

**Organic anion-to-acid ratio influences pH change of soils differing in initial pH**

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**Abstract**

**Purpose** This study aimed to investigate the effect of initial soil pH and organic anion-to-acid ratio on changes in soil pH.

**Materials and methods** Two soils (Podosol and Tenosol) along with two carboxylic acids (malic and citric acid) and their anions (sodium malate and sodium citrate), commonly found in plant residues, were used in this study. Stock solutions of either malic acid and disodium malate or citric acid and tri-sodium citrate were added to pre-incubated soils at anion-to-acid ratios of 0:100, 10:90, 25:75, 50:50, 75:25, 90:10, 100:0 and at 0.25 g C kg<sup>-1</sup> soil. Soils were adjusted to 80% field capacity and mixed thoroughly, and 3 replicates of 50 g of each soil were transferred into individual plastic cores and incubated at 25 °C in the dark for 30 days. Soil pH, respiration, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were determined.

**Results and discussion** Soil pH increased linearly with increasing organic anion-to-acid ratio. The addition of organic anions to soil resulted in net alkalisation. However, the addition of organic acids immediately decreased soil pH. During subsequent incubation, soil pH increased when the organic anions were decomposed. Alkalinity generation was lower in the Podosol (initial pH 4.5) than in the Tenosol (initial pH 6.2), and was proportional to anion-to-acid ratio across all the treatments. Cumulative CO<sub>2</sub>-C release was approximately 3 times lower in the Podosol than the Tenosol at day 2 due to lower microbial activity in the low-pH Podosol.

**Conclusions** Increasing anion-to-acid ratio of organic compounds increased soil pH. Increases in soil pH were mainly attributed to direct chemical reactions and decomposition of organic anions. Low pH decreased the amount of alkalinity generated by addition of organic compounds due to incomplete decomposition of the added compounds. This study implies that organic anion-to-acid ratio in plant residues plays an important role in soil pH change.

**Keywords** Carbon cycle • Decomposition • Nitrogen transformation • Plant residue • Organic acid • Organic anion • Soil pH

**1 Introduction**

Soil organic matter (SOM) governs biogeochemical cycles of carbon (C), nitrogen (N) and other primary nutrients, and therefore, the beneficial effects of SOM have been widely recognised for plant growth and maintenance of pH status and structure in soils. However, the effect of organic matter and its cycling on change in soil pH reported after the addition of plant residues vary markedly between studies (Marx et al. 2002; Noble and Randall 1999; Tang et al. 1999; Xu et al. 2006a, b; Yan and Schubert 2000). The variability in direction and magnitude of pH change may differ due to differences in biochemical composition of plant residues and types of soils used in the different studies.

Carbon and N cycle processes, and association/dissociation reactions of H<sup>+</sup> primarily contribute to change in soil pH following the addition of organic residues to soils (Brady and Weil, 2002; Xu et al. 2006b). In general, plant residues contain an excess of cations over inorganic anions and the concentration of excess cations reflects the concentration of organic anions within the residues, since the charge balance is

maintained by synthesis of organic anions (Haynes and Mokolobate 2001; Xu et al. 2006b). Most of the alkalinity generated (pH increase) following the addition of organic residues to soil occurs via decarboxylation of organic anions which consumes  $H^+$  during microbial decomposition (Marschner and Noble 2000; Mengel 1994).

In addition to C cycle processes, nitrogen transformation is also responsible for changes in soil pH following organic residue addition (Bolan et al. 1991; Mao et al. 2010). During mineralization, organic N is mineralised to ammonium ( $NH_4^+$ ) via ammonification and produces an  $OH^-$  ion which increases soil pH. Subsequent nitrification of  $NH_4^+$  to nitrate ( $NO_3^-$ ) releases  $H^+$  ions while denitrification consumes  $H^+$  (Bolan et al. 1991; Xu and Coventry 2003; Xu et al. 2006; Tang et al. 2012). Further to C and N cycling, association/dissociation reactions of  $H^+$  occur between functional groups of organic compounds within plant residues and the soil matrix which lead to an immediate change in soil pH (Ritchie and Dolling 1985; Tang et al. 1999). The magnitude of pH change due to  $H^+$  association/dissociation reactions depends on the acid dissociation constant ( $pK_a$  value) of organic functional groups within the plant residues, the initial soil pH and soil pH buffer capacity (Ritchie and Dolling 1985; Rukshana et al. 2012; Wang et al. 2012).

