8 d to recover the soil microbial biomass. After pre-incubation, a set of cores were injected with either labelled or non-labelled solutions using a needle (1.25 G; Terumo Medical Corporation, Melbourne, Australia). Soils used to determine the fate of C (soil respiration, microbial biomass carbon and extractable organic C) were injected with 3 ml of labelled ^{14}C-glucose or ^{14}C-malic acid stock solutions (equivalent to 1 g C kg^{-1} soil; 100 Bq g^{-1} soil or 5.0 kBq $vial^{-1}$). Soils for pH and N analyses were amended the same except that unlabelled stock solutions were used. Unamended controls were injected with an equivalent amount of Milli-Q water. In each case, 9 injections were made across the soil surface at approximately 0.6 cm depth for all sets of samples. After pre-incubation, soils were then adjusted to 90% of field capacity using Milli-Q water and placed into separate glass jars (Ball® Quart Wide Mouth jars, Jarden Corporation, USA) containing an alkali trap (10 ml 1 M NaOH) to quantify CO_2 production and a water reservoir (10 ml CO_2 free water) to maintain headspace humidity. For controls, 2 soil vials were placed per incubation jar to ensure that basal respiration could be detected. Incubation jars without soil were included as blanks. Three replicates were maintained unless specified. Jars were incubated in the dark at 25 °C and gently agitated twice daily by hand to maximize the efficiency of the alkali trap. Soil vials were destructively sampled at 3 and 15 d for analysis. Alkali traps for the final sampling time (15 d) were replaced with new traps at 3 d.

2.4. Soil respiration

Cumulative soil respiration (μg CO_2-C g^{-1} soil) was determined by quantifying the amount of CO_2 trapped by alkali at 3 and 15 d. Within 48 h of each sampling time, 4 ml aliquots from each NaOH trap were titrated using 1 M HCl in the presence of excess 0.5 M $BaCl_2$ and 1 drop phenolphthalein indicator (5% w/w) using micro burette (Zibilske, 1994). Each trap was analysed in duplicate. The $^{14}CO_2$-C concentration within NaOH traps was determined by quantifying ^{14}C activity in a 1-ml aliquot with 4 ml scintillation cocktail (Ultima Gold XR alkali compatible; Sigma-Aldrich) using a liquid scintillation analyser (Tri-Carb 2810 TR; PerkinElmer, Massachusetts, USA). Sample counts were background corrected.

2.5. Soil chemical analyses

Total C and N concentrations were determined on air-dry soil using an Elementar Vario EL analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Particle-size distribution was analysed using a Laser Particle Size Analyser (Malvern Mastersizer 2000, Worcestershire, UK).

Soil pH and concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) were quantified immediately after sampling (3 d and 15 d) using moist soil. Soil pH was determined using a pH meter (Thermo Orion 720A+, Beverly, USA) after extraction in 0.01 M $CaCl_2$ (1:5 soil: solution) by shaking end-over-end for 1 h followed by centrifugation at 2000 rev min^{-1} (492 *g*) for 10 min. Soil pH buffer capacity of the soils were determined by shaking soil in 0.01 M $CaCl_2$ (1:5) with varying amounts of HCl or K_2CO_3. Soil pH buffer capacity (pHBC) was calculated from the slope of a linear regression determined between +/- 1 mmol OH^-/H^+ from the original soil pH. The amount of alkalinity produced in soil was estimated from pHBC and pH change resulting from addition of C compounds.

Nitrogen (N) extractions were performed using 15 g soil with 2 M KCl (1:1) by shaking end-over-end for 1 h followed by centrifuging at 3500 rev min^{-1} (1507 *g*) for 5 min and passing the extracts through Whatman No. 1 filter papers (Whatman International, Maidestone, England). Filtered extracts were stored frozen and concentrations of NH_4^+ and NO_3^- were later determined using a Flow Injection Analyser (LACHAT QuickChem 8500, Loveland, USA).

Microbial biomass C was determined at the end of the experiment (15 d) by chloroform fumigation-extraction according to Vance et al., (1987) with the following modifications. Briefly, ^{14}C compound-amended soils and non-amended controls were fumigated using ethanol-free chloroform and incubated in the dark for 24 h at 25 °C. On the following day chloroform vapours were removed by repeated evacuation and 7 g soil (dry weight equivalent) was extracted with 28 ml 0.5 M K_2SO_4 by shaking end-over-end for 1 h followed by centrifugation at 500 rpm (31 *g*) for 5 min. The extracts were then passed through Whatman No. 42 filter papers (Whatman International, Maidestone, England). Non-fumigated samples were extracted in the same way but immediately following sampling. Extracts were stored frozen and later analysed for ^{14}C activity as described above. Total organic C in the extracts was determined following dichromate oxidation (Heanes, 1984; Conyers et al., 2011). Briefly, 10 ml of each K_2SO_4 extract was transferred to a 100 ml glass digestion tube with 10 ml $K_2Cr_2O_7$ (49.0 g l^{-1}; BDH) and 20 ml H_2SO_4 (98%; Merck) was gradually added before transferring tubes to a pre-heated digestion block. Sucrose standards were included for calibration. Extracts were digested at 130 °C for 30 min, allowed to cool at room temperature and incrementally adjusted to a final volume of 100 ml using Milli-Q water mixing thoroughly each time. Total organic C was determined colorimetrically at 600 nm using a UV-Visible Spectrophotometer (Cary 50 Bio, Varian Australia Pty Ltd). Total microbial biomass C was calculated as the difference in total organic C concentration between fumigated and non-fumigated soils and using a k_{EC} of 0.37 (Sparling and Zhu, 1993; Joergensen, 1996).

2.6. Statistical analysis

For each soil, a 3-way analysis of variance (ANOVA) was used to determine the effects of soil depth × compound × incubation time on pH, NH_4^+ and NO_3^- concentration, net alkalinity, N cycle alkalinity, alkalinity balance, total CO_2-C, substrate derived CO_2-C, % substrate C decomposed, % CO_2-C derived from substrate and % CO_2-C derived from soil using GENSTAT 11th Edition (VSN International, Hemel Hempstead, England). For extractable organic C, substrate-derived extractable organic C, microbial biomass C and substrate derived microbial biomass C, a 2-way ANOVA was used with soil and compound as the main factors. For all significant main effects and interactions a post-hoc Tukey honest significant difference (HSD) test was used to determine significant differences ($P \leq 0.05$) between means.

