

## BELOWGROUND RESPONSES TO CLIMATE CHANGE

# Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorous inputs to soils

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## Summary

1. The soil sink for carbon is important in regulating climate and soil fertility. The sink strength is dependent on the balance of soil carbon decomposition and formation. Variation in the rates of these processes to manipulations of resource availabilities under global change, such as elevated atmospheric carbon dioxide, is not explained by soil microbial theory.

2. To investigate disparate responses of soil carbon dynamics in field investigations, to altered carbon, nitrogen and phosphorous availability, we couple fractionation, isotope and mesocosm techniques to quantify soil carbon decomposition and formation under different resource regimes. These regimes involve addition of multiple levels of carbon, nitrogen and phosphorous, alone and in combination.

3. We hypothesize that: (i) there is no net effect of labile carbon input rate on soil carbon stocks because reductions in soil carbon decomposition are offset by reductions in soil carbon formation; (ii) with simultaneous nutrient addition soil carbon stocks will increase because nitrogen will inhibit further soil carbon decomposition, and mitigate reductions in soil carbon formation observed under elevated labile carbon availability alone; (iii) this increase in soil carbon stocks will be a product of greater formation and decreased decomposition of slower-cycling, mineral-associated, soil carbon, whereas less stable, particulate soil carbon will simply turnover faster (due to greater soil carbon decomposition and formation).

4. In contrast to our predictions formation of soil carbon is positively correlated with labile carbon input rates. In addition, nutrient amendment does not interact with carbon amendment to affect total soil carbon contents. However, there are significant interactive effects when the formation and decomposition responses of different soil carbon fractions are considered. For nitrogen alone, its effects on soil carbon fractions follow our hypotheses. However, phosphorous amendment increases decomposition of the soil carbon fraction that constitutes a longer-term sink.

5. Our results highlight the need for rhizodeposition, phosphorous and soil carbon fractions to be explicitly considered when interpreting potential soil organic carbon responses to altered resource availability. In the discussion, we make four recommendations for future investigations to improve our understanding of soil carbon responses to altered carbon, nitrogen and phosphorous availabilities.

**Key-words:** carbon sequestration, carbon sink, decomposition, nitrogen, nitrogen deposition, nutrient availability, phosphorous, priming effect, rhizodeposition, soil organic carbon

## Introduction

Global changes such as elevated atmospheric CO<sub>2</sub> concentrations and nitrogen (N) deposition rates are altering the input

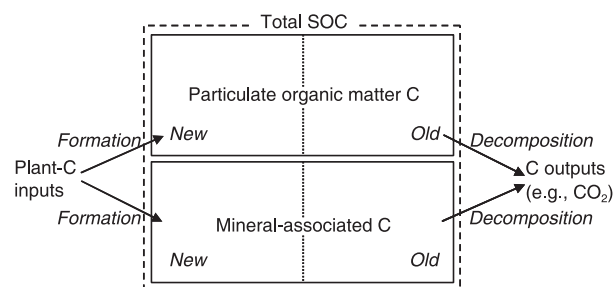
rates of carbon (C) and N to soils (van Groenigen *et al.* 2006; Hyvönen *et al.* 2007). There is experimental evidence that the resulting changes in C and N availabilities in soils alter the magnitude of the imbalance between soil organic carbon (SOC) decomposition and formation processes, thereby affecting total SOC contents (Neff *et al.* 2002; Fontaine *et al.*

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2004; van Groenigen *et al.* 2006). An improved understanding of how altered C and N input rates to soils will affect SOC decomposition and formation processes may enable more accurate prediction of how SOC stocks will respond to global change, and feedback to atmospheric CO<sub>2</sub> concentrations and soil fertility (Lal 2004).

Mechanistic investigations typically treat SOC as consisting of a continuum of C-containing fractions whose cycling rates vary from < 1 year to millennia (Olk & Gregorich 2006; von Lützow *et al.* 2007). It is often defined as material containing, at least partially, humified matter (Paul, Morris & Böhm 2001). Following this definition then, new C-inputs to soils (e.g. plant litter) do not increase SOC stocks; instead these inputs must first be processed by decomposers before 'formation' of SOC. The size of SOC fractions is determined by the balance of SOC 'formation' and 'decomposition'. The latter is often used as a catch-all term (e.g. Cardon *et al.* 2001) to include loss processes such as mineralization of SOC to CO<sub>2</sub>, as well as leaching of C solubilized from SOC as it is further humified (Fig. 1).

That we cannot reliably explain varying responses of SOC stocks in different environments to increased inputs rates of C and N to soils, is perhaps the best evidence that we do not have the required mechanistic understanding of SOC formation and decomposition processes. For example, the positive



**Fig. 1.** Conceptual representation of the fractions and processes that determine the size of the total SOC pool (broken-line box). This representation is an operational definition for the purposes of the current study; SOC fractions and processes may be conceptualized a number of different ways. Here, total SOC is separated into two fractions (solid-line boxes: particulate organic matter and mineral-associated C), which have different turnover times and hence C sink capacities. SOC formation occurs when C inputs to the soil are humified (e.g. through microbial activity); they may enter either SOC fraction. It is also likely that SOC in the more rapidly cycling fraction (i.e. the particulate matter) moves into the mineral-associated fraction across the experiment but this arrow is not shown. Carbon that is added to the soils during the experiment, and that enters either SOC fraction, is classified as 'new' SOC. In contrast, C present prior to the experiment is classified as 'old' SOC; the net decrease in the size of this pool of old C occurs through processes referred to collectively as 'decomposition'. The operational definitions used in the current study neither permit movement of old C between fractions to be estimated nor the 'decomposition' of new SOC that occurred during the study. This is because, by definition, no new SOC was present in the soils at the start of the experiment. Decomposition of old SOC therefore represents treatment effects on the *net* decrease in old SOC within a fraction; formation represents the *net* increase in new SOC within a fraction. See text for further details.

relationship between soil N fertilization rate and SOC stocks under elevated CO<sub>2</sub> (van Groenigen *et al.* 2006) mirrors the situation in cropping systems where increases in residue inputs under N fertilization result in higher SOC contents (Gregorich *et al.* 1996; McLauchlan 2006). However, effects of N fertilization on SOC stocks in non-agricultural grasslands and forests have not necessarily translated to greater SOC stocks, despite increases in plant inputs to soils. Indeed, positive (Waldrop *et al.* 2004), negative (Mack *et al.* 2004; Waldrop *et al.* 2004) and negligible (Neff *et al.* 2002) effects of N fertilization on SOC stocks have been observed.

