



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 CO₂ priming. Our previous studies showed that such CO₂ priming enhanced alkalinity release but the mechanisms behind this are unknown. This study used ¹⁴C-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. ¹⁴C-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 CO₂ in the Podosol while 56–74% was mineralised in other two soils with malic acid being mineralised more than glucose. The CO₂ 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 NO₃ immobilization while the effect of malic acid via ammonification, NO₃ immobilization and decarboxylation/decomposition of native soil organic matter. This study confirmed that alkalinity priming occurred with concurrent CO₂ priming as a result of C compound addition. This alkalinity priming depended on added C source, initial soil pH and soil organic matter content.

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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 CO₂ 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 CO₂ 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

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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 ; immobilized 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 ($35^{\circ}02'\text{S}$, $147^{\circ}20'\text{E}$); Frankston, Victoria ($38^{\circ}14'\text{S}$, $145^{\circ}22'\text{E}$) and Shepparton, Victoria ($36^{\circ}28'\text{S}$, $145^{\circ}36'\text{E}$). 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 ($\text{R}-\text{COOH}$) functional groups ($\text{pK}_{\text{a}1} = 3.4$, $\text{pK}_{\text{a}2} = 5.13$). 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 $30 \mu\text{l}$ of ^{14}C -glucose (5.55 MBq ml^{-1} ; D-Glucose-UL- ^{14}C ; Sigma–Aldrich) and $22.5 \mu\text{l}$ ^{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 ($4.2 \text{ cm ID} \times 5.5 \text{ cm high}$) 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 $5.0 \text{ kBq vial}^{-1}$). 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 ($\mu\text{g CO}_2\text{-C g}^{-1}$ 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 $^{14}\text{CO}_2\text{-C}$ concentration within NaOH traps was determined by

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 | 5.7 | 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 |
| Tenosol | 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 $\text{mmol H}^+/\text{OH}^- \text{ pH}^{-1} \text{ kg}^{-1}$ soil.

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