3. Results

3.1 Soil pH

Glucose addition significantly ($P < 0.05$) increased soil pH at 3 d in the Kandosol topsoil and subsoil, and the Tenosol topsoil whereas pH remained constant in the Podosol topsoil and subsoil, and the Tenosol subsoil (Figure 1). In subsequent incubation, the soil pH then decreased with the magnitude of pH decrease being greatest in the Podosol. At 15 d after glucose addition, the pH of Kandosol topsoil and subsoil and Tenosol topsoil was greater than the control while the pH of other soils was lower than the control. Changes in pH followed a similar pattern in topsoils and subsoils.

The effect of malic acid on soil pH change depended on soil type and soil layer (Figure 1). The addition of malic acid to the Kandosol topsoil and subsoil, and the Tenosol topsoil increased pH at 3 d. However, soil pH decreased at 3 d after malic acid addition in the Podosol topsoil and subsoil, and the Tenosol subsoil. Soil pH of the malic acid treatments decreased at 15 d compared to 3 d in the Kandosol topsoil and subsoil, and the Tenosol topsoil. The pH of Podosol amended with malic acid was increased during the incubation period, but was lower than that of the control. The Tenosol topsoil and subsoil, and Kandosol topsoil and subsoil with malic acid had a higher pH than the control soil at 15 d. However, the Tenosol subsoil had the greatest increase in soil pH over the control soil at 15 d.

3.2 Nitrogen transformation

Concentrations of NH_4^+ were low in the non-amendment controls ranging from 0.8 to 5.6 mg kg^{-1} soil, and were stable during the incubation period for all soil types and layers except the Tenosol and Podosol topsoil (Figure 2). Glucose addition decreased NH_4^+ concentration at 3 d for all soils except the Tenosol subsoil. However, NH_4^+ concentration remained unchanged over time after glucose addition except for the Kandosol topsoil and Podosol subsoil where NH_4^+ concentration was higher at 15 d than at 3 d. The addition of malic acid significantly ($P \leq 0.05$) increased the NH_4^+ concentration in the Kandosol topsoil, and the Tenosol topsoil and subsoil at 3 d. In the Tenosol topsoil, the NH_4^+ concentration was approximately 4 times greater than the control soil. However, the NH_4^+ concentration was decreased in the Kandosol topsoil, and the Tenosol topsoil and subsoil at 15 d. In the Podosol, NH_4^+ concentration was similar to the control soil.

At 3 d, the concentration of NO_3^- in the non-amended control was highest (51 mg kg^{-1} soil) in the Kandosol, followed by the Tenosol and Podosol (Figure 3). Glucose addition decreased NO_3^- concentration to the detection limit in the Kandosol and in the Tenosol. With incubation time, glucose amendment significantly ($P \leq 0.05$) increased NO_3^- concentration in the Kandosol topsoil and Tenosol topsoil but did not in the respective subsoils. In the Podosol similar NO_3^- dynamics occurred between the topsoil and the subsoil. Similar to glucose, the addition of malic acid also decreased the NO_3^- concentration in the Kandosol and Tenosol at 3 d. However, the magnitude of decrease was lower in the malic-acid-amended soils compared to the glucose-amended soil at 3 d. In the Podosol, the NO_3^- concentration was very low for all treatments during the incubation period.

3.3. Alkalinity production

Net alkalinity production, alkalinity generated through the N cycle and alkalinity balance after addition of glucose and malic acid to soils at 3 d and 15 d are presented in Table 2. After glucose amendment, a net increase in alkalinity occurred in the Kandosol and Tenosol at 3 d but net acidification occurred in the Podosol. The changes in alkalinity between 3 to 15 d were relatively small when glucose was added. Alkalinity production was greater for malic acid than glucose for all soils. The greatest alkalinity production (8.5 mmol kg^{-1} soil) was observed in the Kandosol topsoil with malic acid at 3 d. More than half of the alkalinity was balanced via the processes that were not attributed to added C or the N cycle (Table 2). However, with malic acid, net alkalinity production significantly ($P \leq 0.05$) decreased between Days 3 and 15 except for the Podosol. In general, the net alkalinity/acidity production was smaller in the subsoil compared to the topsoil.

3.4 Carbon mineralisation

As expected, C amendments increased the cumulative soil respiration detected as CO_2-C (Table 3). However, mineralisation patterns following amendment depended on the C source and soil type. For both glucose-amended and malic-acid-amended soils, greater amounts of added C were mineralised during the first 3 d of incubation than the rest of the incubation time. Further, greater amounts of C were mineralised in the malic-acid-amended soil (up to 1415 µg CO_2-C g^{-1} soil) compared to the glucose-amended soil (up to 1102 µg CO_2-C g^{-1} soil) during the first 3 d. At 15 d the cumulative CO_2-C exceeded the amount of added C (1 g C kg^{-1} soil) in the Kandosol and Tenosol. However, in the Podosol, cumulative CO_2-C was far below the amount of added C, indicating low microbial activity and that most of the added C remained in the soil and was not mineralised.

A higher proportion of malic acid-C was decomposed than the glucose-C as indicated by a higher proportion of substrate-derived CO_2-C (Table 3). In the Kandosol topsoil, % decomposition of glucose and malic acid was 42% and 62%, respectively by 3 d. Decomposition was the lowest in the Podosol than any other soil. In the Podosol, only 27% of malic acid was mineralised by the end of the incubation, whereas 73% was mineralised in the Kandosol for the same treatment. A higher proportion of CO_2-C was derived from substrate during the first 3 d of incubation. For example, in the Kandosol topsoil 40% CO_2-C was derived from added glucose during the first 3 d whereas this decreased to 22% during 3 to 15 d. This indicates that added C became depleted towards the end of incubation study and a greater proportion of the C mineralised was from the soil.

3.5 Extractable organic C

At the end of the 15-d incubation, K_2SO_4-extractable organic C was only slightly higher in the substrate-amended than non-amended soils in the Kandosol and Tenosol, indicating that most of the added C (1 g C kg^{-1} soil) was mineralised. However, the greatest amount of extractable organic C was present in the Podosol, indicating that mineralisation was restricted in this soil. Extractable organic C concentration tended to be greater in the glucose- than malic acid-amended soils. Similar amounts of extractable organic C were observed between the topsoil and subsoil (Figure 4).

The concentration of substrate-derived (glucose and malic acid) extractable organic C was very low in the Kandosol and Tenosol, ranging from 4 to 35 µg C g^{-1} soil. The amount of substrate-derived extractable organic C was greatest in the Podosol (376 to 630 µg C g^{-1} soil) and was similar between the glucose and malic acid treatments, and between topsoils and subsoils (Figure 4).