While a number of studies have determined the fate and decomposition of organic acids and anions after addition to soil (Hamer and Marschner 2005; Jones and Brassington 1998; Oburger et al. 2009; van Hees et al. 2003), only a few have examined changes in soil pH (Li et al. 2008; Yan et al. 1996). Our previous study showed that organic acid addition to soil resulted in an immediate decrease in soil pH, followed by a period in which the soil pH increased and recovered to the original level (Rukshana et al. 2012). In contrast, the addition of organic anions increased soil pH (Rukshana et al. 2011). It is important to investigate the combined effect of acid and anion forms on changes in soil pH since plant residues contain both forms of organic compounds at different proportions.

This study aimed to investigate the effect of percent dissociation of organic acids on soil pH change during a 30-day incubation period using two carboxylic acids commonly found in plant residues. Organic acids and their anions were added at different ratios to achieve different percentages of dissociation. For this, malic acid and citric acid, and their sodium salts (sodium malate and sodium citrate) were added at various anion-to-acid ratios to two soils differing in initial pH. It was hypothesized that 1) the magnitude of the pH change would be proportional to the anion-to-acid ratio of organic compounds added due to net alkalisation by organic anions, and 2) the magnitude of soil pH change would be greater in soils with higher than lower initial pH as decomposition of added C was limited in soils with low initial pH.

## 2 Materials and methods

### 2.1 Soils

Two soils were collected at 10-30 cm depth (A2) from Frankston (38°14'S, 145°22'E) and Shepparton (36°28'S, 145°36'E) Victoria, Australia. These soils are classified as Podosol and Tenosol (Isbell, 2002), or Podzol and Cambisol (FAO/ISRIC/ISSS, 2006), respectively. The Podosol was collected from a site under native vegetation whereas the Tenosol was collected from permanent grass pasture. Soils were sieved (<2 mm), thoroughly mixed and air-dried for subsequent analysis and the incubation study. The Podosol had the following properties: initial pH 4.5 (0.01 M  $CaCl_2$ ), sand 94.8%, silt 0.2%, clay 5.0%, total C 2.89 g  $kg^{-1}$  and total N 0.11 g  $kg^{-1}$ , pH buffer capacity (pHBC) 0.69 cmol  $pH^{-1} kg^{-1}$  soil and field capacity 12% (w/w). The Tenosol had: initial pH 6.2 (0.01 M  $CaCl_2$ ), sand 88.1%, silt 3.9%, clay 8.0%, total C 1.90 g  $kg^{-1}$  and total N 0.21 g  $kg^{-1}$ , pHBC 0.53 cmol  $pH^{-1} kg^{-1}$  soil and field capacity 18%. Soil chemical and physical properties were shown in Rukshana et al. (2011).

### 2.2 C compounds

Two carboxylic acids ( $R-COOH$ ) (malic acid and citric acid) and their anions (sodium malate and sodium citrate), commonly found within plant residues, were selected for this study. Malic acid and citric acid contain two ( $pK_{a1} = 3.4$ ,  $pK_{a2} = 5.13$ ) and three ( $pK_{a1} = 3.15$ ,  $pK_{a2} = 4.77$ ,  $pK_{a3} = 6.40$ ) carboxyl functional groups, respectively. Stock solutions of malic acid (55.82 g  $l^{-1}$ ) (Sigma-Aldrich), di-sodium malate (74.12 g  $l^{-1}$ ) (Merck), citric acid (58.32 g  $l^{-1}$ ) (BDH Chemicals) and tri-sodium citrate (81.62 g  $l^{-1}$ ) (Chem Supply) were prepared in reverse osmosis (RO) water.