One reason why positive, above-ground plant productivity responses to N fertilization may not necessarily translate to greater SOC stocks (Neff *et al.* 2002) is that the majority of plant-C in non-cropped systems enters mineral soils via roots (van Groenigen *et al.* 2006). There is now substantial evidence that of the root-C entering these soils, the dominant input is in the form of labile-C compounds (i.e. rhizodeposition) (van Hees *et al.* 2005; Högberg & Read 2006; Boddy *et al.* 2007). Enhanced rhizodeposition rates under elevated atmospheric CO<sub>2</sub> are likely to increase supply rates of labile plant-C to mineral soils (Pendall, Mosier & Morgan 2004). Undoubtedly, some of this rhizodeposited C will be incorporated into SOC as microbially derived compounds (Lundberg, Ekblad & Nilsson 2001). Yet despite evidence for elevated rhizodeposition, Heath *et al.* (2005) observed decreasing SOC formation of root-derived C under increasing atmospheric CO<sub>2</sub> concentrations. If this effect is observed in other systems, then the negligible or positive effects of elevated atmospheric CO<sub>2</sub> on SOC stocks (Jastrow *et al.* 2005; van Groenigen *et al.* 2006) suggest that enhanced root inputs under elevated CO<sub>2</sub> must also reduce rates of SOC decomposition. That is, for decreased SOC formation to be associated with positive or negligible effects on total SOC stocks requires that SOC decomposition must simultaneously decrease.

The possibility that enhanced rhizodeposition might reduce rates of SOC decomposition needs to be explored. Indeed, some experimental observations suggest that labile C inputs to soils result in no SOC decomposition (Dalenberg & Jager 1981; Wu, Brookes & Jenkinson 1993; Fontaine & Barot 2005). The apparent absence of a 'priming effect' (Cheng 1999; Kuzyakov, Friedel & Stahr 2000) on SOC in such instances has been attributed to the utilization of labile C inputs by microbes which do not mineralize SOC and out-compete those that do (Fontaine *et al.* 2004; Fontaine & Barot 2005). This 'preferential substrate utilization' theory (Dalenberg & Jager 1981; Wu *et al.* 1993; Fontaine & Barot 2005) contrasts with priming effect theory because the latter proposes that the increase in microbial activity with elevated labile C inputs will concomitantly stimulate SOC decomposition. Notably, Heath *et al.* (2005) observed greater SOC formation of root-derived C with N fertilization, mitigating decreases in SOC formation under elevated CO<sub>2</sub>. This effect, combined with the possibility that exogenous N addition inhibits SOC decomposition (Franklin *et al.* 2003; Hyvönen *et al.* 2007), may explain why SOC contents increase when systems are exposed simultaneously to N and CO<sub>2</sub> fertilization

(van Groenigen *et al.* 2006), but not necessarily N or CO<sub>2</sub> fertilization alone (Neff *et al.* 2002; van Groenigen *et al.* 2006). To understand the mechanisms underlying these responses requires studies that quantify both SOC formation and decomposition, and not just the net balance of these processes, under experimentally controlled, labile C and N input rates to soils.

Much of the empirical work examining how changes in plant-C inputs and N availability will impact SOC stocks measures rates of CO<sub>2</sub> loss from soils across short time-scales (Cheng 1999; Kuzyakov *et al.* 2000; Fontaine *et al.* 2004). However, soil CO<sub>2</sub> efflux responses do not necessarily predict how SOC stocks will respond to global change factors (Melillo *et al.* 2002; Knorr *et al.* 2005b). The reason for this is that the magnitude and variability of soil CO<sub>2</sub> efflux is largely dependent on the dynamics of a rapidly cycling pool of C (Gu, Post & King 2004; Kirschbaum 2004) and this highly labile SOC pool constitutes only a tiny fraction of the total SOC. Indeed, SOC is not a single entity but rather a conglomeration of multiple pools of C cycling at different rates (Ågren & Bosatta 2002; Kirschbaum 2004; Eliasson *et al.* 2005; Knorr *et al.* 2005b), each of which may respond differently to changes in resource availability (Cardon *et al.* 2001; Neff *et al.* 2002; Manning *et al.* 2006). If we want to understand how SOC stocks will respond to variation in N fertilization and labile-C input rates, we need to measure directly the responses of individual SOC fractions.

Here, we amended intact soil monoliths with C, N and phosphorous (P) across 1 year. We manipulated P, as well as N, because much of our understanding of how N fertilization affects SOC comes from studies where P is added simultaneously with N (e.g. Cardon *et al.* 2001; Mack *et al.* 2004; Heath *et al.* 2005); also P may both affect SOC dynamics itself and modify the impacts of N alone (Cleveland & Townsend 2006). Furthermore, as with C and N, human activities are enhancing P availabilities in soils (Wassen *et al.* 2005). We amended soils chronically (twice-weekly) across a range of input levels to investigate the potential for input rate dependent effects of C and nutrients (e.g. as seen in van Groenigen *et al.* 2006). We utilized <sup>13</sup>C-techniques to partition the effects of C, N and P additions into those on SOC formation and decomposition (Fig. 1). We resolved these dynamics for the total SOC pool and for individual SOC fractions which differ in their sink potentials. Following from our current understanding of labile C and N effects on SOC processes, we hypothesized that (i) there is no net effect of labile C input rate on SOC stocks because decreased SOC decomposition is offset by decreased SOC formation. Conversely, we hypothesized that (ii) with simultaneous nutrient addition total SOC stocks will increase because N will inhibit SOC decomposition, and also mitigate decreases in SOC formation observed under elevated labile-C inputs alone. We hypothesized that (iii) this increase in SOC stocks will be a product of increased SOC formation and decreased decomposition of slower-cycling, mineral-associated SOC fractions; whereas less stable, particulate SOC fractions will simply turnover faster (i.e. greater formation balanced by greater decomposition).

## Materials and methods

### EXPERIMENTAL DESIGN

Intact soil monoliths (28.5 cm deep × 15.3 cm diameter encased in PVC piping) were collected from a mixed hardwood stand dominated by *Quercus alba* L. within the Duke Forest Teaching and Research Laboratory, North Carolina, USA (35°97' N, 79°08' W, elevation 150 m, MAT 14.6 °C, MAP 1100 mm). The soil was a low-fertility, sandy loam of the White Store series. This highly weathered series falls within the general classification of Ultisols, which dominate the southeastern US and enormous parts of tropical and warm temperate regions, and are characterized by their low inherent fertility (Richter & Markewitz 2001). Specifically, at our site total percentage C, N and P were 1.200 ± 0.0495, 0.046 ± 0.0019 and 0.012 ± 0.0020, respectively (mean ± SE; *n* = 8 spatial replicates). The soil texture was classified as loamy sand, with 15% silt and 5% clay; base saturation was 16% and CEC (meq 100 g dry soil<sup>-1</sup>) 2.1. Litter was removed from the monoliths (or soil cores) to standardize surface available nutrients and then maintained under constant environmental conditions (17.5 °C) in a controlled-environment greenhouse of the Duke University Phytotron. We used controlled-conditions to tightly control resource inputs to soils.