3.6 Microbial biomass C

The addition of C substrate increased total microbial biomass C by the end of 15-d incubation (Figure 5). This microbial biomass C was partly derived from soil organic C and partly from added C (substrate-derived). A greater amount of total microbial biomass C was observed in the Kandosol (106 to 365 µg C g^{-1} soil) than the Tenosol (17 to 227 µg C g^{-1} soil). Total microbial biomass C was greater with glucose-amended soils compared to malic-acid-amended soils. In the Kandosol, total microbial biomass C was greater in the topsoil than the subsoil for both amended and non-amended treatments. In the Tenosol, the amount of microbial biomass C was greater in the topsoil than the subsoil.

Substrate-derived microbial biomass C was greater in the Kandosol than the Tenosol. Moreover, the amount of glucose-derived microbial biomass C was slightly greater than malic-acid-derived microbial biomass C except for the Tenosol topsoil. Further, for both soils, substrate-derived microbial biomass C was greater in the topsoil than the subsoil.

In the Podosol, microbial biomass C in the non-amended control soil was almost at detection limits. Further, with C amendments, no data were obtained for both total microbial biomass C and the substrate-derived microbial biomass C due to the lack of repeatability of the measurement among replicates and the presence of negative values.

Irrespective of soil type, there was a close relationship between alkalinity balance and the amount of CO_2 derived from soil at Day 3 with a slope of the correlation line being 8-fold greater for malic acid than for glucose (Figure 6). Such a correlation was not found at Day 15.

4. Discussion

This study confirmed that carbon priming and alkalinity priming occur in soil as a result of the addition of carbon compounds that are commonly present in decomposing crop residues or root exudates. The magnitude of these processes depended on the type of compound added, content of native organic matter, clay content and clay minerals in soil. In general, mineralisation of added and native soil organic C was greater in soils with higher than lower C content. Alkalinity generation was also greater in soils with a higher than with a lower C content while alkalinity priming, as indicated by alkalinity balance, was positively correlated with decomposition of native organic matter (carbon priming) in soil, particularly when malic acid was added (Figure 6). Furthermore, the majority of the added C was mineralised to CO_2 in soils, resulting in low amounts of substrate-derived extractable organic C and microbial biomass C after the 15-d incubation.

4.1. N cycle alkalinity

The contribution of the N cycle to alkalinity generation varied with soil type and sampling depth. The N cycle contributed to alkalinity generation in the Kandosol and Tenosol but not in the Podosol, and the contribution of this alkalinity was greater in the topsoil than subsoil (Table 4). These findings could result from both ammonification and NO_3^- uptake by microbes. There was a net increase in NH_4^+ concentrations in the malic acid treatment but a net decrease in NO_3^- concentrations in both glucose and malic acid treatments relative to the corresponding controls. Ammonification, an alkalinising process (Bolan et al., 1991), appeared to mainly contribute to the alkalinity increase in the Tenosol and Kandosol topsoil with malic acid

(Figure 2). While nitrification of NH_4^+ to NO_3^- produces two protons (Bolan et al., 1991), NO_3^- immobilization is associated with the release of one OH^- (Gonzalez et al., 2006; Xu et al., 2006). In this study, the net NO_3^- immobilization would partly contribute the increased alkalinity in both topsoil and subsoil of the Tenosol and Kandosol amended with glucose and malic acid (Figures 2 and 3). Moreover, NO_3^- immobilization and ammonification were greater in the topsoils and may be a result of greater soil organic C and N than the equivalent subsoil. In contrast, NO_3^- immobilization and ammonification were limited in the Podosol probably due to the lack of nitrifying and ammonifying microorganisms. Furthermore, the low total N content in the Podosol might also be responsible for limited N transformation. Therefore, no contribution of the N cycle to alkalinity production was observed in the nutrient-poor Podosol (low C and N, and low initial pH).

Malic acid and glucose had different impacts on alkalinity generation via the N cycle. The relative contribution of the N cycle to alkalinity generation was much greater with glucose than malic acid (Table 2). For glucose addition, most of this alkalinity was derived via NO_3^- immobilization by microbes (Figure 3). However, malic acid contributed to alkalinity generation through the ammonification process. These observations can be explained by the following reasons. Glucose decomposition starts with the glycolysis metabolic pathway where one molecule of glucose produces two molecules pyruvate and 2 molecules adenosine triphosphate (ATP). Also under aerobic conditions, pyruvate can be completely oxidised to CO_2 to generate more ATP (Hua and Shimizu, 1999). In addition to energy production, a number of studies have shown that glucose addition to soil enhances microbial growth and NO_3^- uptake (Recous et al., 1990; Zagal and Persson, 1994; Myrold and Posavatz, 2007). Therefore, in this study the added glucose might have been assimilated by microorganisms for cell formation or alternatively used for energy production and release of CO_2. In comparison, malic acid firstly dissociated, acidifying the soil. Subsequent decarboxylation of the malate anion generates alkalinity. In contrast to glucose, malic acid might have been used for energy production, releasing CO_2 rather than incorporation into microbial cells. Alkalinity generation with glucose was mainly derived from the N cycle while malic acid generated alkalinity from both the N cycle (mainly ammonification) and the C cycle. In addition, microbial immobilization of NO_3^- is suppressed by high NH_4^+ concentration (Recous et al., 1990; Myrold and Posavatz, 2007). Therefore, it is consistent that lower NO_3^- uptake with malic acid than glucose addition occurred as ammonification was greater with malic acid addition.

4.2. The fate of added C

The fate of added C differed among soil types. In the Kandosol, 56-73% of added C was mineralised to CO_2-C and 9-20% was incorporated to microbial biomass C by the end of the 15 d incubation, resulting in negligible C remaining in the soil as extractable organic C. The Kandosol was the most favourable soil environment for microorganisms because of its higher pH and organic C content. Biological processes including ammonification, nitrification, NO_3^- immobilization and decarboxylation were all greater in the Kandosol compared with other soils. Moreover, a greater portion of the added C was mineralised to CO_2-C, indicating that a greater amount of the added C was used for energy production and not growth. The fate of added C in the Tenosol was similar to the Kandosol for similar reasons as outlined above.

Mineralisation of added C was low in the Podosol. Only 21-27% of C added to the Podosol was mineralised to CO_2 and most remained in the soil solution as extractable organic C (Table 3 and Figure 4). The low mineralisation of added C in the Podosol is most likely due to the low C and N content and low initial pH (4.5) that limit microbial activity.

