

# Nonlinear root-derived carbon sequestration across a gradient of nitrogen and phosphorous deposition in experimental mesocosms

MARK A. BRADFORD\*, NOAH FIERER†, ROBERT B. JACKSON‡, THOMAS R. MADDIX\* and JAMES F. REYNOLDS‡

\*Odum School of Ecology, University of Georgia, Athens, GA 30602, USA, †Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA, ‡Department of Biology, Nicholas School of the Environment and Earth Sciences, Center on Global Change, Duke University, Durham, NC 27708, USA

## Abstract

Enhanced sequestration of plant-carbon (C) inputs to soil may mitigate rising atmospheric carbon dioxide (CO<sub>2</sub>) concentrations and related climate change but how this sequestration will respond to anthropogenic nitrogen (N) and phosphorous (P) deposition is uncertain. We couple isotope, soil C fractionation and mesocosm techniques to assess the sequestration of plant-C inputs, and their partitioning into C pools with different sink potentials, under an experimental gradient of N and P deposition (0, 10, 30, 60 and 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>; and 0, 2, 6, 12 and 20 kg P ha<sup>-1</sup> yr<sup>-1</sup>). We hypothesized that N deposition would increase sequestration, with the majority of the C being sequestered in faster cycling soil pools because N deposition has been shown to accelerate the turnover of these pools while decelerating the turnover of slower cycling pools. In contrast to this hypothesis, sequestration into all soil C pools peaked at intermediate levels of N deposition. Given that P amendment has been shown to cause a net loss of soil C, we postulated that P deposition would decrease sequestration. This expectation was not supported by our data, with sequestration generally being greater under P deposition. When soils were amended simultaneously with N and P, neither the shape of the sequestration relationship across the deposition gradient, nor the observed sequestration at the majority of the deposition rates, was statistically predictable from the effects of N and P in isolation. The profound nonlinearities we observed, both for total sequestration responses and the partitioning of C into soil pools with different sink potentials, suggests that the rates of N and P deposition to ecosystems will be the critical determinant of whether they enhance or decrease the long-term sequestration of fresh plant-C inputs to soils.

*Keywords:* carbon sink, elevated N deposition, elevated P deposition, eutrophication, microbial biomass, nutrient availability, nutrient limitation, rhizodeposition, root turnover, soil organic carbon

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## Introduction

Increasing atmospheric carbon dioxide (CO<sub>2</sub>) concentrations and global climate change are focusing attention on mitigation strategies that sequester carbon (C) in long-term sinks (Schimel *et al.*, 2001; Lal, 2004). The largest terrestrial biogenic sink is soil organic C (SOC). Because the current size of the SOC sink is far less than its achievable maximum for the majority of the Earth's soils (Lal, 2004), SOC sequestration is likely to be an

important mitigation strategy. However, recent evidence suggests that sequestration of fresh plant-C in SOC will decline as CO<sub>2</sub> concentrations increase above the current ambient (Heath *et al.*, 2005; Gill *et al.*, 2006), with similar declines expected in response to climate-related soil warming (Gu *et al.*, 2004; Knorr *et al.*, 2005). To our knowledge, there is no comparable study that assesses how sequestration of fresh plant-C inputs will respond to the global increase in nitrogen (N) deposition from anthropogenic sources (Vitousek *et al.*, 1997; Fenn *et al.*, 2003; Galloway *et al.*, 2004; Holland *et al.*, 2005).

Correspondence: Mark A. Bradford, tel. +1 706 542 7829, fax +1 706 542 3344, e-mail: markb@uga.edu

N fertiliser application to cropping systems typically increases SOC concentrations (Gregorich *et al.*, 1996; Jarecki & Lal, 2003; McLauchlan, 2006) due to an increase in the biomass of residues returned to cropped soils (Jarecki & Lal, 2003). In contrast to cropped systems, the impacts of elevated N deposition on SOC stocks in natural and seminatural ecosystems are not consistently positive. Although experimental N deposition typically leads to an increase in aboveground plant-C inputs to soils in noncropping systems, positive (Nadelhoffer *et al.*, 1999; Waldrop *et al.*, 2004), negative (Mack *et al.*, 2004; Waldrop *et al.*, 2004) and negligible (Neff *et al.*, 2002) effects on total SOC contents are observed. The inconsistency between SOC responses in cropping and other systems may occur because the majority of plant-C in noncropped systems enters mineral soils via roots (van Groenigen *et al.*, 2006). There is now substantial evidence that the dominant form of this root-C input is labile, rhizodeposited material (van Hees *et al.*, 2005; Högberg & Read, 2006; Boddy *et al.*, 2007). Relatively few studies (e.g. Heath *et al.*, 2005) have followed sequestration of root-C inputs under global change drivers and there is a need to redress this paucity to increase our understanding of how sequestration of root-C inputs is affected by altered resource availability. This is especially relevant given the expectation that the C:nutrient balance of soil microorganisms will play a critical role in determining SOC decomposition and formation rates (Hessen *et al.*, 2004).