Resource amendment treatments were assigned randomly within blocks (*n* = 3) and mesocosm locations within blocks were re-assigned every 14 days. Treatments were initiated after 3 months of acclimation to controlled-environment conditions and consisted of twice-weekly, surface additions of C, N and/or P in 175 mL of deionized H<sub>2</sub>O solution as sucrose, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and/or KH<sub>2</sub>PO<sub>4</sub>, respectively. Note that the sucrose C had a different <sup>13</sup>C value to the SOC and so their independent fates (i.e. formation and decomposition, respectively; Fig. 1) could be resolved (see below). Sucrose was used because disaccharides are the dominant constituent of rhizodeposited C (van Hees *et al.* 2005), and SO<sub>4</sub><sup>2-</sup> was used as the counterion to NH<sub>4</sub><sup>+</sup> because it has minimal effects on soil microbes and pH (Gulledge, Doyle & Schimel 1997). Given the regularity of the resource amendments, and as the soils were mostly free-draining, soil moisture was relatively constant across time and no water-logging was observed (mean ± 1 SE = 17 ± 0.4%; from weekly measurements of four additional monoliths, receiving only water as for the controls, using a Campbell Scientific Inc. (Logan, UT) CD620 and CS620 TDR probe with 12 cm rods).

Our design consisted of three, discrete experiments. In the first, the 'Factorial Experiment', three levels of C were added (0, 150 and 800 g C m<sup>-2</sup> year<sup>-1</sup>) in a factorial combination with three levels of N : P (0 : 0, 3 : 0.6 and 10 : 2 g N : P m<sup>-2</sup> year<sup>-1</sup>). In the second, the 'C Regression Experiment', five levels of C were added (0, 70, 150, 350 and 800 g C m<sup>-2</sup> year<sup>-1</sup>) in the absence of any N or P. The third, the 'NP Regression Experiment', involved addition of five levels of N, (0, 1, 3, 6 and 10 g N m<sup>-2</sup> year<sup>-1</sup>), five levels of P (0, 0.2, 0.6, 1.2 and 2 g P m<sup>-2</sup> year<sup>-1</sup>), and the same five levels of N and P added together (0 : 0, 1 : 0.2, 3 : 0.6, 6 : 1.2 and 10 : 2 g N : P m<sup>-2</sup> year<sup>-1</sup>). Additions were made across 1 year, providing 104 addition events per monolith.

We selected the C amendment rates to reflect the potential range of labile C addition rates observed in soil. We estimated labile C addition rates by assuming that soil heterotrophic respiration is dominated by the mineralization of labile C pools (Gu *et al.* 2004; Knorr *et al.* 2005b). Published soil respiration rates vary substantially across ecosystems and average 1139 g C m<sup>-2</sup> year<sup>-1</sup> (Rustad *et al.* 2001). We divided the published C fluxes by two under the assumption that root respiration contributes to half of total soil CO<sub>2</sub> efflux (Hanson *et al.* 2000). N amendment rates were selected to

span the global range of atmospheric N deposition levels (Fenn *et al.* 2003; Galloway *et al.* 2004; Holland *et al.* 2005). Phosphorous deposition rates are less well quantified but, when done so, range from 0.07 to 2 kg P ha<sup>-1</sup> year<sup>-1</sup> for total wet and dry deposition (Newman *et al.* 1995; Anderson & Downing 2006; Oelmann *et al.* 2007) to as much as 88 kg P ha<sup>-1</sup> year<sup>-1</sup> for net fertilizer accumulation (i.e. fertilizer P input – crop P removal; Bennett, Carpenter & Caraco 2001). On average, in the European Community net P-fertilizer accumulation is 12.8 kg P ha<sup>-1</sup> year<sup>-1</sup> (Bennett *et al.* 2001). Our chosen P amendment rates therefore span from high atmospheric deposition rates to low to high net fertilizer accumulation rates.

#### SOIL ANALYSES

After 12 months of resource amendment, the top 10 cm of the upper mineral horizon of each monolith was harvested, sieved to 2 mm, and homogenized. We chose 0–10 cm as a depth because (i) the soils were relatively stone-free across this depth and thus more homogeneous across experimental units; (ii) responses in surface soils tend to occur more rapidly than at depth due to greater biotic activity; and (iii) it was a manageable volume of soil. We removed the organic soil (i.e. the horizon typically designated 'H' or 'O') prior to soil processing because it was too shallow (approximately 0.5–1-cm deep) to work with alone and, in addition, its inclusion in the mineral sample would have introduced additional variability in soil carbon contents across experimental units. The bulk density of the mineral soil layer was 1.245 ± 0.0223 g soil cm<sup>-3</sup> ( $n = 63$ ; mean ± 1 SE) and was unaffected by treatment. Hence, all C data are presented as concentration of C per unit volume of soil.

A subsample of the upper mineral horizon soil was air-dried and used for SOC determinations. Fractionation into particulate organic matter (POM) and mineral-associated C pools was achieved using a chemical dispersant (sodium hexametaphosphate: NaHMP) followed by physical fractionation; we classified material that passed through a 53-µm sieve as mineral-associated and material retained on this sieve as POM C (Paul *et al.* 2001). Specifically, 30 g of air-dried soil was dispersed with 100 mL of NaHMP for 18 h in rectangular 175 mL Nalgene bottles placed on their side in an end-to-end shaker at high velocity; the approach is effective at fully dispersing soils, including breaking-up microaggregates. After 18 h, soils were passed through the 53 µm sieve. We ensured that all mineral-associated material was rinsed through using DI water and then both fractions were dried to constant weight at 105 °C; air-dried soil was also dried at this temperature to permit air-dry to oven-dry weight conversions. Fractions were ball-milled to a fine powder prior to chemical determinations (see below).

While there is no one method for determining meaningful SOC fractions (Olk & Gregorich 2006; von Lützow *et al.* 2007), mineral-associated C pools are expected to have slower turnover times and greater long-term sink capacity than POM C pools (Schlesinger & Lichter 2001). This expectation was supported by the higher  $\delta^{13}\text{C}$  values (Collins *et al.* 2000) for the mineral-associated C ( $n = 3$ ; mean ± 1 SE =  $-27.5 \pm 0.03\text{‰}$ ) than for the POM C ( $n = 3$ ; mean ± 1 SE =  $-28.0 \pm 0.06\text{‰}$ ), which suggests that mineral-associated C has been processed to a greater extent. Further, recent work shows that C in the POM fraction is largely plant-derived, while that in the mineral-fraction is largely more recalcitrant, microbially derived material (Grandy & Robertson 2007; von Lützow *et al.* 2007). Soil C and N concentrations, and  $\delta^{13}\text{C}$  values, were determined using elemental analysis and continuous flow, isotope ratio mass spectrometry (Thermo, San Jose, CA). Analytical precision was ± 0.1  $\delta^{13}\text{C}\text{‰}$ . Working gas and soil standards were calibrated to

PDB using NIST-SRM 1577b Bovine Liver as a reference standard. The absolute ratio (R) for  $^{13}\text{C}/^{12}\text{C}$  'locked-into' the THERMO software is 0.011180199.