In the Podosol, the microbial biomass C data were not considered due to the lack of repeatability of the measurement among replicates and the presence of negative values. The following reasons could account for this. Firstly, microbial biomass C in the non-amended Podosol soil was very low. Since only a small portion of added C was decomposed, extractable organic C levels were very high following amendment. Microbial biomass is calculated as the difference between these two values and the variability in extractable organic C in the amended soils was greater than the additional C released following fumigation. Secondly, at low pH, 0.5 M K_2SO_4 might act as a flocculant and C solubilised via fumigation might adsorb to flocculated soil colloids, hence underestimating microbial C (Haney et al., 1999). Moreover, Pietri and Brookes (2008) also reported that microbial biomass C determination at low pH (3.81) might have a negative value using the fumigation-extraction method.

A greater amount of CO_2-C was released from added substrate and from native soil organic matter in the topsoil compared to the subsoil (Table 3). These findings could be explained by the following reasons. Firstly, as expected C content was greater in the topsoils than subsoils (Table 1) since the topsoils receive plant organic C input. Secondly, C in the topsoil is likely to have a greater bioavailability for microorganisms (Kemmitt et al., 2006) and be composed of less recalcitrant materials (Hamer and Marschner, 2005). Thirdly, total microbial biomass C was greater in the topsoil than the subsoil (Figure 5) which is consistent with other studies (e.g. Salome et al., 2010). Salome et al. (2010) have suggested that microbial communities in the topsoil can use simple substrates more rapidly than those in the subsoil.

In this study, CO_2-C priming occurred in the Kandosol and Tenosol, but not in the Podosol. Hamer and Marschner (2005) reported that CO_2 was released from the labile soil organic C pool and not from the

stable C pool. A greater amount of CO_2-C derived from soil than added C in the Kandosol and the Tenosol, indicates that soil organic C pool was more labile in these soils. On the contrary, organic C in the Podosol might be more resistant to biodegradation resulting in lower mineralisation. The Podosol used in this study was a virgin soil (with native vegetation) and recent addition of organic matter from plants would have been low and therefore any C in this soil is likely to be more stable against decomposition than the other soils. Therefore, even though the Podosol topsoil had the greater organic C than the Tenosol subsoil, soil-derived CO_2-C was lower in the Podosol topsoil.

The decomposition of malic acid was greater than glucose as indicated by total CO_2-C release (Tables 2 and 3). A lag phase in malic acid decomposition observed in our other study (unpublished) indicates that this compound is degraded by specialised microorganisms. Malic acid might first activate specific dormant microbial species, which then grew and became dominant, ultimately resulting in greater decomposition than glucose substrate. In comparison, microorganisms may use added glucose for both microbial growth and energy as discussed earlier.

4.3. Alkalinity priming and the C cycle

Alkalinity priming and CO_2 priming differ between soil types. Alkalinity priming occurred in the Kandosol and Tenosol, but not in the Podosol. Similarly, total C mineralisation (C mineralisation from substrate and from native soil organic C) was much greater in the Kandosol and Tenosol, than the Podosol (Table 3). These findings could be explained by the native organic matter content of these soils. In the Kandosol and Tenosol the decomposition of soil organic matter induced by added substrate might result in release of organic compounds from native soil organic matter which contributes to the generation of alkalinity. For example, carboxylic acids or anions generated via the decomposition of native soil organic matter might undergo decarboxylation generating alkalinity and CO_2 release. In this study, alkalinity not derived from either added substrate C or the N cycle was termed alkalinity balance (Table 2) and is alkalinity derived via priming of native soil organic matter.

Soil organic C had more influence on alkalinity priming than initial soil pH. Alkalinity priming occurred in the Kandosol and Tenosol with greater CO_2-C release from native soil organic matter but not occurred in the Podosol. Although the pH of Tenosol topsoil (4.7) was much lower than that of Kandosol topsoil (5.8), the alkalinity priming for both soils was greater especially for net alkalinity production and alkalinity balance in the malic acid than glucose treatment (Table 2). The greater alkalinity priming of the topsoils of the Kandosol and the Tenosol had resulted from greater organic content of these soils. However, the Podosol (low initial pH with low organic C) restricts microbial respiration and decomposition, and no alkalinity priming was observed at the end of incubation. This indicates that soil organic C in the Podosol is recalcitrant. Organic C can be protected against decomposition by interaction with the mineral particles in soil (Baldock and Skjemstad, 2000; von Lutzow et al., 2006) and this is generally exacerbated at low initial soil pH (von Lutzow et al., 2006) and low organic C soil. Alkalinity priming and CO_2 priming also differ between added C compounds. Greater alkalinity priming was observed for malic acid than glucose. The majority of the alkalinity generated after malic acid addition was derived from the C cycle, and for glucose addition this was predominantly derived from the N cycle as discussed earlier. Glucose is a readily mineralised by microorganisms and is used for growth and energy depending on their energy state and growth stage (Raubuch et al., 2010). Therefore, microorganisms used the glucose C and soil NO_3^- for cell formation and/or energy production, and consequently NO_3^- uptake led to increased soil pH. However, for malic acid alkalinity priming occurred via both C (decarboxylation) and N (ammonification) cycles as discussed earlier. Furthermore, in the malic acid treatment, the great decomposition could solubilise additional soil organic C which in turn contributes to alkalinity production.

5. Conclusions

This study confirmed that alkalinity priming occurred with concurrent CO_2 priming. Alkalinity priming occurred from the decomposition of labile soil organic C compounds. The majority of the added C was mineralised to CO_2 in the soils with high C content, resulting in low amounts of substrate-derived extractable organic C and microbial biomass C in soil. However, in the soils with low C and low initial pH, most of the added C remained in the soil as extractable organic C rather than being mineralised. Therefore, further organic C inputs in these soils lead to an increase soil C storage. Malic acid resulted in greater alkalinity priming than glucose. With glucose addition, alkalinity was derived via N cycle, while malic-acid-induced alkalinity via both C and N cycles and resulted in greater alkalinity priming. Microbial NO_3^- immobilisation appeared to be the mechanism for alkalinity priming following glucose addition. For malic-acid-treated soils alkalinity generation occurred via decarboxylation of added substrate, ammonification and decomposition of native soil organic matter. Therefore, simple sugars such as glucose in plant residues would not result in the same magnitude of soil pH increases than organic residues dominated by malic acid.

Acknowledgements

This research was supported under Australian Research Council's *Discovery Projects* funding scheme (DP0877882 and DP120104100).