### 2.3 Soil incubation

Soils were adjusted to 60% field capacity and pre-incubated in the dark at 25°C for 10 days. Stock solutions of either malic acid and disodium malate or citric acid and tri-sodium citrate were added to pre-incubated soil at anion:acid ratios of 0:100, 10:90, 25:75, 50:50, 75:25, 90:10, 100:0 and at 0.25 g C  $kg^{-1}$  soil. The sodium concentration was balanced in each treatment by adding  $Na_2SO_4$  (118.26 g  $l^{-1}$ ) (Ajax Finechem) where necessary. After adding stock solutions, soils were adjusted to 80% field capacity and mixed thoroughly, and 3 replicates of 50 g (dry weight equivalent) of each soil were transferred into individual plastic cores (3.7 cm ID × 5 cm high, IPLEX Pipelines) fitted at one end with nylon mesh (0.75  $\mu m$ , Australian Filter Specialists) and packed to bulk density of 1.4 g  $cm^{-3}$ . Soils receiving only water were included as controls. In total, 180 cores comprised of 168 C-amended soil cores (2 soils × 7 compounds × 2 ratios × 2 sampling times × 3 replicates) and 12 control soil cores (2 soils × 2 sampling times × 3 replicates) were included.

Cores were placed into glass incubation jars (Ball® Half Pint Wide Mouth jars, Jarden Corporation, USA) fitted with gas-tight lids to allow sampling of headspace gases and containing a small water reservoir (8 ml) to reduce soil drying and maintain headspace humidity (Butterly et al. 2009). Cores were incubated at 25°C in the dark for 30 days and maintained 80% of field capacity throughout the incubation period.

#### 2.4 Soil respiration

Soil respiration was determined by quantifying CO<sub>2</sub> concentration within the headspace of incubation jars at 2, 4, 7, 15 and 30 days using an infra-red gas analyser (Servomex 4210 Industrial Gas Analyser, Cowborough, UK). The set of jars from the final sampling time (30 days) were used for soil respiration measurements. Soil respiration ( $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil h}^{-1}$ ) was estimated by converting sensor readings into CO<sub>2</sub> concentration using calibration jars with known volumes of pure CO<sub>2</sub>. After each measurement all jars were opened, and to allow the headspace to exchange with ambient air. Net CO<sub>2</sub> release was calculated as the difference in CO<sub>2</sub> concentration between sampling times minus the background CO<sub>2</sub> concentration measured when the jars were closed. Cumulative respiration over the entire incubation period was calculated for each soil core.

#### 2.5 Soil analytical methods

Initial total C and nitrogen (N) concentrations were determined using an Elementar Vario EL analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Particle-size distribution was analysed following the hydrometer method (Gee and Bauder, 1986).

At 2 and 30 days, a set of cores ( $n = 90$ ) was destructively sampled, and pH and N concentration were analysed using moist soil. Soil pH was measured using a pH meter (Thermo Orion 720A+, Beverly, USA) after extraction of 5 g soil with 0.01 M CaCl<sub>2</sub> (1:5) by shaking end-over-end for 1 h followed by centrifugation at 2000 rev min<sup>-1</sup> (492 g) for 10 min. The electrical conductivity (EC) was measured in water extract (1:5 soil: water) with an EC meter (CDM 210, Copenhagen, Denmark).

Soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were extracted using 12 g soil with 2 M KCl (1:1) by shaking end-over-end for 1 h followed by centrifuging at 3500 rev min<sup>-1</sup> (1507 g) for 5 min and passing the sample through Whatman 1 filter papers (Whatman International, Maidstone, England). Filtered extracts were stored frozen, and NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations were later determined using a flow injection analyser (LACHAT QuickChem 8500, Loveland, USA).

#### 2.6 Statistical analysis

For each soil, a 3-way analysis of variance (ANOVA) was used to determine the effect of compound  $\times$  ratio  $\times$  time on pH, cumulative CO<sub>2</sub>-C and NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentration using GENSTAT 11<sup>th</sup> Edition (VSN International, Hemel Hempstead, England). For all significant main effects and interactions, a post-hoc Turkey honest significant difference (HSD) test was used to determine significant differences ( $P \leq 0.05$ ) between means.