For those studies (Neff *et al.*, 2002; Mack *et al.*, 2004; Waldrop *et al.*, 2004) that have directly measured SOC contents in natural and seminatural ecosystems undergoing experimental N deposition, the N amendment rate is typically at the highest end of what ecosystems experience (e.g. 100 kg N ha<sup>-1</sup> yr<sup>-1</sup> in Neff *et al.*, 2002). There is, therefore, uncertainty as to whether the same SOC responses (i.e. greater sequestration of fresh plant-C inputs to soils; Nadelhoffer *et al.*, 1999; Neff *et al.*, 2002) will be realized under more typical deposition rates. To advance our understanding of fresh plant-C sequestration dynamics in natural and seminatural ecosystems exposed to elevated N deposition, what is required is an approach where sequestered fresh plant-C is quantified separately from the existing, large background of SOC. This needs to be carried out under a range of contemporary N deposition rates.

The only natural ecosystem study (Mack *et al.*, 2004) to show large declines in total SOC in response to experimental N addition did so when phosphorous (P) was also added. Changes in soil P availability, due to either natural variability or anthropogenic inputs, can modify ecosystem responses to N deposition (Matson *et al.*, 1999; Wassen *et al.*, 2005; Cleveland & Townsend, 2006) and P deposition alone has the potential to

directly alter SOC dynamics (Cleveland & Townsend, 2006). Studies that seek to predict how N deposition will impact fresh plant-C sequestration need to consider how changes in the availability of soil P will modify N deposition effects.

Much of the empirical work examining how changes in plant-C inputs and nutrient availability will impact SOC stocks is based on measurements of CO<sub>2</sub> loss from soils across short-timescales (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.*, 2005) because the magnitude and variability of soil CO<sub>2</sub> efflux is largely dependent on the dynamics of a rapidly cycling pool of labile C (Gu *et al.*, 2004; Kirschbaum, 2004) that 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.*, 2005), 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 sequestration will respond to variation in N and P availabilities, we need to measure directly the responses of individual SOC fractions.

Here, we experimentally impose five N and P deposition rates to intact plant-soil monoliths maintained under constant environmental conditions for 1 year. The monoliths receive N and P amendments singly or simultaneously, permitting us to investigate potential interactive effects of N and P deposition on sequestration. We couple <sup>13</sup>C techniques with SOC fractionation techniques to quantify directly the impacts of the deposition on the sequestration of fresh plant-C inputs into SOC pools that differ in their long-term sequestration potentials (Schlesinger & Lichter, 2001). Our range of N deposition rates, which include a zero N control, are representative for much of the conterminous United States (10 kg N ha<sup>-1</sup> yr<sup>-1</sup>), Western Europe and Asia (30 kg N ha<sup>-1</sup> yr<sup>-1</sup>), and areas throughout the globe in the vicinity of industrialized centres and intensive agriculture (60 and 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>) (Ineson *et al.*, 1998; Fenn *et al.*, 2003; Galloway *et al.*, 2004; Holland *et al.*, 2005). The primary objective of our study is to increase the understanding of how altered N and P availability affects the sequestration rates of root-C inputs to soils; not how total SOC stocks themselves respond.

## Materials and methods

### *Mesocosm and 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. Total percentage C, N and P were  $1.200 \pm 0.0495$ ,  $0.046 \pm 0.0019$  and  $0.012 \pm 0.0020$ , respectively (mean  $\pm$  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 to standardize surface available nutrients. Monoliths were planted with a monoculture of the grass *Andropogon virginicus* L. at a density of 265 individuals m<sup>-2</sup>; juvenile plants were collected from open-canopy areas, on the same soil type, adjacent to the hardwood stand. The experimental mesocosms were maintained under constant environmental conditions (17.5 °C, 14 h photoperiod) in a controlled-environment (CE) greenhouse of the Duke University Phytotron. We used controlled conditions to tightly control resource inputs to soils. The dominant plant-C inputs to the soils across the experiment were in the form of root-C as we observed minimal foliar senescence and death.