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Table 1: Selected physiochemical properties of the soils used in this study

Soil Classification[a]	Soil Classification[b]	Soil layer	Depth (cm)	pH[c]	pHBC[d]	Sand (%)	Silt (%)	Clay (%)	Total organic C (g kg^{-1})	Total N (g kg^{-1})
Kandosol	Luvisol	Top	0-10	5.8	11.1	45.2	18.7	36.1	12.41	1.15
		Sub	10-15	5.4	6.3	35.3	18.6	46.1	8.94	0.83
Podosol	Podzol	Top	10-30	4.4	6.1	97.1	1.3	1.6	2.89	0.11
		Sub	30-50	4.5	3.2	97.3	1.1	1.6	1.49	0.05
Tensosol	Cambisol	Top	0-10	4.7	5.9	73.4	8.2	18.4	10.89	0.93
		Sub	10-30	6.1	2.9	81.0	5.8	13.2	1.90	0.21

[a] The Australian Soil Classification (Isbell, 2002)
[b] World Reference Base for Soil Resources (FAO/ISRIC/ISSS, 2006)
[c] 0.01 M $CaCl_2$ (1:5 soil:solution)
[d] mmol H^+/OH^- pH^{-1} kg^{-1} soil

Table 2: Net alkalinity production, alkalinity generated by the N cycle and alkalinity balance ($mmol_c\ kg^{-1}$ soil) during 0-3 and 3-15 d of incubation.

Soil	Compound	[a] Net alkalinity production		[b] N-Cycle alkalinity		[c] Alkalinity balance	
		0-3 d	3-15 d	0-3 d	3-15 d	0-3 d	3-15 d
Kandosol topsoil	glucose	3.94	3.49	3.43	3.94	0.51	-0.45
	malic acid	8.50	2.36	2.78	1.67	5.72	0.69
Kandosol subsoil	glucose	2.36	1.19	1.51	1.55	0.85	-0.36
	malic acid	4.64	1.83	1.61	1.32	3.03	0.51
LSD (P = 0.05)		0.33		0.21		0.42	
Podosol topsoil	glucose	-0.36	-2.53	-0.04	-0.03	-0.32	-2.50
	malic acid	-5.70	-3.19	-0.03	-0.05	-5.67	-3.15
Podosol subsoil	glucose	-0.11	-1.62	-0.03	0.00	-0.07	-1.63
	malic acid	-4.01	-3.35	0.00	-0.05	-4.01	-3.30
LSD (P = 0.05)		0.23		0.21		0.23	
Tenosol topsoil	glucose	1.94	1.45	0.73	3.20	1.21	-1.75
	malic acid	8.07	4.93	1.17	3.79	6.90	1.14
Tenosol subsoil	glucose	0.28	-0.09	0.56	1.10	-0.28	-1.19
	malic acid	-0.99	2.83	0.76	1.08	-1.75	1.75
LSD (P = 0.05)		0.34		0.81		0.94	

[a] Net alkalinity production = (pH of the amended soil) – (pH of the non-amended) × pHBC
[b] N-cycle alkalinity = (ΔNH_4^+ - ΔNO_3^-)
$\Delta\ NH_4^+$ = (NH_4^+ in amended soil - NH_4^+ in non-amended soil)
$\Delta\ NO_3^-$ = (NO_3^- in amended soil - NO_3^- in non-amended soil)
[c] Alkalinity balance = Net alkalinity production - N cycle alkalinity
Negative values indicate acidity production

Table 3: Total CO_2-C, substrate derived CO_2-C, and soil derived CO_2-C during 0-3 and 3-15 d of incubation.

Soil	Compound	Total CO_2-C		Substrate derived CO_2-C				Soil derived CO_2-C			
		µg CO_2-C g^{-1} soil		µg CO_2-C g^{-1} soil		%		µg CO_2-C $^{g-1}$ soil		%	
		0-3 d	3-15 d	0-3 d	3-15 d	0-3 d	3-15 d	0-3 d	3-15 d	0-3 d	3-15 d
Kandosol	control	60	102	*	*	*	*	60	102	100	100
topsoil	glucose	1055	621	422	134	40	22	633	487	60	78
	malic acid	1415	488	624	101	44	21	790	387	56	79
Kandosol	control	140	323	*	*	*	*	140	323	100	100
subsoil	glucose	977	526	408	180	42	34	569	346	58	66
	malic acid	1255	425	592	128	47	30	663	297	53	70
LSD (P = 0.05)		37.3		12.3				32.2			
Podosol	control	102	245	*	*	*	*	102	245	100	100
topsoil	glucose	141	322	120	109	85	34	21	213	15	66
	malic acid	90	501	21	245	23	49	69	256	77	51
Podosol	control	69	75	*	*	*	*	69	75	100	100
subsoil	glucose	142	211	134	80	94	38	8	130	6	62
	malic acid	69	348	6	230	9	66	63	118	91	34
LSD (P = 0.05)		56.3		12.6				32.5			
Tenosol	control	29	21	*	*	*	*	29	21	100	100
topsoil	glucose	1102	491	495	128	45	26	607	363	55	74
	malic acid	1399	457	655	81	47	18	744	375	53	82
Tenosol	control	32	7	*	*	*	*	32	7	100	100
subsoil	glucose	530	872	327	308	62	35	202	564	38	65
	malic acid	737	738	444	246	60	33	294	492	40	67
LSD (P = 0.05)		53.7		13.3				56.1			

% CO_2-C derived from substrate = (Substrate derived CO_2-C/total treatment derived CO_2-C) x 100

% CO_2-C from soil = (Soil derived CO_2-C)/total treatment derived CO2-C) x 100

Substrate-derived biomass C was the difference in ^{14}C-CO_2 between fumigated and non-fumigated soil samples.