### 3 Results

#### 3.1 Soil pH

The addition of organic compounds linearly increased soil pH with increasing anion-to-acid ratios for both soils (Fig. 1 and Table 1). In the Tenosol, the pH was higher than the initial pH for all anion-to-acid ratios at 2 days. However, in the Podosol, the pH was lower than the initial pH where up to 50% and 40% of C was added as malic and citric acid, respectively at day 2. For 100% malate, the relative increase in pH was higher in the Tenosol than the Podosol.

Changes in soil pH over 30 days varied depending on soil type, C source and anion-to-acid ratio. Soil pH increased significantly ( $P < 0.001$ ) from day 2 to day 30 at all ratios in the Podosol. For example, soil pH increased from 4.08 to 4.57 units between 2 and 30 days where 100% malic acid was added and from 5.16 to 5.82 units where 100% malate was added. However, in the Tenosol pH change varied between the ratio treatments. For citrate/ citric acid, the pH decreased between 2 and 30 days for citrate-to-citric acid ratios <40:60 while it increased at the ratios >40:60 during the same period. A similar trend was observed for malate although soil pH was generally not significantly affected by sampling time.

#### 3.2 Soil respiration

The addition of organic compounds increased soil respiration (Fig. 2). Maximum soil respiration was approximately 3 times greater in the Tenosol than in the Podosol. In the Tenosol, soil respiration was the highest at day 2 for all anion-to-acid ratios. In the Podosol, soil respiration was the highest at day 2 at anion-to-acid ratios >50:50, and at day 4 at the ratios <50:50. This indicated a lag phase where microorganisms adjusted to the pH drop following acid addition to the Podosol. For both soils, soil respiration declined during subsequent incubation irrespective of anion-to-acid ratio until they reached similar levels of CO<sub>2</sub> release to those of the non-amended controls (generally up to 15 days).

The addition of malate/citrate increased cumulative CO<sub>2</sub> release (Fig. 3 and Table 1). Cumulative respiration was greater in the Tenosol than the Podosol and increased over time up to 15 days and remained constant between 15 and 30 days in all treatments. However, anion-to-acid ratio did not consistently affect CO<sub>2</sub> release.

### 3.3 Nitrogen transformation

In the Podosol, the addition of organic compounds, relative to the control, decreased NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations (Tables 1 and 2). Increasing anion-to-acid ratios generally decreased NH<sub>4</sub><sup>+</sup> concentration at day 2 but increased it at day 30. The NH<sub>4</sub><sup>+</sup> concentration thus decreased over time when the anion-to-acid ratio was <25:75 and increased when the anion-to-acid ratio was >50:50. Increasing anion-to-acid ratio generally decreased NO<sub>3</sub><sup>-</sup> concentration at day 2, especially for malate/malic acid treatments, and did not affect it at day 30.

In the non-amended controls, Tenosol had lower NH<sub>4</sub><sup>+</sup> concentrations but 9 to 17-fold higher NO<sub>3</sub><sup>-</sup> concentration than the Podosol (Table 2). In Tenosol, NH<sub>4</sub><sup>+</sup> concentration increased over time and was not affected by addition of organic compounds. The NO<sub>3</sub><sup>-</sup> concentration also increased over time but decreased when organic compounds were added. Increasing anion-to-acid ratios did not affect NO<sub>3</sub><sup>-</sup> concentration at day 2 but increased it at day 30 when the anion-to-acid ratio was >50:50.

## 4 Discussion

### 4.1 Effect of anion-to-acid ratio

Soil pH increased linearly with increasing organic anion-to-acid ratio, which supports the first hypothesis. This trend could result from either the direct chemical reactions between soil and organic anions, or via organic anion decomposition. Yan et al. (1996) observed an immediate (2 h) increase in soil pH during direct shaking of malate with soil and attributed this pH increase to exchange reactions of H<sup>+</sup> with the organic anion. However, this pH increase was much less compared to that after moist incubation. During incubation, organic anion decomposition consumes H<sup>+</sup> ions via decarboxylation thereby increasing soil pH. The net soil alkalisation resulted from organic anion addition is consistent with our previous findings (Rukshana et al. 2011). In contrast, organic acid addition immediately decreased soil pH due to the dissociation of H<sup>+</sup> from acid functional groups but the soil pH subsequently increased when the organic anions were decomposed.

