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Model organic compounds differ in priming effects on alkalinity release in soils through carbon and nitrogen mineralisation

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ABSTRACT

The influence of organic matter and its cycling on soil pH change is still unclear. This study investigated the effect of organic compounds on carbon and nitrogen dynamics and their relationship with pH changes in two soils differing in initial soil pH (Podosol of pH 4.5 and Tenosol of pH 6.2). Seven organic compounds representing common compounds in decomposing plant residues or root exudates were added to the soils and incubated for 60 d. The largest cumulative soil respiration occurred when glucose, malic acid and citric acid were added. In addition, the Tenosol had the greater respiration compared to the Podosol. The addition of organic acids (acetic, malic, citric, ferulic and benzoic acid) instantly decreases soil pH due to the dissociation of H^+ from the acids. The pH of both soils was then restored over time, which was positively correlated with decomposition % of these compounds. The pH of the Tenosol amended with all the organic acids and of the Podosol with malic acid exceeded that of the control, and net alkalization occurred, with the degree of alkalization being greater with malic and citric acid. Adding organic acids to the Tenosol generally increased NH_4 concentrations but decreased NO_3 concentrations. The addition of glucose decreased pH in Podosol but slightly increased it in the Tenosol. The addition of glucosamine hydrochloride decreased pH due to significant nitrification. The results suggest that the addition of organic acids stimulates microbial NO_3 uptake, and ammonification and decomposition of indigenous soil organic matter, resulting in a priming effect on alkalinity release, and that the degree of the priming effect is influenced by type of organic acid and initial soil pH.

Key words: acidification, alkalinity priming, soil respiration, decomposition, nitrogen transformation, mineralisation, indigenous organic matter.

1. Introduction

Soil acidification including subsoil acidification is a widespread problem in agricultural soils around the World (Vonuexkull and Mutert, 1995; Ma and Ryan, 2010). The removal of agricultural products containing ash alkalinity (Marschner and Noble, 2000) and nitrogen derived from ammonium-based chemical fertilisers will result in a net acidification of soil (Wang et al., 2009; Tang et al., 2011). Ensuring that unharvested plant residues are retained and added back to the soil will reduce the magnitude of this acidification. The ash alkalinity of the plant residues, and the mineralisation of any residual nitrogen they contain, can neutralise acidity within the soil layer to which they are added. Ash alkalinity is positively correlated with the amount of excess cations present in the residues (Noble et al., 1996) and therefore is also related to the chemical composition of the residues (Noble et al., 1996; Tang et al., 1999; Tang and Yu, 1999; Xu et al., 2006a).

Differences in alkalinity between plant residues can be attributed to differences in biochemical composition (Yan and Schubert, 2000; Xu et al., 2006a). Principal organic compounds in plant tissue include carbohydrates, nitrogen compounds, lipids and lignin (Kogel-Knabner, 2002). Plants also release a range of low-molecular-weight carboxylic acids/anions including malic, citric, oxalic, acetic, succinic, benzoic, ferulic, and vanillic acids into the rhizosphere (Shen et al., 1996; Jones, 1998; Strobel, 2001; Xu et al., 2006a). The amount and biochemical composition of plant residues and the composition of elements and biochemicals released during plant residue decomposition will determine the impact of plant residues on soil pH change.

Soil pH change induced by the addition of organic residues to soil occurs primarily through H^+ association/disassociation reactions and carbon (C) and nitrogen (N) cycle processes (Xu et al., 2006b). Association/dissociation reactions with H^+ occur more rapidly than C and N cycling processes and may lead to an immediate alteration of soil pH upon addition of plant residues. Organic compounds in plant residues contain functional groups which may be a source or a sink for H^+ (Brady and Weil, 2002). The magnitude of the pH change derived from H^+ association/dissociation reactions depends on the acid dissociation constants (pKa values) of organic functional groups within the plant residues and the initial soil pH (Ritchie and Dolling, 1985; Tang et al., 1999; Xu et al., 2006b; Rukshana et al., 2011). Where the pKa values of the functional groups are higher than the soil pH, a net association of H^+ with the functional groups will occur resulting in an increase in soil pH. Whether this increase can be detected will depend on the concentration of functional groups present on the plant residues, the amount of residue added and the buffering capacity of the soil. Generally plant material contains an excess of cations over inorganic anions and the charge balance is maintained by synthesis of organic anions (Haynes and Mokolobate, 2001; Xu et al., 2006b). However, most of the alkalinity produced (pH increase) after organic matter is added to soil is associated with the decarboxylation process which consumes H^+ during microbial decomposition of organic anions (Mengel, 1994; Yan et al., 1996; Tang et al., 1999; Marschner and Noble, 2000).

Nitrogen transformations during decomposition can also influence soil pH change. Ammonification of organic N produces OH^- which increases soil pH (alkalisation) (Bolan et al., 1991; Haynes and Mokolobate, 2001; Xu et al., 2006b). Nitrification of ammonium to nitrate releases 2 moles of H^+ per mole of N transformed, which will decrease soil pH (acidification) (Haynes and Mokolobate, 2001). Thus, the combined effect of ammonification of organic N and its subsequent nitrification generates one mole of H^+ per mole of N transformed (Bolan et al., 1991;

Haynes and Mokolobate, 2001). Subsequent uptake of nitrate by plants or soil microbes releases one mole of OH^- per mole of N uptake which balances the net result of mineralisation and nitrification. In a system where no ammonium fertiliser N is added and no losses of N occur, no net change in acidity/alkalinity will occur during N cycling. The addition of ammonium-based fertilisers in agricultural systems and the subsequent uptake and removal of added N in agricultural products or losses due to conversion and leaching or denitrification of nitrate will result in a net acidification because a portion of the N cannot move through the full cycle of N transformations. If nitrate-based fertilisers are used or soil conditions do not favour or delay nitrification of mineralised N, an increase in soil pH will result (Haynes and Swift, 1989; Haynes and Mokolobate, 2001; Tang et al., 2011).

A number of studies have examined the effect of plant residues on soil pH, and the presence of low-molecular-weight carboxylic acids in soil and plants, but few have investigated the influence of specific compounds present within plant materials, and the contribution of their functional groups and pKa to soil pH change. In our previous study (Rukshana et al., 2011), we observed that acidic functional groups immediately decreased soil pH and the magnitude of the decrease depended on the pKa of the acids, the rate of compound addition, the initial soil pH and soil pH buffer capacity. Furthermore, amine and hydroxyl functional groups had no immediate effect on pH. However, the study focused on the contribution of organic compounds on chemical processes that can change in soil pH.