Two competing processes can influence the size of the SOC pool, the conversion of C inputs into SOC (i.e. formation; Fig. 1) and the decomposition of extant SOC. To quantify these two processes we used isotope mixing models (e.g. Ineson *et al.* 1996). The native SOC had a C<sub>3</sub>-photosynthetic value ( $n = 3$ ; mean ± 1 SE =  $-27.6 \pm 0.09\text{‰}$ ), whereas the sucrose-C had a C<sub>4</sub>-photosynthetic value ( $-11.59$ ), the difference in the C isotope composition between the two being sufficient to discriminate sources (Staddon 2004). SOC formation was calculated as follows (*sensu* Ineson *et al.* 1996):  $C_{\text{formation}} = C_{\text{fraction}} \cdot (\delta^{13}\text{C}_{\text{final}} - \delta^{13}\text{C}_{\text{native}}) / (\delta^{13}\text{C}_{\text{input}} - \delta^{13}\text{C}_{\text{native}})$ , where  $C_{\text{fraction}}$  is the measured size of the fractions (total, POM, or mineral-associated SOC),  $\delta^{13}\text{C}_{\text{final}}$  and  $\delta^{13}\text{C}_{\text{native}}$  are the  $\delta^{13}\text{C}$  values of these fractions (at the end of the experiment) from the C-amended, and water only-amended soils, respectively, and  $\delta^{13}\text{C}_{\text{input}}$  is the C isotope value of the sucrose.

Duplicate subsamples of fresh soil were used to determine pH, measured after mixing soil with H<sub>2</sub>O 1 : 1 by volume (Allen 1989), gravimetric soil moisture (24 h at 105 °C) and microbial biomass. The latter was measured using modified substrate-induced respiration (SIR) (West & Sparling 1986) and a chloroform fumigation-extraction (CFE) protocol described in Fierer & Schimel (2003). SIR primarily measures active microbial biomass (Wardle & Ghani 1995) while CFE estimates the biomass of both active and inactive microbes. Triplicate subsamples of fresh soil were used for determinations of K<sub>2</sub>SO<sub>4</sub>-extractable NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations on a Lachat autoanalyzer (Milwaukee, WI) using Lachat methods 31-107-06-5-A and 12-107-04-1-B, respectively. Potential CO<sub>2</sub> production rates were determined over 30 day incubations at 20 °C, using the method described in Fierer *et al.* (2005). The measurement of C respiration rates in this manner provides a coarse assessment of microbially available resource concentrations.

#### STATISTICAL ANALYSES

To analyse the Factorial Experiment we constructed a full interaction linear model with two, discrete factors (C effect and NP effect). As in the regression models used to analyse the C and NP Regression Experiments, block was included as a non-interacting factor. For statistical significance we assumed an  $\alpha$ -level of 0.05. In none of our analyses did we detect marginally significant (i.e.  $P < 0.1$  but  $> 0.05$ ) treatment effects and so when non-significance is reported as  $P > 0.05$  it can be read as  $P > 0.1$ . Statistical analyses were performed in S-Plus 7.0 (Insightful Corporation, Seattle, WA). Data were tested for assumptions of normality and homogeneity of variance; when violated data were log<sub>e</sub>-transformed to meet these assumptions.

## Results

#### FACTORIAL EXPERIMENT

There were significant effects of both C and NP amendments on total SOC contents but the interaction term was not significant (Table 1). Given this non-significant interaction, the effects of C amendment are presented pooled across the NP amendments (Fig. 2a) and the NP amendment effects pooled across the C amendment (Fig. 2b). Low rates of C amendment (150 g C m<sup>-2</sup> year<sup>-1</sup>) reduced the sum (i.e. old + new) SOC content, but high rates of C amendment (800 g C m<sup>-2</sup> year<sup>-1</sup>) increased it, relative to the no C amendment

**Table 1.** ANOVA results for the effects of C and NP amendments on SOC in the Factorial Experiment

SOC pool	SOC fraction	C effect		NP effect		C × NP effect		Transform
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
Old + new	Total	5.82	0.013	5.36	0.017	ns	ns	none
	POM	30.9	< 0.001	ns	ns	4.16	0.017	log <sub>e</sub>
	Mineral	ns	ns	6.35	0.009	ns	ns	log <sub>e</sub>
Old	Total	4.30	0.032	6.12	0.011	ns	ns	none
	POM	24.4	< 0.001	ns	ns	4.33	0.015	log <sub>e</sub>
	Mineral	ns	ns	7.28	0.006	ns	ns	none
New	Total	186	< 0.001	14.3	0.001	ns	ns	log <sub>e</sub>
	POM	131	< 0.001	7.22	0.011	ns	ns	log <sub>e</sub>
	Mineral	52.3	< 0.001	11.5	0.003	4.16	0.049	none

SOC is divided into two pools (new and old), which can be summed to estimate the amount of C in the total SOC, or in the fractions (POM and mineral) of which it consists (Fig. 1). Examination of the old SOC permits an understanding of treatment impacts on SOC decomposition; and of new SOC on SOC formation (see text). *F* and *P* values are reported for significant ( $P < 0.05$ ) model terms; ns: not significant. Degrees of freedom (d.f.) were 2 and 4 for the sum (old + new) and old pool main and interaction effects, respectively; residual d.f. was 16. For the new pool, d.f. was 1 for the main C effect and 2 for the main NP and interaction effects; residual d.f. was 10. Data transforms are shown in the last column.

treatment (Fig. 2a). At the low C amendment rate, the lower sum SOC largely resulted from decomposition of the old SOC. That is, the amount of old SOC was markedly lower under low C amendment, compared to the other C amendment rates, and formation of new SOC was too small to compensate for this decomposition (Fig. 2a). In contrast, under high C amendment the amount of decomposition of old SOC was similar to that observed in soils receiving no additional C (i.e. they had similar old SOC contents). The greater sum SOC under high C amendment, when compared to the no C amendment treatment, was therefore the result of new SOC formation (Fig. 2a).

The addition of N and P reduced the sum SOC contents, relative to the control non-NP amended soils (Fig. 2b). In contrast to the C amendment effects, the effects of N and P on the sum SOC were unaffected by the amendment rate (i.e. low and high rates had similar effects on old and new SOC; Fig. 2b). The net decrease in the sum SOC occurred despite the greater formation of new SOC under NP amendment; that is, the increased decomposition of old SOC with NP amendment 'swamped-out' the positive effects on new SOC formation (Fig. 2b).