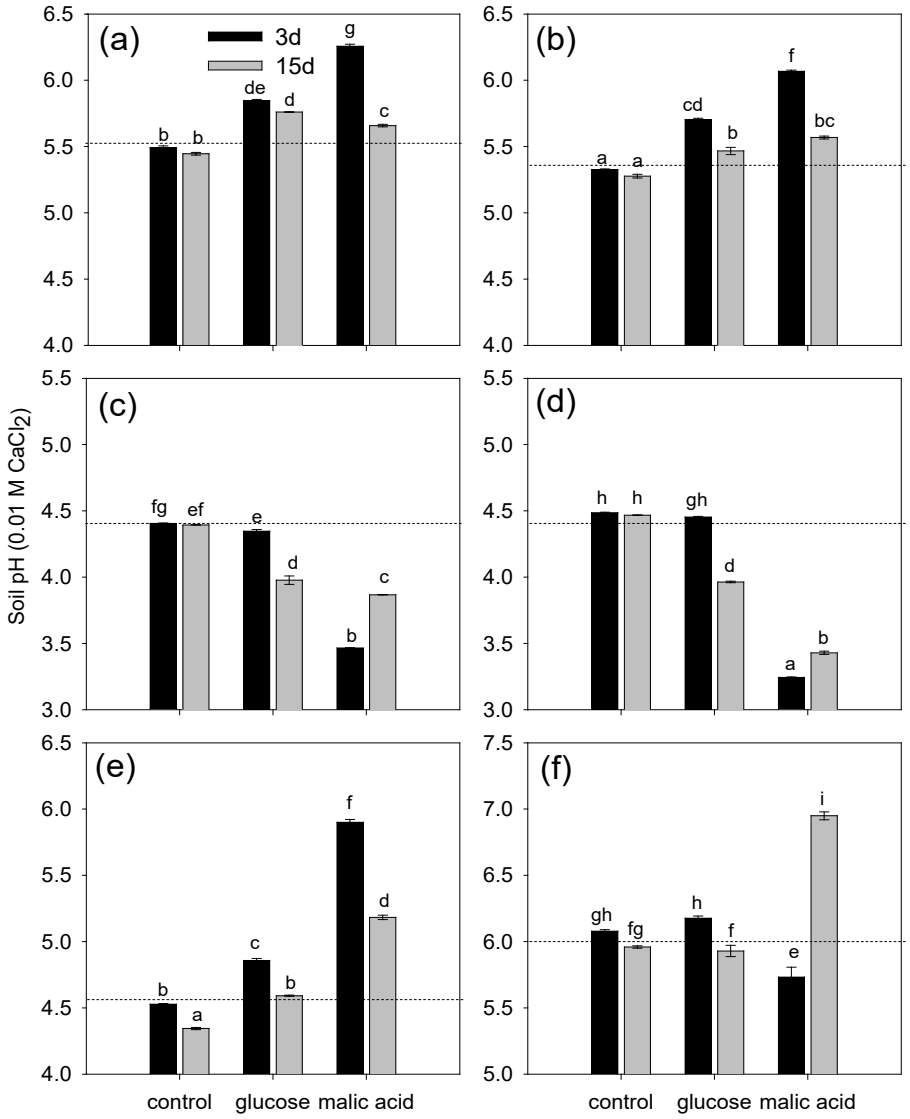

Figure 1. Soil pH at 3 d and 15 d in the Kandosol (a) topsoil and (b) subsoil, Podosol (c) topsoil and (d) subsoil, Tenosol (e) topsoil and (f) subsoil with malic acid and glucose and non-amended control. Bars represent the standard error of the mean (n = 3). Dotted lines indicate the initial soil pH. Note the different scales on the Y axis. Means with the same letter are not significantly ($P \leq 0.05$) different using a post-hoc Tukey HSD test.

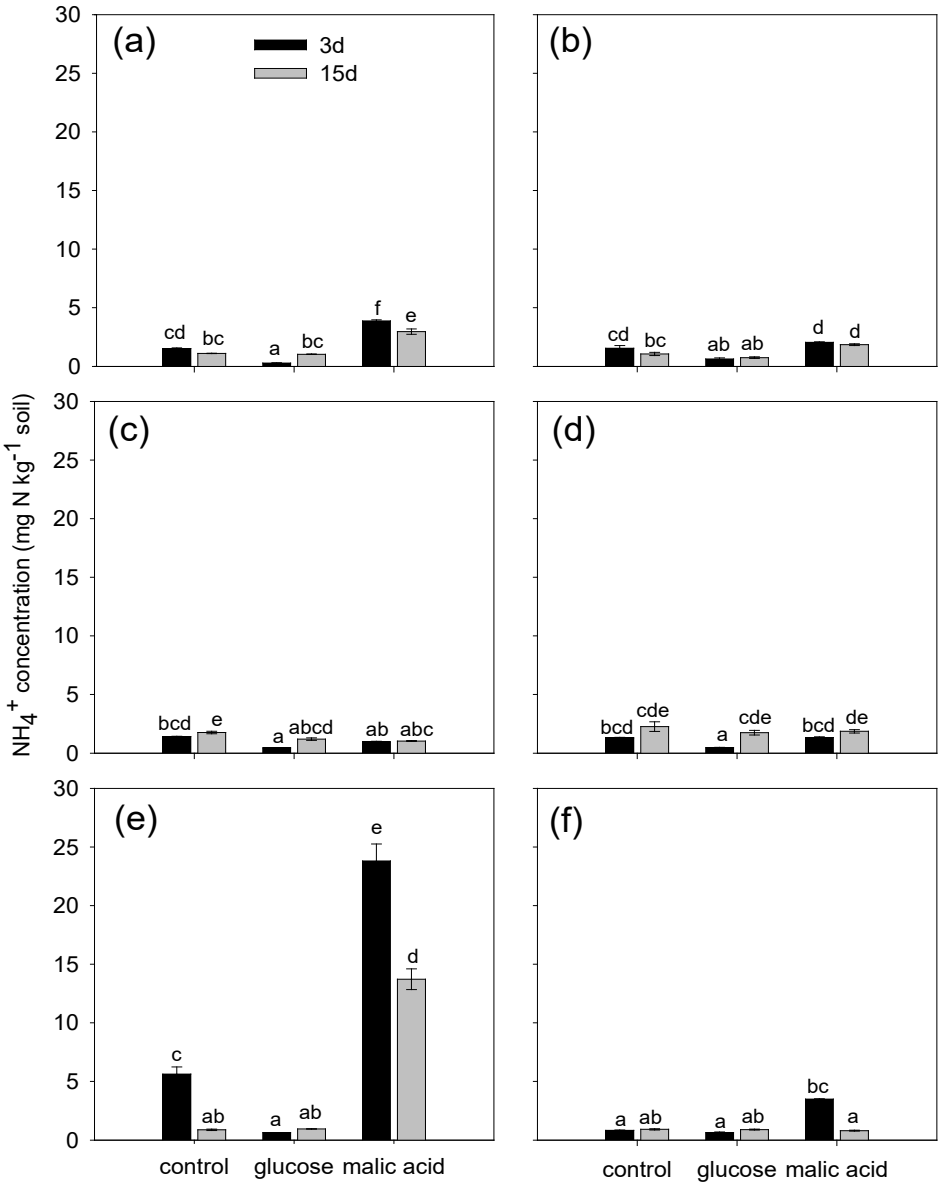

Figure 2. Ammonium (NH_4^+) concentration at 3 d and 15 d in the Kandosol (a) topsoil and (b) subsoil, Podosol (c) topsoil and (d) subsoil, Tenosol (e) topsoil and (f) subsoil with glucose, malic acid and non-amended control. Bars represent the standard error of the mean (n = 3). Means with the same letter are not significantly ($P \leq 0.05$) different using a post-hoc Tukey HSD tests.