Several studies have shown that the addition of organic substrates to soil can stimulate the mineralisation of native soil organic matter with extra CO_2 release (Hamer and Marschner, 2005; Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010). Enhanced cycling of native organic C and N in response to organic substrate addition also has the potential to alter soil pH through decarboxylation, association/dissociation of H^+ and N transformations. However, the impact of stimulating soil organic matter mineralisation on acid/alkalinity release by organic substrates has not yet been examined.

The experiment described in this paper aimed to investigate the effect of model organic compounds on biochemical processes as well as the contribution of C and N dynamics to change in soil alkalinity during a 60-d incubation period. Seven major organic compounds commonly found in decomposing plant residues and in the rhizosphere were added at 0.5 g C kg^{-1} to two soils of initial pH 4.5 and 6.2. We hypothesized that 1) soil pH change after organic compound addition would be related to the contribution of chemical functional groups to biochemical processes, 2) alkalinity generated by organic acids would be related to their decomposition (CO_2 release) and 3) the net change in alkalinity results from the combined effects of the C and N cycles.

2. Materials and methods

2.1 Soils and C compounds

Composite samples of soil were collected from the 10-30 cm layers (A2) of a Podosol and a Tenosol (Isbell, 2002) (Podzol and Cambisol (FAO/ISRIC/ISSS, 2006), respectively located near Frankston (38°14'S, 145°22'E) and Shepparton (36°28'S, 145°36'E) Victoria, Australia. Soils were sieved (<2 mm), thoroughly mixed and air-dried for subsequent analysis and the incubation study. The initial pH values of the Podosol and Tenosol were 4.5 and 6.2 (0.01 M $CaCl_2$), respectively. The Podosol had 94.8% sand, 0.2% silt, 5.0% clay, 2.89 g C kg^{-1} and 0.11 g N kg^{-1}, and the Tenosol had 88.1% sand, 3.9% silt, 8.0% clay, 1.90 g C kg^{-1} and 0.21 g N kg^{-1}. Detailed soil physical and chemical properties are shown in Rukshana et al. (2011).

Five low-molecular-weight carboxylic acids and two carbohydrates were selected and added to the soils as these are commonly found in decomposing plant residues and in the rhizosphere, and differ in the type and number of chemical functional groups. Acetic acid, malic acid, citric acid, benzoic acid and ferulic acid contain acidic carboxyl (R-COOH) functional groups. The number of carboxyl groups and pK_a of these organic acids are: acetic acid (pK_{a1} = 4.76); malic acid (pK_{a1} = 3.4, pK_{a2} = 5.13); citric acid (pK_{a1} = 3.15, pK_{a2} = 4.77, pK_{a3} = 6.40), benzoic acid (pK_{a1} = 4.21) and ferulic acid (pK_{a1} = 4.52). Glucose is a simple sugar with a neutral hydroxyl groups (R-OH) and glucosamine hydrochloride is a basic amino sugar with a single amine group ($R\text{-}NH_2$). Among these compounds, benzoic and ferulic acid are aromatic while the rest are aliphatic in nature.

2.2 Soil incubation

Air-dried soil was pre-incubated at 60% field capacity in the dark at 25 °C for 10 d. At the end of the pre-incubation period, stock solutions of acetic acid (62.5 g L^{-1}, Rhone-Poulenc), malic acid (69.8 g L^{-1}; Sigma-Aldrich), citric acid (72.9 g L^{-1}, BDH Chemicals), ferulic acid (40.4 g L^{-1}, MP Biomedicals), glucosamine hydrochloride (74.8 g L^{-1}, Sigma-Aldrich) and glucose (62.5 g L^{-1}, Ajax Finechem) were added to each soil at 0.5 mg C g^{-1} soil. Benzoic acid (Ajax Finechem) was added at the same C rate to the two soils but as a powder due to its low solubility in water. Soils without any added compound were included as control. Soils were mixed thoroughly and 3 replicates of 25 g soil were packed into individual PVC cores (3.7 cm × 5 cm, IPLEX Pipelines) fitted at one end with nylon mesh (0.75 µm, Australian Filter Specialists) with a bulk density of 1.4 g cm^{-3}. Cores were placed into 1-L glass

incubation jars (Ball® Quart Wide Mouth jars, Jarden Corporation, USA) fitted with gas-tight lids to allow sampling of headspace gases, and containing a small reservoir with 8 ml of water to reduce soil drying and maintain headspace humidity (Butterly et al., 2009). The soils were incubated for 60 d at 25 °C and maintained at 80% of field capacity throughout the incubation period. At 0, 1, 3, 7, 15, 30 and 60 d, a set of cores were destructively sampled for analyses.

2.3 Soil respiration

Soil respiration was determined by quantifying the carbon dioxide (CO_2) concentration within the headspace of incubation jars at 7, 15, 30 and 60 d using an Infra-red Gas Analyser (Servomex 4210 Industrial Gas Analyser, Cowborough, UK). Soil respiration (μg CO_2-C g^{-1} soil) was estimated by converting sensor readings into CO_2 concentration using calibration jars with known volumes of pure CO_2. After each measurement all jars were opened to allow the headspace to exchange with ambient air. Net CO_2 release was calculated as the difference between CO_2 concentration when the jars were closed and that taken prior to opening. The headspace humidity of the standard jars was kept the same as the sample jars to account for the presence of any water vapour and its potential effect on the CO_2 measurements. The percentage of added C respired as CO_2 was calculated by expressing the difference in CO_2 emission between the organic amended soils and their corresponding non-amended control soils as a function of the amount of organic C added. Any priming effects of the added substrates on the total amount of CO_2 respired was not considered in this calculation.

2.4 Soil chemical analyses

Soil pH of moist samples 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. The concentrations of total C and N in the soils were determined using an Elementar Vario EL analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Soil pH buffer curves were established between pH 1.8 and 7.8 for the Podosol, and 2.1 and 7.8 for the Tenosol, by shaking 5 g of soil in 25 ml of 0.01 M $CaCl_2$ with varying amounts of HCl or K_2CO_3 for 7 d as described in Rukshana et al. (2011). The amount of alkalinity generated by each organic amendment was estimated from the measured differences in pH between the organic-compound-treated soils and their corresponding control soil using the pH buffer curves obtained for non-amended soils (Equations 1 and 2).

Podosol, $y_1 = -59.794 + 31.46\ x_1 - 5.477\ x_1^2 + 0.318\ x_1^3$ (1)

Tenosol, $y_2 = -33.780 + 18.41\ x_2 - 3.399\ x_2^2 + 0.210\ x_2^3$ (2)

Where, y_1 and y_2 are the added alkali in cmol OH^- kg^{-1} soil, and x_1 and x_2 are the corresponding pH values measured after alkali additions.