The SOC pool is not homogeneous. By examining the effects of C and NP amendments on the POM and mineral-associated C fractions, we can better understand how the changes in the total SOC will affect shorter- to longer-term SOC storage (see Fig. 1). The effects of C and NP amendments on the POM and mineral-associated C fractions were distinct from the effects observed for the total SOC. Specifically, no significant interactive effects of C and NP amendment were observed for the total SOC (Fig. 2 and Table 1) but interaction effects between the amendments were observed on the POM and mineral-associated fractions (Table 1).

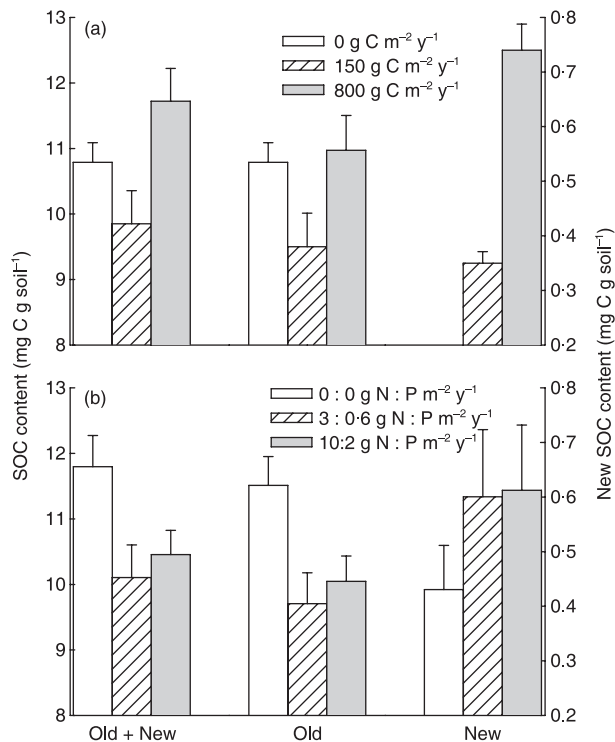
For the sum (old + new) POM fraction, the significant C × NP interaction (Table 1) resulted from the response of the old POM C. Specifically, NP amendment increased

decomposition of old POM C under low C amendment but had no effect under high C amendment (Table 1, Fig. 3a). Despite positive effects of both C and NP amendment on formation of new POM C (Table 1; see also Supplementary Table S1 in Supplementary Material), these gains of new C were too small to offset the influence of old POM C decomposition on the sum POM C response (Supplementary Table S1).

Decomposition of the mineral-associated C was significantly greater under NP amendment (Table 1, Supplementary Table S1). Means ± 1 SE (mg C g soil<sup>-1</sup>) for this fraction (pooled across C amendment levels given a non-significant interaction) for 0 : 0, 3 : 0.6 and 10 : 2 g m<sup>-2</sup> year<sup>-1</sup> N : P amendment rates were: 8.2 + 0.31, 6.5 + 0.28 and 7.1 + 0.37, respectively. In contrast, NP amendment effects on formation of new mineral-associated C were dependent on C amendment rates (significant C × NP interaction; Table 1). That is, positive effects of NP amendment on formation were apparent at both NP amendment rates under high C amendment but only apparent at the highest NP amendment rate under low C amendment (Fig. 3b). Note that for investigating C addition effects on new SOC formation, only data from the two C amendment levels (i.e. 150 and 800 g C m<sup>-2</sup> year<sup>-1</sup>) and not the water-only amendment were included in the statistical analyses.

#### REGRESSION EXPERIMENTS

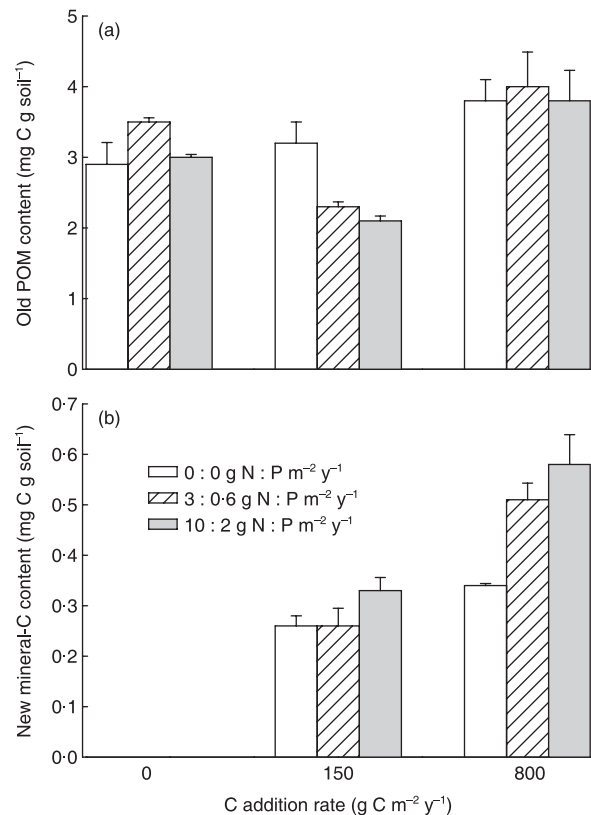
The Regression Experiments confirmed and further elucidated the SOC responses to C and NP amendments observed in the Factorial Experiment. In the C Regression Experiment the decomposition of old POM C was more or less linearly retarded with increasing rates of C amendment ( $F_{1,11} = 7.39$ ,  $P < 0.05$ ; Fig. 4). Across the same gradient there was a positive, linear response of new POM C formation ( $F_{1,11} = 80.2$ ,  $P < 0.001$ ; Fig. 4). No significant effects of C amendment on decomposition of mineral-associated C were observed



**Fig. 2.** Main effects of C (a) and NP (b) amendment on total SOC contents in the Factorial Experiment. The total SOC is divided into three pools: the sum SOC (old + new) which shows the net result of SOC decomposition and formation on total SOC content, the old SOC which shows the effects of decomposition and the new SOC which shows SOC formation during the experiment. The left y-axis is to be read for the sum and old SOC pool responses, and the right y-axis for the new SOC pool responses. There were significant ( $P < 0.05$ ) main effects of C and NP amendment but no significant interactions (see Table 1 and text). In (a) C amendment rates in  $\text{g C m}^{-2} \text{ year}^{-1}$  are 0 (white bars), 150 (striped bars) and 800 (grey bars). In (b) NP amendment rates in  $\text{g N : P m}^{-2} \text{ year}^{-1}$  are 0 : 0 (white bars), 3 : 0.6 (striped bars) and 10 : 2 (grey bars). Values are means  $\pm 1$  SE ( $n = 9$ ; data pooled across N : P amendment levels in (a) and C amendment levels in (b) given a non-significant interaction between these factors). At the start of the experiment, the mean  $\pm 1$  SE for the total SOC was  $12.2 \pm 0.56 \text{ mg C g soil}^{-1}$ . The y-axis does not start at zero.

( $P > 0.05$ ; data not shown) but there was a positive, saturating C formation response for this fraction ( $F_{1,8} = 10.0$ ,  $P < 0.05$ ; Fig. 4).