In the Podosol, increasing organic anion-to-acid ratio tended to decrease concentrations of both NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> at day 2 and increased NH<sub>4</sub><sup>+</sup> concentration at day 30 (Table 2). While a decrease in NH<sub>4</sub><sup>+</sup> concentration is associated with H<sup>+</sup> production (and vice versa), a decrease in NO<sub>3</sub><sup>-</sup> concentration may indicate denitrification/microbial immobilization and is associated with H<sup>+</sup> consumption (Fan et al. 2002; Gonzalez et al. 2006; Xu et al. 2006b). However, the contribution of N cycle as affected by anion-to-acid ratio to soil pH change was negligible (<0.012 pH units). In the Tenosol, increasing organic anion-to-acid ratio increased NO<sub>3</sub><sup>-</sup> concentration, which potentially contributed to a pH decrease of up to 0.05 units.

### 4.2 Influence of incubation time

Soil pH increased from day 2 to day 30 in the Podosol when the organic compounds were added. As discussed earlier, this increase in soil pH could be attributed to decarboxylation of organic anions and also ammonification of organic N. In addition, soil pH at day 30 was similar to initial pH when 100% of compound was added as an acid, and hence no net change in soil pH was observed, indicating that by day 30 the H<sup>+</sup> ions dissociated from acid groups were counteracted by the OH<sup>-</sup> ions generated via decomposition of organic anions. Increases in soil pH to values exceeding the initial pH at day 30 for the other ratios (>10:90) were mainly attributed to organic anion decomposition and ammonification (H<sup>+</sup>-consuming process) (Haynes and Mokolobate 2001; Xu et al. 2006b). However, the contribution of the N cycle to pH change was minimal where net ammonification was observed between 2 and 30 days in the Podosol.

In contrast, the pH change over time in the Tenosol depended on the anion-to-acid ratio. From days 2 to 30, soil pH decreased at lower anion-to-acid ratios whereas it increased at higher anion-to-acid ratios. A pH decrease at lower ratios might have resulted from net nitrification as NO<sub>3</sub><sup>-</sup> concentration increased from 2 to 30 days, and the relative change in NH<sub>4</sub><sup>+</sup> was less than NO<sub>3</sub><sup>-</sup> from 2 to 30 days at all anion-to-acid ratios. However, even though nitrification increased with increasing anion-to-acid ratio at 30 days, soil pH increased at higher anion-to-acid ratios. This increase in soil pH at higher anion-to-acid ratios mainly resulted from the C cycle during the decomposition of organic anions. The contribution of direct chemical reactions between soil and organic anions to the soil pH increase would have diminished over time because most of added anions in Tenosol would be decomposed by day 30, as indicated by the data of cumulative CO<sub>2</sub> release in Fig. 3.

### 4.3 Impact of initial soil pH

The effect of anion-to-acid ratio was influenced by the initial soil pH. In the Podosol (initial pH 4.5), soil pH at day 2 was below the initial pH when organic compounds with anion-to-acid ratios <50:50 were added. In comparison, in the Tenosol (initial pH 6.2), pH at day 2 was above the initial pH for all the anion-to-acid ratio treatments. These findings can be explained as follows. The Podosol was an acidic soil that was made more

acidic when a high proportion of organic acids relative to their anions were added. The resulting pH was too acidic for microbial activity as indicated by the low levels of soil respiration. Therefore, the recovery to the initial pH was slower in this soil, and at lower anion-to-acid ratios soil pH did not fully recover to the original pH by day 2. In contrast, in the Tenosol, soil respiration was 3 times greater than the Podosol, indicating faster decomposition of added C by microbes in a more favourable soil pH (pH 6.2). In addition, a lower pHBC of the Tenosol than the Podosol might also contribute to its faster pH recovery.

This study confirmed that soil respiration was lower in the soil with a lower than the soil with higher initial pH. This finding is consistent with other studies that soil respiration and organic matter decomposition were limited at low soil pH (Kemmitt et al. 2006; Khalil et al. 2005; Kyveryga et al. 2004; Motavalli et al. 1995; Xu et al. 2006a; Wang et al. 2012). The lower C decomposition in the Podosol is also likely to be associated with its higher C/N ratio. However, the amount of cumulative CO<sub>2</sub>-C released by day 30 was lower than the amount of added C for the Podosol, indicating incomplete decomposition of the added C. Similar to this study, Hoyle et al. (2008) observed that respired CO<sub>2</sub>-C was less than the added C as glucose in soils of pH between 5.1 and 6.1. They attributed their results to low microbial biomass and low metabolism of added C due to low organic matter and clay contents of the soils.