The extractions of mineral N were performed immediately (<3 h) after removing the samples from the incubation. Mineral N was extracted by shaking 12 g soil with 2 M KCl (1:1) end-over-end for 1 h followed by centrifuging at 2000 rev min^{-1} (492 *g*) for 5 min and passing the supernatant through Whatman 1 filter papers (Whatman International, Maidestone, England). Filtered extracts were stored frozen and ammonium (NH_4^+) and nitrate (NO_3^-) concentration were later determined using a Flow Injection Analyser (LACHAT QuickChem 8500, Loveland, USA). The contribution of ammonification to soil alkalisation was calculated based on one OH^- produced per NH^+_4 mineralised from organic N and the contribution of nitrification to soil acidification according to one H^+ produced per organic N transformed to NO_3^-. Alkalinity balance was calculated as a difference between the measured alkalinity and the amount of alkalinity resulting from N cycle.

2.4 Statistical analyses

A three-way analysis of variance (ANOVA) was first used to examine the effects of soil × compound × incubation time using GENSTAT 11th Edition (VSN International, Hemel Hempstead, England) on pH, concentrations of ammonium and nitrate, decomposition (assessed by quantifying cumulative respiration of CO_2) and measured alkalinity. In addition, this analysis demonstrated the presence of a significant 3-way interaction, and large and significant differences between the two soils. In an effort to improve the interpretation of the differences within each soil, a 2-way ANOVA was performed for each soil using compound and incubation time as main factors on the measured parameters. The reduction in df associated with the 2-way analysis conducted for each soil would rendered the significance testing more conservative. For cumulative respiration over the duration of the incubation period, 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 test was used to determine significant differences ($P < 0.05$) between means.

3. Results

3.1 Soil pH

The addition of organic acids (acetic, malic, citric, benzoic and ferulic acid) immediately decreased soil pH due to H^+ dissociation from carboxyl functional groups (Fig. 1). The magnitude of the pH decrease at 0 d was in the order of citric acid > malic acid > acetic acid > benzoic acid > ferulic acid in the Podosol and for the Tenosol, malic acid > citric acid > acetic acid > benzoic acid > ferulic acid. Over the subsequent 60-d incubation period, soil pH increased as organic anions were mineralised and H^+ ions were consumed. The rate of pH recovery was greater in the Tenosol than the Podosol. For example, recovery to the original pH in Podosol receiving malic and citric acids occurred at 60 d, whereas in the Tenosol this had occurred by 3 d (Fig. 1a, d). Glucosamine hydrochloride did not have any immediate effect on pH in either soil. However, during subsequent incubation glucosamine hydrochloride decreased the soil pH by up to 1.5 pH units in the Tenosol (Fig. 1f). Glucose addition to the Podosol slightly decreased the soil pH during the incubation period whereas it increased the pH of Tenosol by 0.15 units after 1 d incubation (Fig. 1f). However, soil pH changes after glucose addition were small compared with the other compounds. On average, the magnitude of soil pH change in response to addition of organic compounds was greater in the soil with higher initial pH (Tenosol) than lower initial pH (Podosol). For example, in the Tenosol the immediate reduction in pH after addition of malic acid was approximately double that of the Podosol soil.

3.2 Soil respiration

The addition of most of the C compounds increased soil respiration compared to the control in both soils (Fig. 2). The cumulative respiration was higher after the addition of malic acid, citric acid and glucose during the incubation period and there was no significant difference in cumulative respiration between these treatments in each soil. In the Podosol, cumulative respiration ranged from 91 μg CO_2-C g^{-1} in the acetic acid treatment to 420 μg CO_2-C g^{-1} in the glucose treatment. Addition of acetic acid and benzoic acid increased cumulative respiration compared with the control in the Tenosol but not in the Podosol. Moreover, addition of C compounds resulted in higher cumulative respiration in the Tenosol than the Podosol. For example, cumulative respiration in the Tenosol control (161 μg CO_2-C g^{-1} soil) was approximately double the Podosol control (85 μg CO_2-C g^{-1} soil) at the end of the experiment. Further, cumulative respiration was greater than the added C for all treatments except acetic acid and ferulic acid in the Tenosol during the 60-d incubation period. However, cumulative respiration was lower than the added C for all treatments in the Podosol during the same incubation time.

The percentage of substrate carbon respired was estimated, from the difference in cumulative respired CO_2-C between amended and control soils, and the amount of added C, for each soil at 7 d and 60 d (Table 1). Glucose, malic acid and citric acid carbon was respired faster than carbon in the other substrates. Acetic acid and benzoic acid carbon was not respired in the first 7 d in either soil. Ferulic acid carbon was respired faster in the Tenosol than the Podsol in the first 7 d.

3.4 Nitrogen transformation

The NH_4^+ concentration in the control soils increased during the 60-d incubation in the Podosol (0.6 to 3.6 mg kg^{-1}) and the Tenosol (0.5 to 1.9 mg kg^{-1}) (Fig. 3). The addition of malic acid, citric acid and ferulic acid to the Podosol decreased the NH_4^+ concentration, whereas the addition to the Tenosol did not affect the NH_4^+ concentration. Acetic acid and benzoic acid did not change the NH_4^+ concentration in the Podosol; however, it was slightly increased in the Tenosol. Addition of glucosamine hydrochloride to the Podosol had no effect on NH_4^+ concentration up to 7 d, however after this time a rapid increase in NH_4^+ concentration was observed. The NH_4^+ concentrations were 2.1 mg kg^{-1} and 56.4 mg kg^{-1} at 7 d and 60 d, respectively in Podosol soil which accounted for 7% and 174% of added N as nitrogen compound (Fig. 3c). Conversely, in the Tenosol, glucosamine hydrochloride increased NH_4^+ concentration from 3 d and reached a peak at 15 d where ammonium N was 179% of added N in the form of glucosamine hydrochloride (Fig. 3f). NH_4^+ decreased after 15 d in the Tenosol due to nitrification (Fig. 4f).