In the NP Regression Experiment, given that no C was added across the experiment, responses are restricted to decomposition of old SOC. While there were no significant interactions between N and P amendment (Fig. 5), there was a significant, negative effect of N amendment on the decomposition of the total SOC (Fig. 5). This negative effect was also observed for both the POM and mineral-associated SOC fractions ( $F_{1,33} = 11.8$ ,  $P < 0.01$ ;  $F_{1,33} = 4.49$ ,  $P < 0.05$ , respectively; data not shown). P amendment increased decomposition of the total (Fig. 5) and mineral-associated ( $F_{1,33} = 7.21$ ,  $P < 0.05$ ; data not shown) SOC compared to soils with no P amendment. The POM C fraction did not

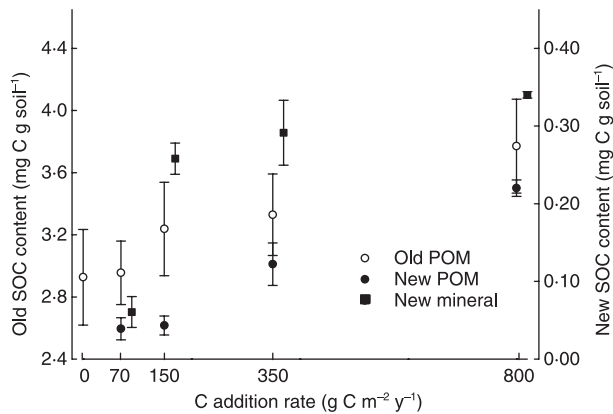


**Fig. 3.** Interactive effects of C and NP amendment on decomposition of old POM C (a) and formation of mineral-associated C (b) in the Factorial Experiment. The significance levels of the C  $\times$  NP interactions are shown in Table 1. NP amendment rates in  $\text{g N : P m}^{-2} \text{ year}^{-1}$  are 0 : 0 (white bars), 3 : 0.6 (striped bars) and 10 : 2 (grey bars). Values are means  $\pm 1$  SE ( $n = 3$ ). At the start of the experiment, the mean  $\pm 1$  SE for the old POM fraction was  $3.7 \pm 0.16 \text{ mg C g soil}^{-1}$ .

respond significantly to P amendment ( $P > 0.05$ ; data not shown). Interestingly, the effects of N amendment (reduced decomposition of SOC) and P amendment (greater decomposition of SOC) appeared independent of amendment rate (Fig. 5); however, the additive consequence of adding N & P together was increasing mitigation of the P effect with increasing N amendment rate (Fig. 5).

#### MICROBIAL AND EDAPHIC RESPONSES

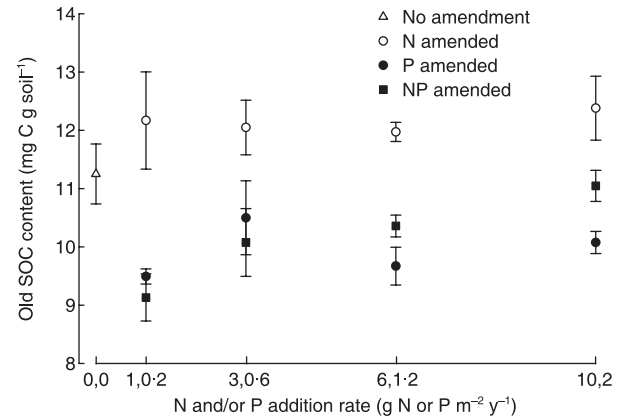
We measured a suite of microbial and chemical soil parameters to investigate the mechanisms underlying the treatment impacts on the SOC dynamics. In the Factorial Experiment, there were no significant interactive effects of C and NP amendment and no main effects of NP amendment on the measured soil properties, except pH (see below). In contrast, C amendment significantly affected every variable measured (Table 2) except soil moisture (data not shown). Since we measured the same variables in the C Regression Experiment, we could examine whether the responses detailed in Table 2 likely represented linear or threshold responses. The higher



**Fig. 4.** Effects of C amendment on old and new C in SOC fractions in the C Regression Experiment. Higher C amendment rates reduced decomposition of old POM C (open circles), while increasing the formation of SOC in both the POM and mineral-associated C fractions (filled circles and squares, respectively). For clarity, values for new, mineral-associated C are offset on the x-axis. There are two y-axes, the left (which does not start at zero) for old SOC values, and the right for new SOC values. Values are means  $\pm$  1 SE ( $n = 3$ ).

pH with high C amendment in the Factorial Experiment (Table 2) was observed in the C Regression Experiment at C amendment rates of  $350 \text{ g C m}^{-2} \text{ year}^{-1}$  and above. CFE biomass, in contrast, was only substantially higher at the highest C amendment rate, whereas SIR biomass increased almost linearly with increasing C amendment rate in the C Regression Experiment; the average rate of C mineralization appeared to map the SIR biomass response (data not shown). The negative effect of C amendment on extractable inorganic N in the Factorial Experiment (Table 2) was exhibited as a negative, saturating (at the  $350 \text{ g C m}^{-2} \text{ year}^{-1}$  amendment rate) response in the C Regression Experiment (data not shown).

In the Factorial Experiment, soil pH was significantly lower under NP addition ( $F_{2,16} = 4.57$ ,  $P < 0.001$ ); means  $\pm$  1 SE for  $0 : 0$ ,  $3 : 0.6$  and  $10 : 2 \text{ g C m}^{-2} \text{ year}^{-1}$  N : P addition levels



**Fig. 5.** Effects of N, P and NP amendment on decomposition of total SOC in the NP Regression Experiment. Decomposition was significantly ( $F_{1,33} = 9.10$ ,  $P < 0.01$ ) reduced by N amendment (open circles). For P (filled circles), decomposition was significantly ( $F_{1,33} = 7.99$ ,  $P < 0.01$ ) greater when P was added compared to when it was not (i.e. 0 compared to all other P amendment rates). The combined effects of N and P amendment were additive (interaction term:  $F_{1,33} = 0.25$ ,  $P = 0.62$ ), giving rise to the phenomenon that the greater SOC decomposition under P amendment was diminished with concurrent and increasing N amendment. The soils which receive only water (no N and/or P) are shown as triangles. Values are means  $\pm$  1 SE ( $n = 3$ ). The y-axis does not start at zero.

were:  $4.91 + 0.221$ ,  $4.83 + 0.275$  and  $4.55 + 0.266$ , respectively. This decrease in soil pH with increasing NP amendment appeared to be a function of the N input: in the NP Regression Experiment a similar decrease was observed (data not shown) and was related only to a significant main effect of N amendment ( $F_{1,33} = 4.40$ ,  $P < 0.05$ ). The only other variable that N amendment significantly affected ( $F_{1,33} = 15.4$ ,  $P < 0.001$ ) in the NP Regression Experiment was total extractable inorganic N, which increased with increasing N amendment rate (data not shown).