Moreover, the amount of alkalinity generated (calculated from soil pHBC and pH increase) in the Tenosol at the two sampling dates was greater than that attributed to the decomposition (assume 100%) of added compounds even when 100% acid was added (see Fig. 1). This additional alkalinity is likely to be derived from the decomposition of native soil organic matter which was termed as 'alkalinity priming' (Rukshana et al. 2012), and could partly result from net decreases in NO<sub>3</sub><sup>-</sup> concentration (equivalent to pH increase of 0.05-0.09 units).

#### 4.4 Effect of C compounds

At a given anion-to-acid ratio, addition of citrate/citric acid to the Tenosol increased soil pH to a greater extent than addition of malate/malic acid at day 2. Two reasons may explain this observation. Firstly, the number of carboxyl groups differs between malate and citrate. Citrate/citric acid contain 3 while malate/malic acid have 2 carboxyl groups. Therefore, greater association of H<sup>+</sup> with citrate and/or greater decomposition of the citrate anion were responsible for greater alkalinity generation than malate/malic acid. Secondly, citrate/citric acid might generate a greater priming effect than malate/malic acid.

### 5 Conclusions

This study demonstrated that increasing organic anion-to-acid ratio linearly increased soil pH. The increases in soil pH were mainly attributed to direct chemical reactions and microbial decomposition of organic anions. This study also confirmed that low initial pH delayed and decreased alkalinity release due to incomplete decomposition of the added compound during 30 days of incubation. Moreover, addition of the organic compounds might have stimulated the decomposition of soil organic matter resulting in 'alkalinity priming'. The results from this study have important implications. Plant residues generally contain an excess of cations over inorganic anions to balance organic anions, e.g. malate, oxalate and citrate. Decomposition of these organic anions will be the major contributor to soil pH increase following plant residue addition. Although organic anion-to-acid ratios in plant residues are unknown, it is expected to differ among residue types and thus be important in determining the effect of residue addition on soil pH change.