The NO_3^- concentration of the control treatment was lower in the Podosol (0.8 to 1.0 mg kg^{-1}) compared to the Tenosol (6.3 to 12.7 mg kg^{-1}) (Fig. 4). In the Podosol, NO_3^- concentration was not affected by the addition of any organic compound, including the N-containing compound (glucosamine hydrochloride) (Fig. 4c). However, in the Tenosol NO_3^- concentrations decreased from 1 d when organic acids (acetic, malic, citric, benzoic and ferulic acid) were added likely due to nitrate immobilisation (Fig. 4d, e). However, the reduction of NO_3^- concentration was slower in soils receiving acetic acid than all other acids. NO_3^- concentration significantly ($P < 0.001$) decreased 1 d after the addition of glucose and remained low for the rest of the incubation period (Fig. 4f). Glucosamine hydrochloride did not significantly ($P < 0.05$) alter NO_3^- concentration in the Tenosol in the first 15 d, however, NO_3^- concentration increased from 15 d to the end of the incubation period (Fig. 4f).

3.5 Alkalinity production

The amounts of measured alkalinity at Days 0, 7 and 60, and alkalinity production through the N cycle and alkalinity balance at Days 7 and 60 after C compound addition to soils are shown in Table 2. The addition of organic acids resulted in acidification at Day 0, and the acidification was greater in the Podosol than the Tenosol. The greatest acidity was produced (36.8 mmol kg^{-1}) with citric acid addition to the Podosol at Day 0. However, such acidification was counter-balanced by alkalinisation during decomposition of the organic acids with incubation time. A net alkalization occurred in some treatments, particularly in the Tenosol during the incubation period. By Day 7, the greatest alkalinity production (9.5 mmol kg^{-1}) was observed in the Tenosol with the addition of citric acid. The amounts of net alkalinity produced were in order of citric acid > malic acid > ferulic acid > glucose in the Tenosol. The addition of glucosamine hydrochloride resulted in acidification which increased over time due to nitrification. However, benzoic acid, glucosamine hydrochloride and acetic acid resulted in net acidification in the Tenosol. Net alkalinity production was higher at 60 d than 7 d in the Tenosol incubated with glucose, acetic acid and benzoic acid. However, malic acid, citric acid, glucosamine hydrochloride and ferulic acid decreased the net alkalinity at 60 d compared to 7 d. The amount of alkalinity generated after the initial acidification upon the addition of organic acids (with the exception of acetic acid) correlated well with respiration of carbon from the acids (Fig. 5).

4. Discussion

4.1 Impact of organic compound type on soil pH change

In this study, addition of organic acids (acetic, malic, citric, benzoic and ferulic acid) significantly decreased soil pH at Day 0 (Fig. 1). This rapid initial decrease in pH was suspected to result from H^+ dissociation of the organic acids. At a given pH, the acid dissociation constant (pK_a) of an organic acid represents the proportion of dissociated and un-dissociated forms of the organic acid. In theory, if the initial soil pH is less than the pK_a of the organic acid, addition of the acid may increase in soil pH due to association of H^+ from the soil. Conversely, if the initial pH is greater than pK_a of the organic acid, soil pH may decrease due to dissociation of H^+ from the acid (Ritchie and Dolling, 1985). We have previously shown that the immediate reduction in soil pH occurred due to the dissociation of H^+ from carboxyl groups (Rukshana et al., 2011). Furthermore, the magnitude of pH decrease depends on the pK_a value of the organic acids and the number of carboxyl groups. In this study, the magnitude of the pH decrease was in the order of citric acid > malic acid > acetic acid > benzoic acid > ferulic acid, except in the Tenosol where the magnitude of pH decrease was greater with addition of malic acid than citric acid. It is possible as the pK_a of the third carboxylic acid group (pK_{a3}) of citric acid was greater than the initial pH of the Tenosol, and therefore may not dissociated. Therefore, in this study organic acids with lower dissociation constants and a greater number of carboxyl groups resulted in a greater decrease in soil pH. Although acetic acid, benzoic acid and ferulic acid all contain a single carboxyl group, the immediate decrease in soil pH was different (acetic acid > benzoic acid > ferulic acid). Because the same amount of C (0.5 g C kg^{-1} soil) was added to the soils in this study, the added molar number of carboxyl groups were in order of acetic acid > benzoic acid > ferulic acid. The magnitude of pH decrease at Day 0 was associated with the number of moles of carboxyl groups added as the organic acids.

During subsequent incubation, soil pH was slowly restored after organic acid addition in this study. This recovery of soil pH mainly resulted from decarboxylation of the dissociated organic acid anions, which consumes H^+ ions as the added organic acids are decomposed and their carbon is respired (Rukshana et al., 2011). It is evident that the generation of alkalinity was correlated with the decomposition of the organic acids (anions) except for acetic acid (Fig. 5). Interestingly, at Days 3 and 7, the pH of Tenosol exceeded its initial pH after the addition of malic acid, citric acid and ferulic acid, which we termed an “alkalinity priming” effect. This observed greater alkalinity release in the Tenosol was counter-balanced by unknown components (Table 2). This excess alkalinity was not derived from the N transformation nor from the decomposition of added C compounds. Therefore, this alkalinity may be derived from decarboxylation during the decomposition of the native soil organic matter. This deserves further investigation.

The pattern of pH change in the acetic acid treatment differed from that in the malic and citric acid treatments. This difference might be related to the difference in metabolism of the two acid groups. Malic acid and citric acid (and their dissociated anions) could be decomposed to CO_2 and also incorporated into microbial biomass C (Jones et al., 1996). However, acetic acid (and the dissociated anions) might be preferably incorporated into microbial biomass (anabolism) rather than decomposition to CO_2 (catabolism) (van Hees et al., 2003; Fischer et al., 2010) and decomposition might be arrested as it is not a part of respiratory cycle like malic acid and citric acid (Fischer et al., 2010). This is supported by the finding that the production of respired CO_2 was much lower in the acetic acid treatment than in the malic and citric acid treatments (Fig. 2).

This study confirmed our previous observation that glucose and glucosamine hydrochloride had no immediate effect on soil pH (Rukshana et al., 2011). Glucose is a neutral compound. It has no charge and is not adsorbed onto soil particles (Jones and Edwards, 1998; Hoyle et al., 2008). Generally, glucose mineralisation

tended to increase the pH of the Tenosol (Fig. 1) which may have resulted from the decomposition of the added glucose or indigenous organic C in the soil. This pH increase might also have resulted from nitrate immobilisation (an H^+-consuming process, see below) by microorganisms (Fig. 4f). Glucosamine hydrochloride could alter soil pH through microbial transformation of N over time (Kemmitt et al., 2006) which was observed in this study (see below).