**Table 2.** Main effects of C amendments in the Factorial Experiment on edaphic and microbial variables

Variable	C addition rate ( $\text{g C m}^{-2} \text{ year}^{-1}$ )		
	0	150	800
pH	$4.62 + 0.343$	$4.69 + 0.172$	$4.96 + 0.418$
SIR ( $\mu\text{g C-CO}_2 \text{ g soil}^{-1} \text{ h}^{-1}$ )	$2.4 \pm 0.17$	$4.0 \pm 0.18$	$8.8 \pm 0.65$
CFE ( $\mu\text{g C g soil}^{-1}$ )	$40 \pm 5.5$	$59 \pm 5.8$	$85 \pm 8.1$
Inorganic N ( $\mu\text{g N g soil}^{-1}$ )	$6.5 \pm 0.5$	$4.7 \pm 0.62$	$1.6 \pm 1.17$
Organic N ( $\mu\text{g N g soil}^{-1}$ )	$9.4 \pm 0.67$	$11.2 \pm 0.32$	$12.0 \pm 0.65$
C min ( $\mu\text{g C-CO}_2 \text{ g soil}^{-1}$ )	$61 \pm 3.1$	$67 \pm 3.8$	$127 \pm 13$

Means  $\pm$  1 SE ( $n = 9$ ) are given, except for pH where values are back-transformed means from  $\mu\text{eq. H}^+$  and so only the positive SE is given. Data are pooled across the N : P amendment factor given a non-significant interaction with C amendment and so  $n = 9$ . There were significant ( $P < 0.05$ ) main effects of C amendment for all variables shown. Main effects of NP amendment are not shown: pH was the only variable that responded significantly ( $P < 0.05$ ) and these values are given in the text. Microbial biomass data (SIR and CFE) are raw values (i.e. they not scaled using correction factors).

## Discussion

Based on the theory of preferential substrate utilization and previous experimental observations (Dalenberg & Jager 1981; Wu *et al.* 1993; Fontaine & Barot 2005; Heath *et al.* 2005; van Groenigen *et al.* 2006), we hypothesized that increasing labile C input rates would slow SOC decomposition and formation rates. This hypothesis was supported to some degree considering that the decomposition of SOC was attenuated at higher C amendment rates (Fig. 2). This observation supports the hypothesis that certain soil microbes utilize only fresh plant-C inputs and that these microorganisms can out-compete SOC decomposing microbes, thereby slowing SOC decomposition (Fontaine & Barot 2005). However, when no C was added the amount of SOC decomposition was similar to that under the high C amendment rate, and low rates of C amendment stimulated SOC decomposition (Fig. 2). These observations suggest that the extent to which SOC decomposers are out-competed, and hence the magnitude of the priming effect, will be dependent on the C input rate.

We predicted that SOC formation would decline with increasing C amendment rate. We made this prediction based on the fact that although elevated CO<sub>2</sub> concentrations increase below-ground C inputs, they have been observed to reduce SOC formation from these inputs (Heath *et al.* 2005) and/or have negligible effects on total SOC stocks (van Groenigen *et al.* 2006). In contrast, we observed greater SOC formation with high C amendment rates (Figs 2–4), which we would expect if much of this new SOC is in the form of microbially derived products (Lundberg *et al.* 2001). Indeed, microbial biomass and activity were greatest at the highest C amendment rate (Table 2). SOC stocks declined at the lower C amendment rate because SOC decomposition was greater than formation, yet increased at the higher C amendment rate because formation was greater than decomposition (Fig. 2). These results emphasize that to predict accurately how changes in below-ground C supply to soils will impact SOC stocks will require quantification of C input rates via rhizodeposition.

Our second hypothesis was that nutrient (i.e. NP) amendment, in addition to C amendment, would increase SOC content because it would both inhibit SOC decomposition (Waldrop *et al.* 2004; Knorr, Frey & Curtis 2005a; Hyvönen *et al.* 2007) and enhance SOC formation. In support of the first part of our second hypothesis we observed lower SOC decomposition with N amendment (Fig. 5). Notably, however, P amendment enhanced SOC decomposition compared to soils which did not receive additional P (Fig. 5). It seems likely that the enhanced SOC decomposition with combined N and P amendment in the Factorial Experiment (Fig. 2) was caused by the added P. The NP Regression Experiment indicated that at high rates of NP amendment the N may ameliorate some of the P effects on decomposition (Fig. 5), which may explain why the NP effect on decomposition in the Factorial Experiment was slightly more pronounced at the low amendment rate (Fig. 2). Thus the first part of our second hypothesis, that nutrient amendment combined with C amendment would

further inhibit SOC decomposition compared to C amendment alone, was not supported. We did, however, observe greater SOC formation with nutrient addition. The absence of an interaction between C and NP amendment suggests that reported interactions between elevated CO<sub>2</sub> and N deposition which increase SOC stocks (van Groenigen *et al.* 2006) likely are additive (as opposed to statistically non-additive).

The expectation that simultaneous NP and C addition would increase SOC content (Hypothesis 2) was based on a differential response by POM and mineral-associated C fractions. Specifically, our expectation was that the POM C fraction would turnover faster (i.e. enhanced decomposition and formation of SOC) but that decomposition of the mineral-associated C fraction would decline and its formation increase (i.e. Hypothesis 3). A number of studies (Ågren & Bosatta 2002; Kirschbaum 2004; Eliasson *et al.* 2005; Knorr *et al.* 2005b) have highlighted the importance of considering SOC as a conglomeration of multiple fractions of C cycling at different rates. Our results support this perspective: as hypothesized we found that the different C fractions did not respond uniformly to C, N or P amendment. However, the responses of each fraction were not necessarily as we hypothesized. For example, in the Factorial Experiment decomposition of POM C at the low C amendment rates was greater with NP amendment, but unaffected by NP amendment at the high C amendment rate (Fig. 2). In contrast, decomposition of mineral-associated C was significantly accelerated by NP amendment but not C amendment (Table 1). Differences in SOC fraction responses to C and NP amendment were also observed for SOC formation. That is, formation of POM C was greater under C and NP amendment but these amendments did not interact to determine the response (Table 1). In contrast, formation of mineral-associated C was dependent on interactions between C and NP amendment (Table 1). Interestingly, Cardon *et al.* (2001) examined impacts of elevated CO<sub>2</sub> and differing soil N availability on decomposition and formation responses of SOC fractions but found that the CO<sub>2</sub> and N factors did not interact. Given our results and those of Cardon *et al.* (2001) it is not clear if interactive (i.e. non-additive) effects of C and N need be considered if we want to predict how altered resource availability will affect decomposition and formation of SOC in fractions of differing sink potential. Further research in this area is required.