Resource amendment treatments were assigned randomly within blocks ( $n = 3$ ) and mesocosm locations within blocks were reassigned every 14 days. Treatments were initiated after 3 months of exposure to CE conditions, which facilitated plant establishment. Treatments consisted of twice-weekly, surface additions of N and/or P in 175 mL of deionized H<sub>2</sub>O solution as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and/or KH<sub>2</sub>PO<sub>4</sub>, respectively. For the N additions, SO<sub>4</sub><sup>2-</sup> was used as the counter-ion to NH<sub>4</sub><sup>+</sup> because it has minimal effects on soil microbes and pH (Gulledge *et al.*, 1997).

For each of the treatment factors (i.e. N and P), we had five addition levels. For N these were 0, 10, 30, 60 and 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>; for P these were 0, 2, 6, 12 and 20 kg P ha<sup>-1</sup> yr<sup>-1</sup>; and for N and P added together these were 0:0, 10:2, 30:6, 60:12 and 100:20 kg N:P ha<sup>-1</sup> yr<sup>-1</sup>. 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). P deposition rates are less well quantified but, when done so, range from 0.07 to 2 kg P ha<sup>-1</sup> yr<sup>-1</sup> for total wet and dry deposition (Newman, 1995; Anderson & Downing, 2006; Oelmann *et al.*, 2007) to as much as 88 kg P ha<sup>-1</sup> yr<sup>-1</sup> for net fertiliser accumulation (i.e. fertiliser P input – crop P removal; Bennett *et al.*, 2001). On average, in the European Community net P-fertiliser accumulation is 12.8 kg P ha<sup>-1</sup> yr<sup>-1</sup> (Bennett *et al.*, 2001). Our chosen P amendment rates therefore span from high atmospheric deposition rates to low to high net fertiliser accumulation rates. Additions were made

across 1 year, providing 104 addition events per monolith.

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  $\pm$  1 SE =  $17 \pm 0.5$  volumetric %,  $n = 4$ ; from weekly measurements of four additional monoliths, receiving only water as for the controls, using a Campbell Scientific Inc. (Logan, UT, USA) CD620 and CS620 TDR probe with 12 cm rods]. We rarely observed any leachate from the base of the monoliths following treatment addition; and soil moistures were invariably below field capacity (which, for four monoliths, ranged from 30% to 35% volumetrically). Additional watering occurred as necessary to ensure plants were not water stressed.

#### *Plant and edaphic measurements*

After 12 months of treatment foliar biomass was cut at the soil surface. Roots were isolated through 2 mm sieving and hand sorting, and then washed. Plant materials were dried at 60 °C before mass determinations. Soils were divided into organic (H, 1 cm deep), surface mineral (Ah, 10 cm deep) and B horizons (remaining soil below the Ah).

Following 2 mm sieving, Ah horizon soils were homogenized and then divided for air-drying or storage fresh at 5 °C. We chose this horizon for SOC and microbial analyses because (a) the soils were relatively stone free across this depth and thus more homogenous across experimental units; (b) responses in surface soils tend to occur more rapidly than at depth due to greater biotic activity; and (c) it was a manageable volume of soil. The bulk density of this soil layer was  $1.278 \pm 0.0111$  g soil cm<sup>-3</sup> ( $n = 39$ ; mean  $\pm$  1 SE) and was unaffected by treatment. Hence, all SOC data are presented as concentration of C per unit volume of soil. We recognize that root-derived C may have been sequestered in soils below the depth we sampled but we have no reason to expect this to alter the relative treatment effects we observed.

Fractionation into particulate organic matter (POM) and mineral-associated SOC pools was achieved using a chemical dispersant (sodium hexametaphosphate) 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). While there is no one method for determining meaningful SOC fractions (Olk & Gregorich, 2006; von Lützwow *et al.*, 2007), mineral-associated C pools are expected to have slower turnover times and greater long-term sink capacities 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  $\pm 1$  SE =  $-27.5 \pm 0.03\%$ ) than for the POM C ( $n = 3$ ; mean  $\pm 1$  SE =  $-28.0 \pm 0.06\%$ ), 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 concentrations and  $\delta^{13}\text{C}$  values were determined using elemental analysis and continuous flow, isotope ratio mass spectrometry (Thermo, San Jose, CA, USA). Analytical precision for the C isotope measurements was  $\pm 0.1\%$ . Working gas and solid 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.