4.2 Decomposition and pH change

The addition of glucose, malic acid and citric acid resulted in larger cumulative soil respiration than the addition of other compounds. The carbon contained in the added compounds, including simple sugars, are readily available to microorganisms, and is usually rapidly converted to CO_2, assimilated into microbial products such as amino acids or lipids (Essington, 2004), adsorbed onto the soil matrix or converted into humified soil organic matter (Hoyle et al., 2008). While glucose addition resulted in the greatest cumulative respiration, only a small effect was observed on soil pH. For Malic acid and citric acid their high decomposition rates were related to the greatest alkalisation, as discussed earlier.

Decomposition of benzoic acid and ferulic acid was much lower than that of other C compounds in this study. It has been reported that aromatic structures are not easily degradable and accumulate during the initial stages of litter decomposition (Sollins et al., 1996; Hamer and Marschner, 2002), and that aromatic acids are degraded slower than aliphatic carboxylic acids (Schwab, 2000; Marschner and Kalbitz, 2003). Such findings are consistent with the results of this study.

This study confirmed that soil respiration and decomposition were lower in low initial pH soil than high initial pH soil. Up to 141% of added C in Tenosol and 84% in Podosol were mineralised to CO_2 over 60 d. The smaller decomposition in the Podosol may be related to its lower initial pH and higher C/N ratio. This finding is consistent with other studies demonstrating that soil respiration and organic matter decomposition may be restricted in low pH soil (Motavalli et al., 1995; Kyveryga et al., 2004; Khalil et al., 2005; Kemmitt et al., 2006; Xu et al., 2006a).

The observed lower recovery of respired CO_2-C in the Podosol in this study, when expressed as a percentage of added C, is probably due to the low clay content, low microbial biomass and poor nutrient supply in this Podosol, which will limit microbial processes. The results are consistent with the findings by Hoyle et al.(2008) that respired CO_2-C was less than the added glucose-C and the authors attributed this to low microbial biomass-C and metabolism-C due to low C and low clay content of the soils studied. In other studies, a greater release of CO_2-C than added amounts of C, especially glucose, was observed (De Nobili et al., 2001; Mondini et al., 2006) which is consistent for the Tenosol examined in this work.

Although additional CO_2-C was released from the Tenosol but not the Podosol, it is not possible to determine the source of CO_2-C. Some of the added C might be adsorbed onto the soil matrix as was shown in other studies (Jones and Edwards, 1998; van Hees et al., 2003; Xu et al., 2006a). The respired CO_2-C might be derived from added C (Hoyle et al., 2008), endocellular utilization of C (De Nobili et al., 2001), increased microbial turnover (Hoyle and Murphy, 2007) and/or enhanced mineralisation of soil organic matter (Hoyle et al., 2008) or a combination of these processes.

A higher pH than the initial pH (alkalinity priming effect) occurred in the Tenosol after the addition of some C compounds in this study (Table 2) was partly derived from decomposition of the added C and the N cycling, and partly from an unknown component(s) as discussed earlier. For example, the amount of acidity produced in the Tenosol through a dissociation of H^+ from citric acid was 4.7 mmol (kg^{-1} soil) at Day 0. This acidity was counter-balanced by the alkalinity released via decarboxylation of citrate during the incubation and at 7 d alkalinity production was 9.5 mmol (kg^{-1} soil). At 7 d, N cycle contributed only 0.7 mmol kg^{-1} of alkalinity and therefore, additional 8.8 mmol kg^{-1} of alkalinity was released via unknown components. It might be possible that some of the alkalinity was generated from decomposition of indigenous soil organic matter.

The priming effect on alkalinity was observed only in the Tenosol but not in the Podosol. The Podosol amended with malic and citric acid had not fully restored its pH throughout the incubation period. The first reason for this difference between the soils was that the lower pH of Podosol decreased microbial activity and prolonged the decarboxylation of added C and indigenous organic matter (biological process) after the initial pH decrease due to the dissociation of added organic acids (chemical process). It was evident that the amount of CO_2 released was much lower and delayed in the Podosol as compared to the Tenosol. The second reason may be related to the protection mechanisms of soil organic C in soil. In this study, ligand exchange may be one of the mechanisms which can reduce the decomposition of organic C in the soil with organic acid addition. Ligand exchange is the anion exchange between simple coordinated OH groups on mineral surfaces, carboxyl groups and phenolic -OH groups of soil organic C (von Lutzow et al., 2006). Complexation of organic compounds on mineral surfaces through ligand exchange depends on the ionic strength and pH. In this study, this complexation of organic C on mineral surfaces could have been greater in the Podosol than the Tenosol as it increases with decreasing pH.

Maximum sorption occurs between pH 4.3 and 4.7 corresponding to pKa values of the most abundant carboxylic acid in soils (Gu et al., 1994).

4.3 N transformation and pH change

Soil N transformation contributed partly to soil pH change in this study. While ammonification of organic N causes soil pH to increase, subsequent nitrification results in pH decrease. Where glucosamine hydrochloride was added to the Tenosol, net ammonification occurred and NH_4^+ concentration increased and reached a peak at 15 d. However, after 15 d NH_4^+ concentration decreased and NO_3^- concentration increased. In the Podosol net ammonification occurred but no appreciable nitrification was observed. This finding is consistent with other studies showing that ammonification is the dominant process in acidic soils and very little nitrification takes place (Haynes and Swift, 1989; Khalil et al., 2005; Xu et al., 2006b).

The decrease of NO_3^- concentration at 1 d with the addition of glucose in the Tenosol indicates a rapid immobilisation of N by soil microbes. This is consistent with other studies that N immobilisation/uptake occurred after glucose addition (Recous et al., 1990; Azam et al., 1993; Hoyle et al., 2008). Moreover, Zagal and Persson (1994) found that the immobilisation of N up to 3 d was followed by re-mineralization after addition of glucose to soil with the presence of ^{15}N whereas there was no immobilisation of N where glucose was not added. Nitrate uptake by microbes is associated with the H^+ influx (Gonzalez et al., 2006). Thus, during the incubation period, the increase in soil pH in the Tenosol with glucose and organic acid could have resulted from NO_3^- uptake by microbes as ammonification was minimal in this soil.

Low rates of net nitrification were observed in all treatments (including the control) applied to the Podosol over the incubation period. This may be due to the lack of favourable conditions for nitrifying microbes (low initial pH soil). Nitrification was smaller in the low initial pH soil compared to high initial pH soil as the soil respiration and organic matter decomposition might be restricted at low pH soil (Motavalli et al., 1995; Kyveryga et al., 2004; Khalil et al., 2005; Kemmitt et al., 2006; Xu et al., 2006a). Moreover, both ammonification and nitrification processes are affected by initial soil pH but generally nitrification is more sensitive to low initial pH soil than ammonification (Robson, 1989).