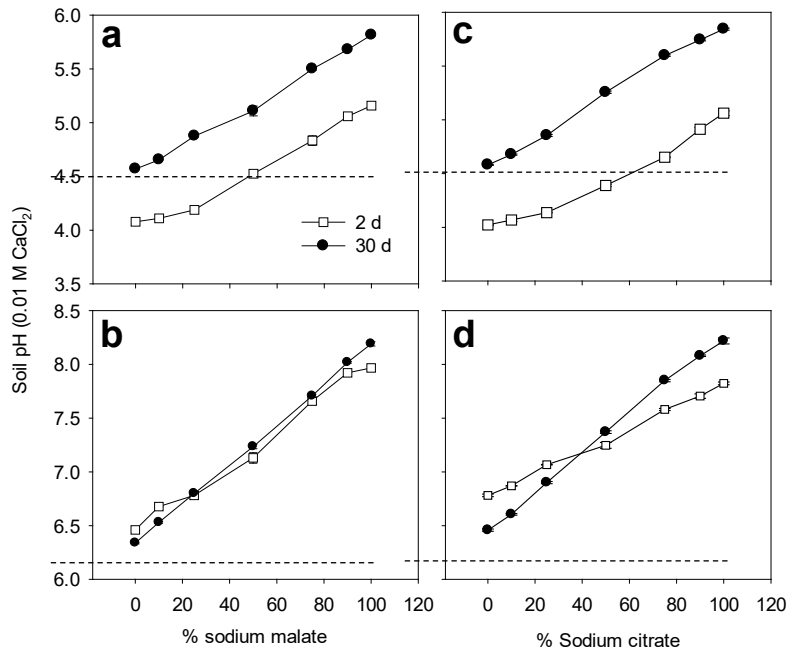
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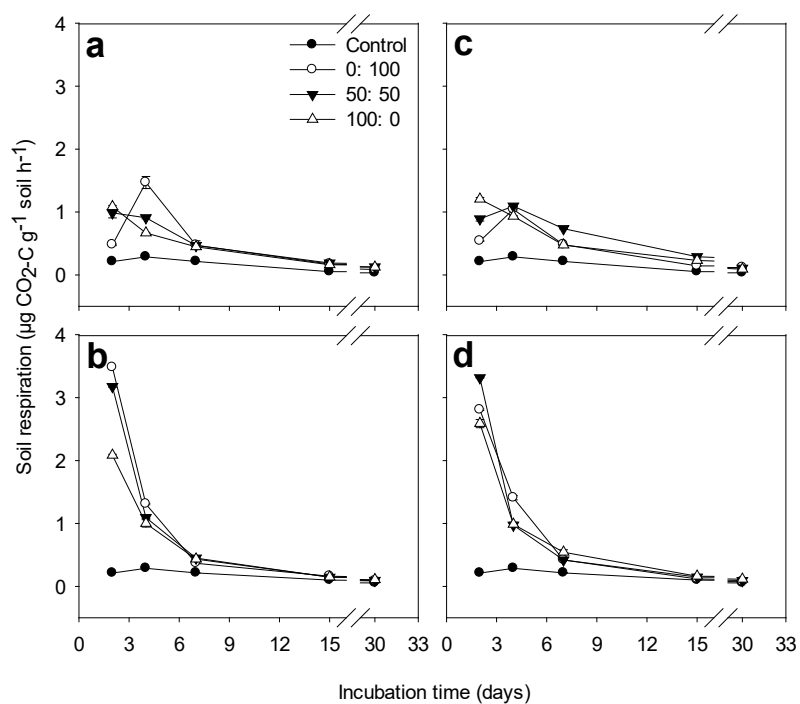
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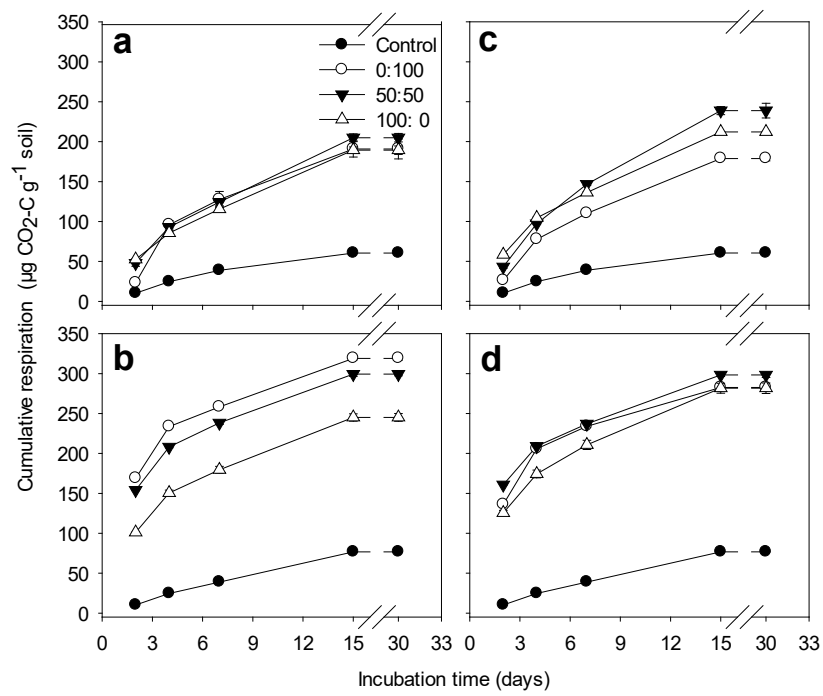


**Fig. 1** Soil pH at 2 and 30 days of incubation of Podosol (a and c) and Tenosol (b and d) with sodium malate and malic acid (a and b) or sodium citrate and citric acid (c and d) at anion-to-acid ratios of 0:100 (0%), 10:90, 25:75, 50:50, 75:25, 90:10 and 100:0 (100%). Bars represent the standard error of the mean ( $n = 3$ ) where they are greater than symbols. Dotted lines indicate the initial soil pH.



