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The accumulation of rhizodeposits in organo-mineral fractions promoted biochar-induced negative priming of native soil organic carbon in Ferralsol

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ABSTRACT

Organo-mineral interactions control the stabilisation of soil organic matter (SOM) in mineral soils. Biochar can enhance these interactions via a range of mechanisms including Al-dominant cation bridging in acidic soils, ligand exchange, H-bonding, and π- π-bonding with polycyclic aromatics. But, field-based evidence of their magnitude is lacking. Here we assessed the role of organo-mineral interactions on the observed biochar-induced negative priming of native soil organic carbon (SOC) in a Ferralsol under annual ryegrass. Using repeated pulse labelling, the magnitude of production and fate of recently photosynthesised ^{13}C was traced amongst: soil plus root respiration, root biomass, soil aggregates and aggregate-associated C fractions. Biochar (*Eucalyptus saligna*, 450 °C) amendment (30 Mg ha^{-1}) increased total belowground ^{13}C recovery by 10% compared to the unamended control over the 12 month sampling period. We detected the greatest quantity of rhizodeposit in the mineral-protected SOM within macroaggregates (250–2000 μm). Through synchrotron-based spectroscopic analysis of bulk soils, we provide evidence of a mechanism for biochar-induced negative priming which is the accumulation of rhizodeposits in organo-mineral (*i.e.* aggregate-protected and silt/clay-bound) fractions.

1. Introduction

Biochar-induced priming and its role in promoting organo-mineral interactions and belowground C allocation and retention remain largely unstudied under field settings. Recent studies have shown that biochar can lower SOC mineralisation by 16–48% cf. unamended controls, a process known as negative priming (Whitman et al., 2015). Many mechanisms have been proposed to explain the observed lowering of native soil organic carbon (SOC) mineralisation following biochar incorporation in soils (Keith et al., 2011; Singh and Cowie, 2014; Fang et al., 2017). Recent biochar studies incorporating plant residues to soils in laboratory incubations (Fang et al., 2017), glasshouse studies (Whitman et al., 2014; Keith et al., 2015) and growing plants in field trials (Weng et al., 2015, 2017) have highlighted the impact of plant-soil-biochar interactions on priming effects. It has been proposed that the development of organo-mineral interactions in mineral soil is the principal mechanism of belowground SOC stabilisation, with up to 91% of total SOC resulting from this interaction (Kleber et al., 2015). Biochar amendment to soil can increase organo-mineral interactions (Liang et al., 2010) and physical protection of native SOC (Hernandez-Soriano et al., 2015; Kerré et al., 2016). Weng et al. (2017) reported that a hardwood biochar aged in Rhodic Ferralsol in the field for nearly a decade lowered SOC mineralisation by 5.5% and stabilised rhizodeposits. Thus short-term field-based mechanistic studies are required to understand the effectiveness and mode of action of biochar-driven organo-mineral interactions in this soil type.

In a subtropical pasture system on a Rhodic Ferralsol, Weng et al. (2015) reported that a freshly-applied *E. saligna* biochar (450 °C, dosed at 30 Mg biochar ha^{-1}) lowered the cumulative native SOC mineralisation by 16% compared to an unamended control over 12 months. In the current study, we assessed the mechanisms through which the biochar resulted in the negative priming of native SOC. Using periodic $^{13}CO_2$ pulse labelling and soil physical fractionation coupled with stable ^{13}C isotope partitioning, the magnitude of production and fate of

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recently fixed ^{13}C was quantified amongst: soil plus root respiration, root biomass, and soil aggregate-associated organic fractions. The soil aggregate-associated fractions consisted of free particulate organic matter (F-POM, < 1600 kg m^{-3}), occluded POM (O-POM, > 53 μm) and mineral-protected soil organic matter (M-SOM, < 53 μm), which were separated by a combination of density fractionation (1600 kg m^{-3}), chemical dispersion and wet sieving. We hypothesised that biochar would facilitate stabilisation and accumulation of rhizodeposits in M-SOM fractions via enhanced organo-mineral interactions thus resulting in negative priming of native SOC.

2. Material and methods

2.1. Experiment description

The experiment consisted of 12 circular microplots (440 mm in diameter) with two treatments (n = 6): 1) soil only control and 2) 30 Mg ha^{-1} *E. saligna* biochar mixed into the 0–100 mm soil profile (ca. 3% w/w equivalent). Within each microplot, two respiration collars were established, one for soil respiration and the other for soil plus root respiration (Weng et al., 2015). In brief, of the six micro-plots within each main plot, three were pulse-labelled with 700 μL L^{-1} of $^{13}CO_2$ (99 atom%) and three with 700 μL L^{-1} of $^{12}CO_2$. Soil sampling was carried out on Day 15 after $^{13}CO_2$ pulse labelling events at 4-, 8- and 12-months following the establishment of the experiment. The detailed $^{13}CO_2$ pulse labelling procedure is documented in Weng et al. (2015). Fresh roots were extracted from the field and incubated in the laboratory to assess respiration over 6 h (Weng et al., 2015). A stainless steel corer (40 mm in diameter) was used to sample three intact soil cores (0–80 mm depth) from each microplot. The initial ^{13}C signatures of soil, root, and respiration of soil plus root were detemined by sampling one day before each labelling event. The $\delta^{13}C$ signatures were analysed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). (Table S1). The soil was frozen at −20 °C and defrosted before sieving.