5. Conclusions

This study demonstrated that the addition of malic, citric and ferulic acid to Tenosol stimulated alkalinity release, resulting in a priming effect. This priming effect was probably due to stimulated microbial NO_3^- uptake, ammonification and decomposition of indigenous organic matter in soil, and was suppressed by low pH. Furthermore, this study showed that the cumulative respired CO_2-C was less than the added C in the Podosol for all treatments, while in the Tenosol this was greater than the added C in some treatments, indicating a priming effect on native soil organic matter. However, it was not possible to determine whether CO_2-C was derived from 1) endocellular utilization of C, 2) increased microbial turnover or 3) enhanced mineralisation of native soil organic matter or 4) a combination of these processes. Future research will use ^{14}C-labelled compounds to determine whether C compounds stimulate the mineralization of native soil organic matter which releases alkalinity.

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Table 1: Estimated percentage of added organic C respired as CO_2 in the Podosol and Tenosol after 7 d and 60 d of incubation.

Compound	Podosol		Tenosol	
	7 d	60 d	7 d	60 d
Acetic acid	0.0[a]	18.2[c]	0.0[a]	69.9[b]
Malic acid	16.5[bc]	84.0[g]	76.8[b]	141.2[e]
Citric acid	13.5[bc]	80.4[fg]	68.7[b]	129.0[de]
Benzoic acid	0.0[a]	30.2[d]	0.0[a]	120.1[d]
Ferulic acid	0.0[a]	72.5[f]	17.7[a]	78.2[b]
[1]Glucosamine	0.0[a]	53.6[e]	61.6[b]	100.9[c]
Glucose	8.9[b]	83.9[g]	64.9[b]	132.7[de]
Significance level				
Compound	***		***	
Time	***		***	
Compound × time	***		***	

[1]Glucosamine hydrochloride
Means with the same letter are not significantly different at $P = 0.05$ using post-hoc Tukey honest significant difference test.
Percentage of added organic C respired as CO_2 (Y) was estimated by Y= (Respired $C_{treatment}$ - Respired $C_{control}$) x 100 / Added C.

Table 2: The amount of measured alkalinity and alkalinity estimated from N cycle, and alkalinity balance (mmol kg^{-1} soil) at 7 d and 60 d after C compound addition to soils.

Soil	Compounds	[1]Measured alkalinity			[2] N cycle alkalinity		[3]Alkalinity balance	
		0 d	7 d	60 d	7 d	60 d	7 d	60 d
Podosol	Acetic acid	-10.28^{c}	-0.36^{jkl}	-0.03^{kl}	0.00^{bc}	-0.04^{bc}	-0.36^{fg}	0.00^{g}
	Malic acid	-24.54^{b}	-7.62^{d}	1.33^{m}	0.00^{bc}	-0.12^{a}	-7.62^{b}	1.45^{h}
	Citric acid	-36.80^{a}	-9.96^{c}	-1.16^{hij}	0.01^{c}	-0.12^{a}	-9.97^{a}	-1.04^{ef}
	Benzoic acid	-6.16^{e}	-1.93^{gh}	-2.03^{gh}	0.00^{bc}	-0.06^{ab}	-1.94^{e}	-1.96^{e}
	Ferulic acid	-2.90^{fg}	-1.36^{hij}	0.11^{kl}	0.00^{bc}	-0.14^{a}	-1.36^{e}	0.24^{g}
	[4]Glucosamine	-0.05^{kl}	0.03^{kl}	-1.48^{hi}	0.10^{c}	3.75^{d}	-0.06^{g}	-5.23^{c}
	Glucose	0.54^{lm}	-3.25^{f}	-0.49^{ijk}	0.01^{bc}	-0.13^{a}	-3.26^{d}	-0.37^{fg}
	Significance level							
	Compound		***			***	***	
	Time		***			***	***	
	Compound x time		***			***	***	
Tenosol	Acetic acid	-4.69^{b}	-1.71^{d}	1.56^{h}	0.12^{b}	0.97^{c}	-1.83^{ab}	0.58^{e}
	Malic acid	-8.75^{a}	6.76^{j}	0.94^{gh}	0.69^{c}	0.56^{bc}	6.07^{g}	0.38^{e}
	Citric acid	-4.75^{b}	9.48^{k}	1.06^{gh}	0.70^{c}	0.56^{bc}	8.78^{h}	0.50^{e}
	Benzoic acid	-2.93^{c}	-1.28^{d}	0.69^{fg}	0.43^{bc}	0.93^{c}	-1.71^{ab}	-0.23^{de}
	Ferulic acid	-1.06^{d}	3.97^{i}	0.63^{fg}	0.58^{bc}	0.81^{c}	3.38^{f}	-0.18^{de}
	[4]Glucosamine	-0.40^{e}	-1.43^{d}	-2.88^{c}	0.87^{c}	-1.52^{a}	-2.30^{a}	-1.36^{bc}
	Glucose	-0.37^{e}	0.07^{ef}	0.76^{g}	0.70^{c}	0.62^{bc}	-0.63^{cd}	0.14^{de}
	Significance level							
	Compound		***			***	***	
	Time		***			**	***	
	Compound x time		***			***	***	

[1]The amount of measured alkalinity was calculated based on the pH buffer curves of the soils and pH changes of C-amended soil relative to the control soil.
[2]Δ NH_4^+ and Δ NO_3 are the difference in concentrations of NH_4^+ and NO_3^- (mmol kg^{-1} soil), respectively, between amended soil and the control soil.
[2]It is assumed that one NH_4^+ and one NO_3^- transformed from organic N produces one OH^- and one H^+, respectively. The N cycle alkalinity was calculated from changes in concentrations in amended soils against the corresponding control soil.
[3]Alkalinity balance = Measured alkalinity - N cycle alkalinity
[4]Glucosamine hydrochloride
Negative values indicate net acidification
Means with the same letter are not significantly different at P = 0.05 using post-hoc Tukey honest significant difference test.