**Fig. 2** Soil respiration during 30-day incubation of Podosol (a and c) and Tenosol (b and d) with sodium malate and malic acid (a and b) or sodium citrate and citric acid (c and d) at anion-to-acid ratios of 0:100, 50:50, and 100:0. Non-amended soils were included as controls. Bars represent standard error of the mean (n = 3) where they are greater than symbols





**Fig. 3** Cumulative respiration during 30-day incubation of Podosol (a and c) and Tenosol (b and d) with sodium malate and malic acid (a and b) or sodium citrate and citric acid (c and d) at anion-to-acid ratios are 0:100, 50:50, and 100:0. Non-amended soils were included as controls. Bars represent standard error of the mean ( $n = 3$ ) where they are greater than symbols

**Table 1** ANOVA summary of the effects of compound, anion-to-acid ratio and incubation time, and their interactions on pH, cumulative respiration (CO<sub>2</sub>-C), and NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations for each soil

Parameter	pH		Cumulative CO <sub>2</sub> -C		NH <sub>4</sub> <sup>+</sup> concentration		NO <sub>3</sub> <sup>-</sup> concentration	
	Podosol	Tenosol	Podosol	Tenosol	Podosol	Tenosol	Podosol	Tenosol
Compound (C)	***	***	***	n.s.	***	n.s.	*	***
Ratio (R)	***	***	***	***	***	n.s.	***	***
Time (T)	***	***	***	***	***	***	*	***
C × R	n.s.	***	***	***	***	n.s.	*	***
C × T	***	*	n.s.	n.s.	***	***	**	***
R × T	***	***	***	***	n.s.	n.s.	***	***
C × R × T	***	***	***	n.s.	***	*	*	***

n.s., \*, \*\* and \*\*\* represent probability of  $P>0.05$ ,  $P\leq 0.05$ ,  $P\leq 0.01$  and  $P\leq 0.001$ , respectively

**Table 2** Concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  ( $\text{mg kg}^{-1}$  soil) at 2 and 30 days in Podosol and Tenosol with various anion-to-acid ratios of either malate/malic acid or citrate/citric acid and non-amended controls

Soil	Compound	Anion:acid ratio	$\text{NH}_4^+$ concentration		$\text{NO}_3^-$ concentration	
			2 day	30 day	2 day	30 day
Podosol	Control	-	1.53	2.36	0.78	0.63
	Malate/malic acid	0:100	0.62	0.55	0.55	0.35
		10:90	0.66	0.53	0.45	0.32
		25:75	0.71	0.69	0.51	0.30
		50:50	0.36	0.54	0.21	0.29
		75:25	0.44	1.07	0.28	0.35
		90:10	0.35	1.35	0.04	0.31
		100:0	0.36	1.40	0.05	0.36
	Citrate/citric acid	0:100	0.99	1.09	0.41	0.33
		10: 90	1.34	0.53	0.40	0.32
		25: 75	1.38	0.79	0.49	0.34
		50: 50	0.75	1.38	0.58	0.32
		75: 25	0.34	1.34	0.40	0.29
		90:10	0.38	1.59	0.36	0.34
		100:0	0.34	1.32	0.29	0.33
	LSD ( $P = 0.05$ )		0.31		0.17	
Tenosol	Control	-	0.44	1.28	7.13	10.81
	Malate/malic acid	0:100	0.32	1.58	0.22	4.88
		10:90	0.32	1.36	0.21	4.78
		25:75	0.39	1.33	0.21	4.88
		50:50	0.36	1.28	0.21	5.28
		75:25	0.38	1.36	0.24	5.99
		90:10	0.34	1.39	0.18	6.56
		100:0	0.34	1.40	0.18	6.81
	Citrate/citric acid	0:100	0.50	1.28	0.37	4.50
		10:90	0.36	1.23	0.25	3.69
		25:75	0.38	1.25	0.22	4.22
		50:50	0.33	1.32	0.24	4.75
		75:25	0.36	1.28	0.22	6.13
		90:10	0.38	1.33	0.24	5.86
		100:0	0.35	1.34	0.21	7.32
	LSD ( $P = 0.05$ )		0.15		0.35	