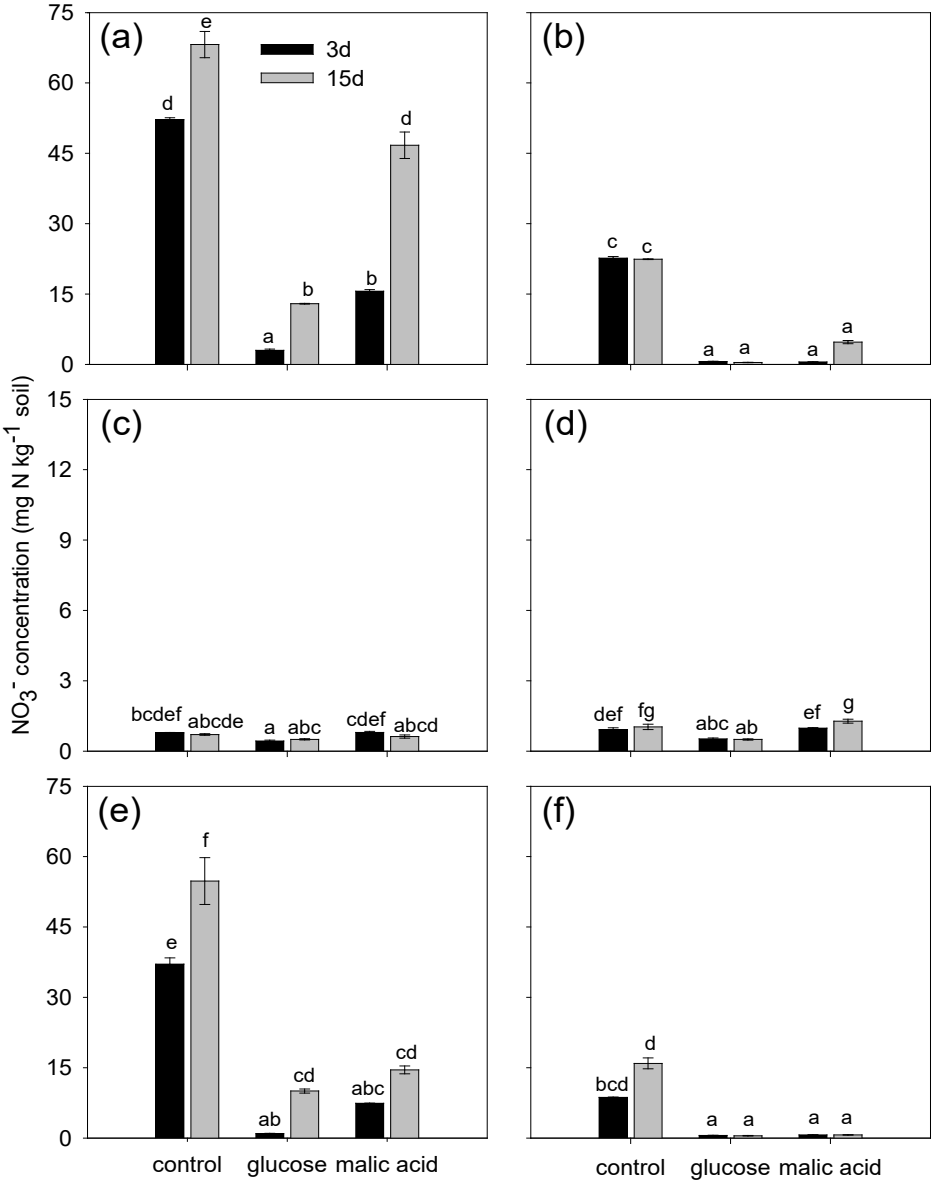

Figure 3. Nitrate (NO_3^-) concentration at 3 d and 15 d in the Kandosol (a) topsoil and (b) subsoil, Podosol (c) topsoil and (d) subsoil, Tenosol (e) topsoil and (f) subsoil with glucose, malic acid and non-amended control. Bars represent the standard error of the mean (n = 3). Note the different scales on the Y axis. Means with the same letter are not significantly ($P \leq 0.05$) different using a post-hoc Tukey HSD tests.