2.2. Aggregate size and density fractionation

Macroaggregates (250–2000 μm) and microaggregates (< 250 μm) were separated by dry sieving through firstly 2000 then 250 μm mesh using a vibratory sieve shaker (FRITSCH, Analysette 3 Pro, Germany) at an amplitude of 1.5 mm for 5 min (Gunina and Kuzyakov, 2014). There were no large macroaggregates (> 2000 μm) obtained from the soil in this study. Further separation of individual aggregate size classes into free POM (F-POM), occluded POM (O-POM), and mineral-protected soil organic matter (M-SOM, combining silt- and clay-bound SOM) was achieved using the method modified from Gunina and Kuzyakov (2014) and Six et al. (1998). Briefly, F-POM was separated as freely floating particles (ρ < 1600 kg m^{-3}) after gently inverting aggregates in a 1600 kg m^{-3} sodium polytungstate solution. The remaining aggregates (ρ > 1600 kg m^{-3}) were then dispersed in 0.5% sodium hexametaphosphate with 18-h shaking on a reciprocal shaker. The dispersed aggregates were then passed through a 53-μm sieve for separation into the M-SOM (< 53 μm) and O-POM (> 53 μm) fractions.

2.3. Quantification of photosynthesized ^{13}C in various belowground C pools

The C and N contents and ^{13}C signatures of bulk soil, aggregates and their associated fractions were quantified by Dumas combustion (Chan et al., 2008) coupled with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) (Weng et al., 2015). The ^{13}C recovery in various C pools at time t (*i.e.* $A^{13}C_{i,t}$, in %) was calculated by dividing the amount of ^{13}C (g m^{-2}) in a specific C pool (*i.e.* C_i) by the initial amount of total added $^{13}CO_2$ (g m^{-2}) at each labelling event (*i.e.* $^{13}C_{added}$):

$$A^{13}C_{i,t} = (^{13}C_{excess,t} \times C_i)/^{13}C_{added} \times 100$$

Where $_i$ represents soil plus root respiration, root biomass, soil aggregates or its associated fractions, $^{13}C_{excess,t}$ indicates the increment of the ^{13}C atom% of an individual C pool from its natural abundance level at a specific sampling time, t. Detailed calculations for ^{13}C atom% and $^{13}C_{excess,t}$ are documented in Hafner et al. (2011) and Fang et al. (2016).

2.4. Synchrotron based edge X-ray absorption fine structure

To better develop our understanding of the quality and characteristics of C accumulated in rhizodeposits, we employed synchrotron-based soft X-ray (SXR) analysis on the bulk soils from 1) planted biochar-amended respiration collars; 2) unplanted biochar-amended collars; and 3) the planted control (nil biochar). Synchrotron based near edge X-ray absorption fine structure (NEXAFS) was implemented at the SXR Spectroscopy beamline (14ID) at the Australian Synchrotron (AS). The spectra were collected over a photon energy range of 275–325 eV with a step size of 0.1 eV. The spectra were collected at an angle of 100° to the beam. The energy calibration was carried out using a graphite standard in the beamline which was collected simultaneously with the I_0 and sample NEXAFS spectra.

2.5. Biometrical analysis

All statistical analyses of ^{13}C recovery in C pools were conducted within the R environment (R Development Core Team, 2012). When significant F-tests were obtained (P = *.05*), the separation of means was achieved using a least significant difference (LSD) test at 5% probability.

3. Results

3.1. Total accumulation of recent C belowground

Biochar amendment resulted in a gradual increase in the total belowground ^{13}C over the 12 month study, whereas belowground ^{13}C recovery in the unamended control remained constant at each pulse labelling event (Table 1). A greater proportion of the total recovered ^{13}C, including soil plus root respiration, root biomass, soil aggregates

Table 1
Proportion of belowground ^{13}C recovery relative to the total allocated ^{13}C enrichment (in percentage) within the control and biochar-amended soil + root respiration collars at 4-, 8- and 12- month pulse labelling events. Standard errors are given in brackets (n = *3*).*, P < *.05*.

Pulse labelling at	4 month				8 month				12 month			
Belowground ^{13}C recovery (%)	Control		Biochar		Control		Biochar		Control		Biochar	
Soil + root respiration	4.3*	(0.1)	2.9	(0.1)	2.4	(0.4)	1.8	(0.4)	5.0*	(0.2)	3.7	(0.3)
Root	26.1	(0.9)	24.3	(1.1)	24.4	(3.4)	21.8	(3.8)	10.0	(1.9)	9.7	(1.6)
Soil	12.7	(2.7)	17.4	(2.7)	22.4	(5.3)	23.4	(2.6)	28.5	(2.4)	40.1*	(2.4)
Sum	43.1	(3.8)	44.6	(3.9)	49.2	(9.1)	47.0	(6.7)	43.5	(4.5)	53.5*	(4.3)