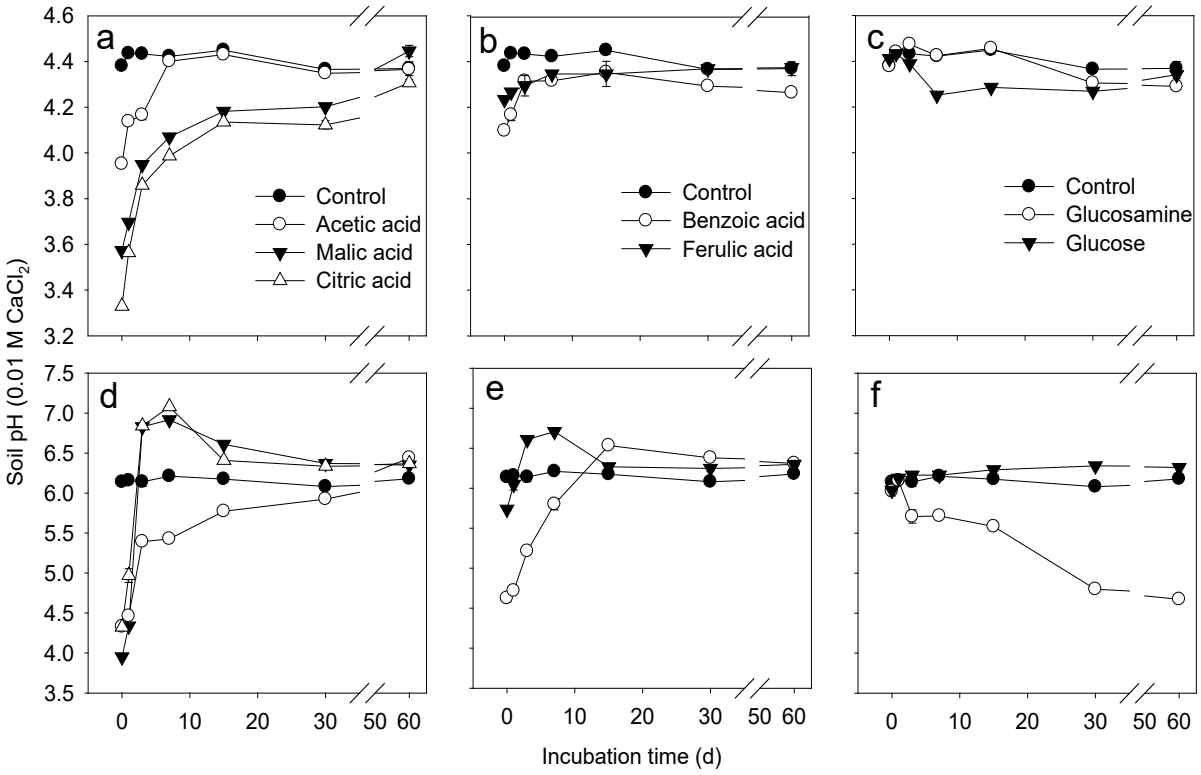

Figure 1. Soil pH changes during 60 d after the addition of model C compounds to a Podosol (a, b, c) and Tenosol (d, e, f). Bars represent the standard error of the mean (n = 3) where they are greater than symbols. Note the different pH scales on the Y axis. Main effects of soil, compound, time and their interactions are all highly significant *(P* ≤ 0.001).

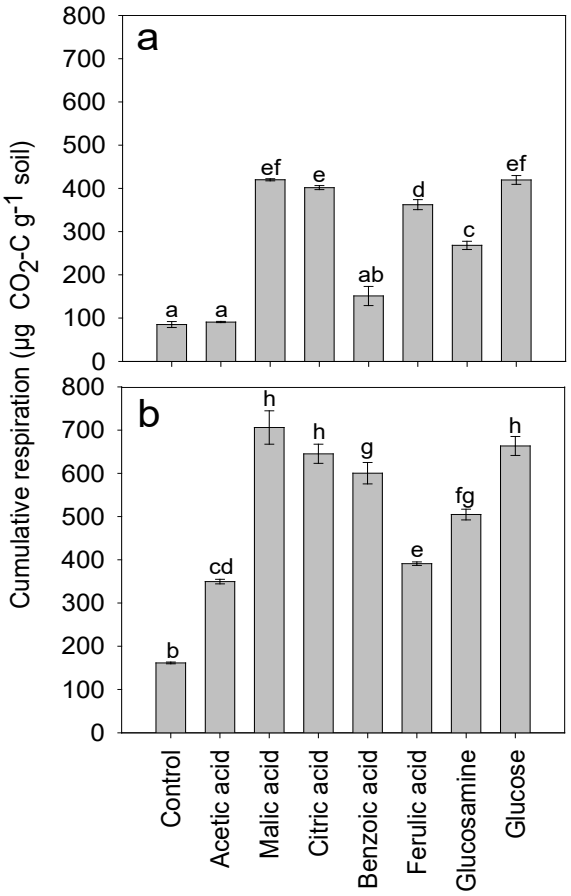

Figure 2. Cumulative respiration (µg CO_2-C g^{-1} soil) during 60 d after addition of model C compounds to a) Podosol and b) Tenosol. The means with the same letter are not significantly different at $P = 0.05$ using post-hoc Tukey honest significant difference test. Main effects of soil, compound and their interactions are all highly significant ($P \leq 0.001$).