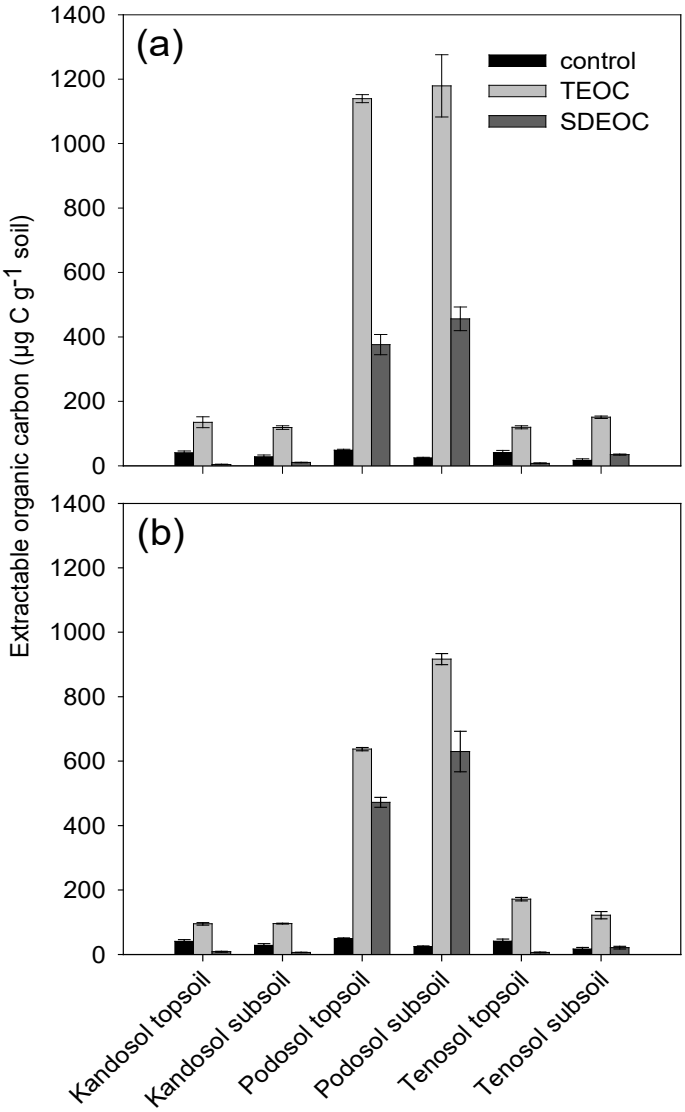

Figure 4. Extractable organic carbon at 15 d in soils with (a) glucose and (b) malic acid. TEOC and SDEOC represent total extractable organic carbon and extractable organic carbon derived from added substrate, respectively. Bars represent the standard error of the mean (n = 3).

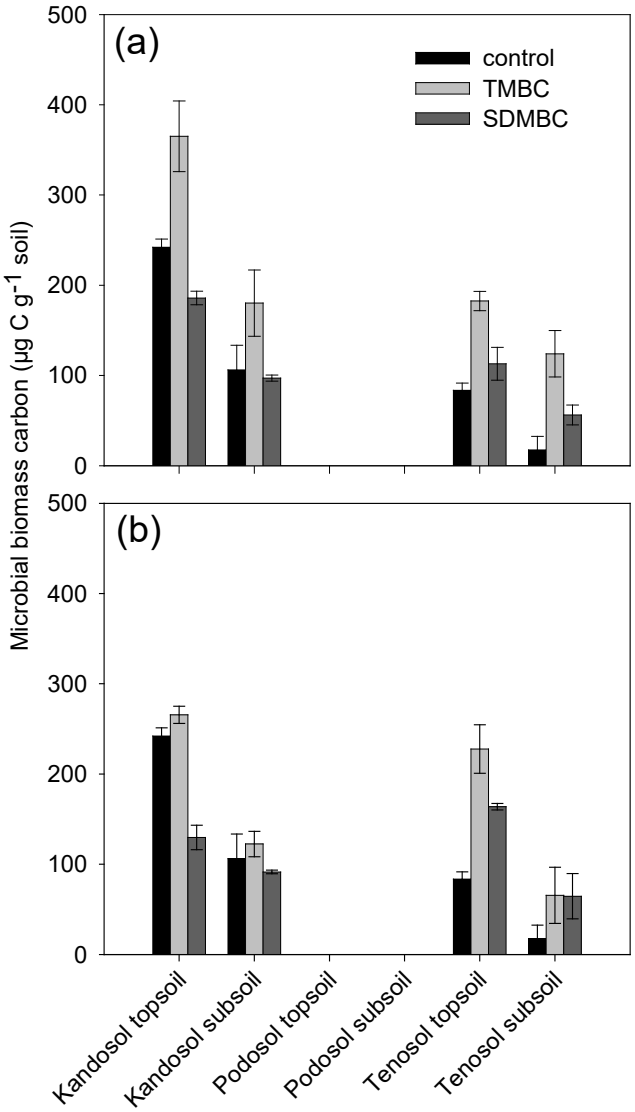

Figure 5. Microbial biomass carbon at 15 d in soils with (a) glucose and (b) malic acid. TMBC and SDMBC represent total microbial biomass carbon and substrate derived microbial biomass carbon, respectively. Bars represent the standard error of the mean (n = 3). Asterisk (*) indicate no data.

SDMBC was the difference in ^{14}C activity between fumigated and non-fumigated soil samples. TMBC was calculated as the difference in organic C extracted from fumigated and from non-fumigated soils, dividing a factor of 0.37.

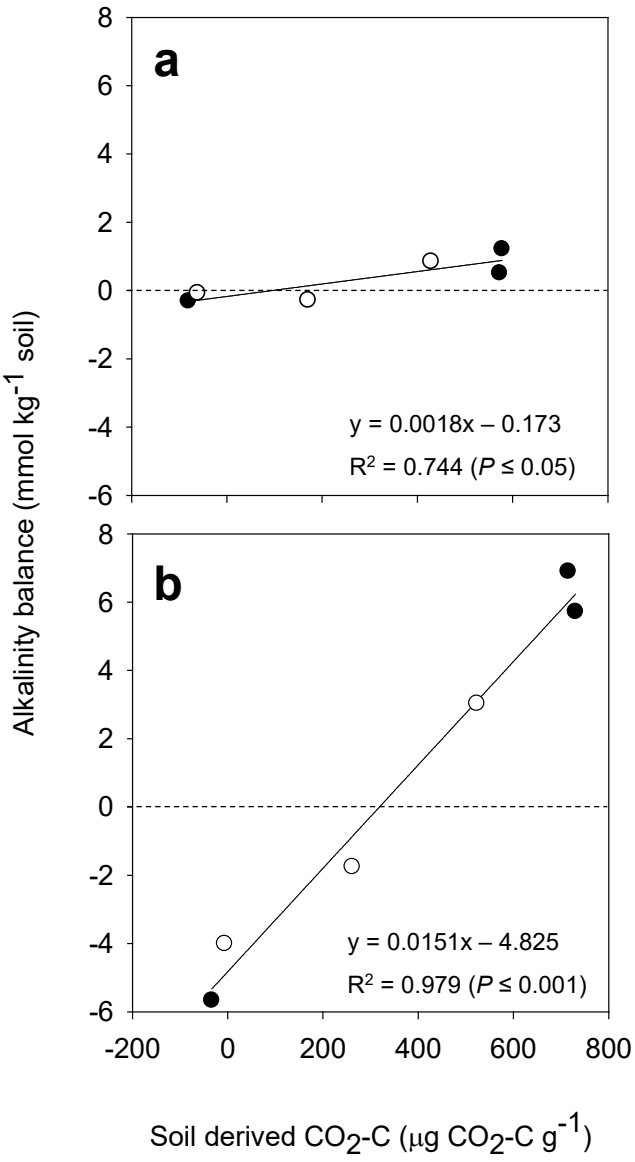

Figure 6. Correlation between soil derived CO_2-C (g CO_2-C g^{-1} soil) and alkalinity balance (mmol kg^{-1} soil) at 3 d in topsoils (black circles) and subsoils (white circles) amended with (a) glucose and (b) malic acid.