Despite positive plant biomass responses, N fertilization of non-agricultural systems has had negative, positive and negligible effects on SOC stocks (Neff *et al.* 2002; Mack *et al.* 2004; Waldrop *et al.* 2004). Neff *et al.* (2002) observed more rapid turnover of faster-cycling SOC fractions under N fertilization but slower turnover of more stable SOC fractions, the net result being no change in total SOC content. In contrast, NP amendment in our Factorial Experiment increased decomposition of mineral-associated C and also, generally, accelerated formation of new SOC in this fraction (Fig. 3). These changes would be expected to be associated with more rapid turnover of this more stable SOC pool. Notably, Neff *et al.* (2002) did not amend with P as well as N and when both were applied in a field experiment SOC stocks were decreased

(Mack *et al.* 2004). Our results, and those of Mack *et al.* (2004), highlight the need for P to be explicitly considered when interpreting SOC responses to altered resource availability. As to the mechanism underlying the mineral-associated C response to P we are at a loss. It may suggest that decomposition of this more recalcitrant SOC fraction requires enzymes that are P-rich, or a high ATP supply rate to provide the energy required for decomposition. Given P eutrophication of soils (Newman *et al.* 1995; Anderson & Downing 2006), elucidation of the mechanism appears essential to predict accurately SOC dynamics. What may also be essential, given those interactions between C and NP amendment on SOC fraction dynamics that we observed, is quantification of how below-ground C input rates are affected by altered N and P availability.

We chronically amended our soils with C in an attempt to simulate conditions that might recreate, at least in part, those of mineral soils receiving rhizodeposition. This process is a dominant pathway through which low-molecular weight, C compounds enter soils but its ecological significance is understudied (van Hees *et al.* 2005; Boddy *et al.* 2007; Pollierer *et al.* 2007). Given that the effects of more recalcitrant C inputs to soils on total SOC contents differed to those we observed, in that they were dependent on nutrient availability (Fontaine *et al.* 2004), we may need to consider two sets of dynamics relating to how SOC will respond to altered resource availability. The first should focus on dynamics associated with discrete litter inputs and the second on more continuous inputs of labile C compounds. The expectation that these inputs are processed differently within soils is already explicit in much of the theory relating soil decomposer food web structure to function (e.g. Hendrix *et al.* 1986). It does not, however, seem to have been coupled with priming effect theory.

Although disaccharides are a dominant constituent of rhizodeposition, there are certainly many more C-compounds found in root exudates, of which sucrose is but one (van Hees *et al.* 2005). An outstanding question, therefore, from our study is whether sucrose is a useful approximate for the general effects of rhizodeposition. Also outstanding is whether the high rates of sucrose amendment (e.g. 800 g C m<sup>-2</sup> year<sup>-1</sup>) we used are realistic; it is likely for example that many of the compounds in fast-cycling C pools (e.g. Gu *et al.* 2004) are significantly more recalcitrant than sucrose. Given that global changes are likely to influence not only the amount but also composition of rhizodeposition, the effects of different rhizodeposits alone and in combination on SOC dynamics would likely be a useful research theme for understanding how the soil C sink may respond to future global change.

As in other studies (e.g. Fontaine *et al.* 2004) that have examined the impacts of C and nutrient amendments on SOC dynamics, our study did not include plants and hence may have missed a critical feedback in determining SOC dynamics (Fontaine & Barot 2005; Luo, Field & Jackson 2006). For example, increasing C amendment was negatively correlated with extractable, inorganic N levels (Table 2), which might be expected to alter plant-C inputs below-ground and hence

SOC dynamics (Fontaine & Barot 2005; Luo *et al.* 2006). Indeed, N addition may itself feedback to decrease C allocation below-ground to roots and rhizodeposition (Bowden *et al.* 2004), although direct effects of N on microbial activity are also common (Bowden *et al.* 2004; Burton *et al.* 2004). The presence of plants might also have served to stimulate competition for N, and would also have introduced other, more recalcitrant C compounds (e.g. cellulose and lignin) to the soil. All of these plant effects might have altered how the microbes utilized SOC and the sucrose inputs. Further, mycorrhizae may play a central role in the turnover of SOC fractions (Manning *et al.* 2006; Osler & Sommerkorn 2007) and in the absence of plants their functional symbiosis was omitted from our experiment. Our work was also restricted to a single soil type; further research is required to evaluate whether our results can be extrapolated to other soils and in the presence of plants. A potentially informative starting point would be to select soils of differing C : N ratios, given the expectation that this ratio will influence SOC responses to C amendments (Osler & Sommerkorn 2007). Even then, caution should be extended in extrapolating the results to the field because periodic amendment of soils with limiting resources may not accurately represent the manner in which C, N and P are made available in soils.

Our results do, however, highlight that our current understanding of SOC dynamics is insufficient for accurate forecasting of how the SOC sink will respond to altered resource availability under global change. They also provide a mechanistic basis for understanding apparently contradictory effects on SOC dynamics of nutrient amendment in the field (e.g. Neff *et al.* 2002 and Mack *et al.* 2004). Based on our findings, we highlight four areas for future investigations to focus on to improve our understanding of SOC responses to altered C, N and P availabilities. First, the response of the total SOC, in terms of both decomposition and formation processes, to variation in C and nutrient amendment rates did not predict that of SOC fractions of differing sink strengths. Thus, there is a need to focus on SOC fractions and not just total SOC contents if we are to determine the soil sink potential for C under global change. Second, we demonstrated that chronic amendment with labile C has the potential to reduce total SOC contents (e.g. Fig. 2), a hitherto unexpected result for low molecular weight C compounds such as mono- and disaccharides (Dalenberg & Jager 1981; Wu *et al.* 1993; Fontaine *et al.* 2004). This finding, in addition to the potential for labile C to constitute the dominant form of below-ground C input (van Hees *et al.* 2005) and the magnitude of its input to vary under global change (Pendall *et al.* 2004), necessitates expansion of priming effect theory to consider the impacts of chronic, labile C amendment on SOC dynamics. Third, in our study the effects of C and nutrient amendment on SOC dynamics, and interactions between these elements, were typically dependent on resource input rates. These data emphasize the need for studies which assess multi-factor and multi-level resource manipulations and hence the recognition that responses to altered resource availability may be non-additive. Fourth, our results and those of others (Mack *et al.*

2004; Cleveland & Townsend 2006) suggest that P availability may play a critical role in determining SOC sink strengths. Notably, P amendment in our study affected decomposition of the SOC fraction of greater not lesser sink strength (i.e. it decreased the mineral-associated SOC fraction). This highlights the pressing need for naturally or anthropogenically varying P availabilities to be explicitly considered when SOC responses to altered C and N availabilities are investigated.

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## Supplementary material

The following supplemental material is available for this article:

**Table S1.** Effects of C and NP amendment in the Factorial Experiment on the decomposition and formation of total, POM and mineral-associated SOC. Pool sizes within the total SOC and its fractions are in units of mg C g soil<sup>-1</sup>

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