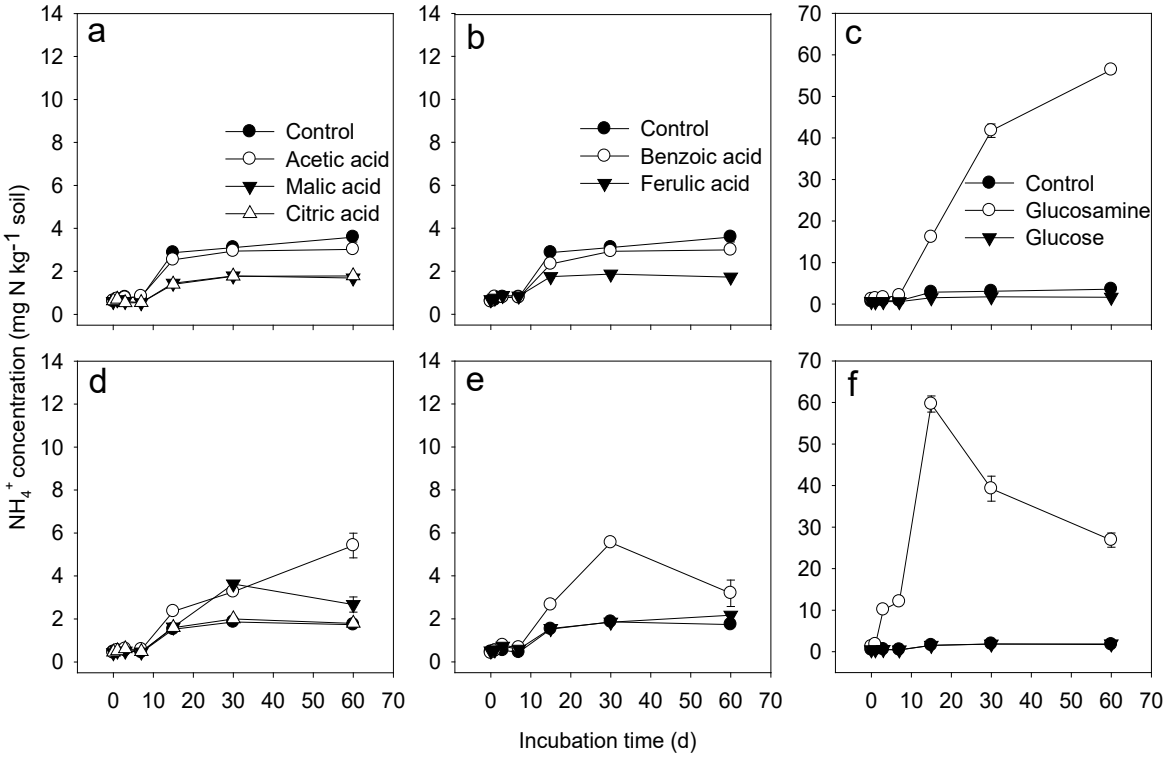

Figure 3. Ammonium (NH_4^+) dynamics during 60 d after the addition of model C compounds to a Podosol (a, b, c) and Tenosol (d, e, f). Bars represent the standard error of the mean (n = 3) where they are greater than symbols. Note the different scales of N concentration on the Y axis. Main effects of soil, compound, time and their interactions are all highly significant *(P* ≤ 0.001).

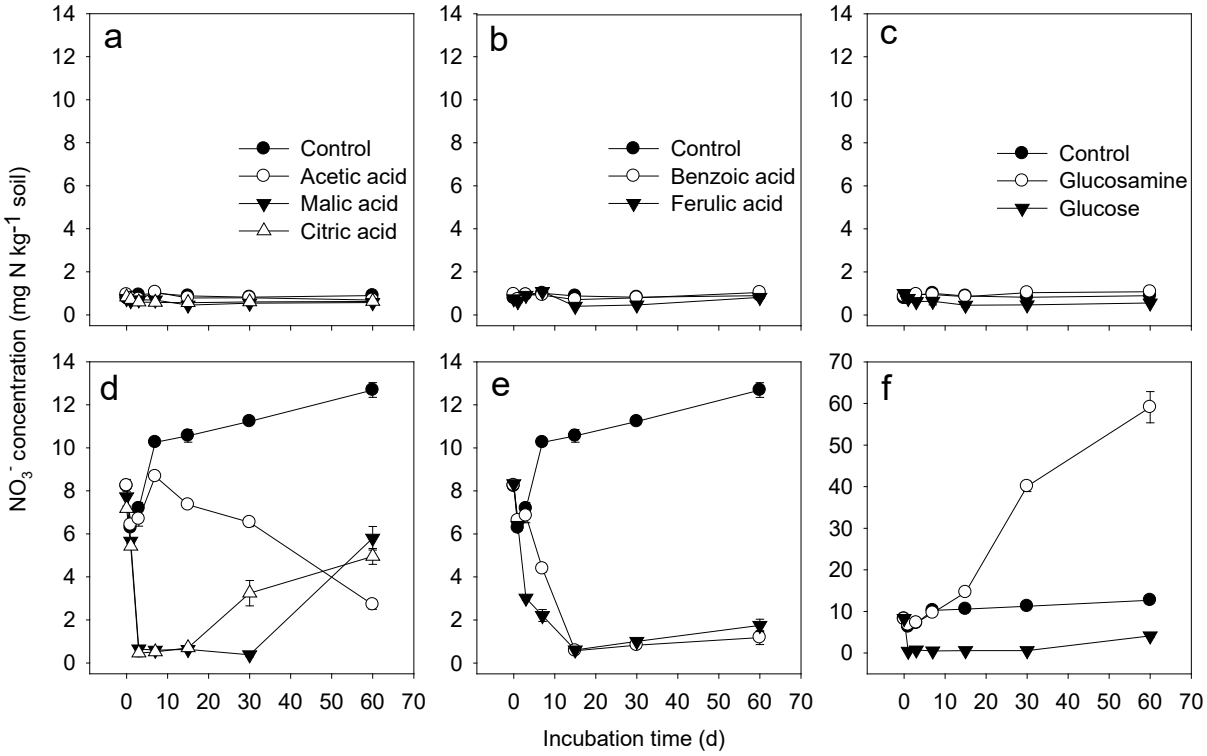

Figure 4. Nitrate (NO_3^-) dynamics during 60 d after the addition of model C compounds to a Podosol (a, b, c) and Tenosol (d, e, f). Bars represent the standard error of the mean (n = 3) where they are greater than symbols. Note the different scales of N concentration on the Y axis. Main effects of soil, compound, time and their interactions are all highly significant *(P* ≤ 0.001).

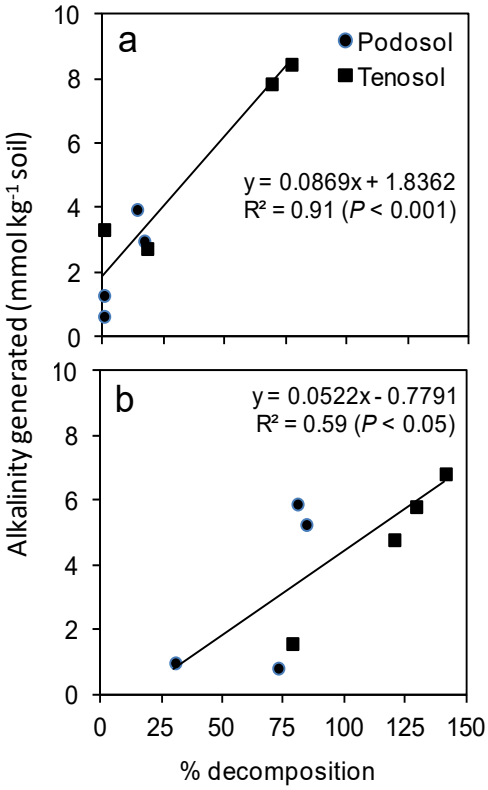

Figure 5. Relationships between % decomposition of added organic compounds (malic acid, citric acid, benzoic acid and ferulic acid) and the amount of alkalinity generated by a) 7 d and b) 60 d after the addition of organic compounds. The amount of alkalinity generated was calculated based on the pH buffer curve of the soils and pH changes of C-amended soil relative to the control soil from 0 to 7 d or 0 to 60 d.