To measure sequestration of fresh plant-C inputs into the total SOC and its fractions, we used the difference in the natural abundance,  $\delta^{13}\text{C}$  values of the plant and soil C. The pre-experimental SOC had a  $\text{C}_3$ -photosynthetic value ( $n = 3$ ; mean  $\pm 1$  SE =  $-27.7 \pm 0.07\%$ ), whereas the grass-C had a  $\text{C}_4$ -photosynthetic value ( $n = 27$ ; mean  $\pm 1$  SE =  $-13.1 \pm 0.07\%$ ), the difference in the C isotope composition between the two being sufficient to discriminate sources (Staddon, 2004). The approach of growing  $\text{C}_4$  plants in  $\text{C}_3$ -derived soils, and vice-versa, has been used effectively to overcome the difficulties in directly measuring changes in C contents of soils, permitting an increased understanding of how root-derived C is sequestered under disturbance (Ayres *et al.*, 2004; Heath *et al.*, 2005). This approach assumes that the  $\delta$  value of the pre-experimental SOC does not deviate significantly during the experimental period and for SOC, this assumption seems likely to be upheld given the relatively slow turnover of the pool. Furthermore, the mean  $\pm 1$  SE ( $n = 39$ ) total, POM and mineral-associated SOC pool sizes, across the experimental units, were  $11.10 \pm 0.214$ ,  $3.36 \pm 0.082$  and  $7.73 \pm 0.168$  mg C g soil $^{-1}$ , respectively. The proportion that grass-derived C contributed to these pools was, at most, 7.2%, 16.1% and 4.7%, respectively, by the end of the experiment. The loss of pre-experimental SOC, which will have occurred across the experiment, was below what was detectable given the variation in SOC contents across space (for eight monoliths, sampled before the experiment, the range for total SOC contents was 10.61–14.35, and the mean  $\pm 1$  SE was  $12.00 \pm 0.495$  mg C g soil $^{-1}$ ).

Sequestration was calculated as follows (sensu Ineson *et al.*, 1996):  $C_{\text{sequestered}} = C_{\text{pool}} \times (\delta^{13}\text{C}_{\text{final}} - \delta^{13}\text{C}_{\text{pre-experimental}}) / (\delta^{13}\text{C}_{\text{plant}} - \delta^{13}\text{C}_{\text{pre-experimental}})$ , where  $C_{\text{pool}}$  is the measured size of the pool (total, POM or mineral-

associated SOC), and  $\delta^{13}\text{C}_{\text{final}}$  and  $\delta^{13}\text{C}_{\text{pre-experimental}}$  are the  $\delta^{13}\text{C}$  values of these pools from the end of, and before initiation of, the experiment, respectively.  $\delta^{13}\text{C}_{\text{plant}}$  is the signature of the grass. We used whole-plant  $\delta^{13}\text{C}$  values because we reasoned that root-derived C likely included both recent photosynthate (as rhizodeposition whose  $\delta^{13}\text{C}$  value we did not measure) and fine-root turnover. The Duke Phytotron CE greenhouse enabled rapid air exchange, maintaining the  $\delta^{13}\text{CO}_2$  signature of air at ambient. Given the large background of pre-experimental SOC and the experimental duration, as expected (Paul *et al.*, 2001) treatment impacts on the pre-experimental SOC could not be resolved from the variation in pre-experimental pool sizes. In this paper, as in others (Ayres *et al.*, 2004; Heath *et al.*, 2005), we therefore report treatment impacts on root-derived C sequestration and not mineralization/loss of pre-existing SOC.

Duplicate subsamples of fresh soil were used to determine pH, measured after mixing soil with  $\text{H}_2\text{O}$  1:1 by volume (Allen, 1989), gravimetric soil moisture (24 h at  $105^\circ\text{C}$ ) and microbial biomass. The latter was measured using modified substrate-induced respiration (West & Sparling, 1986), which primarily measures active microbial biomass (Wardle & Ghani, 1995). Potential  $\text{CO}_2$  production rates were determined over 60-day incubations at  $20^\circ\text{C}$ , 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 C concentrations.

#### Statistical analyses

To evaluate SOC sequestration responses to N and P amendment we used two approaches. To determine the relationship (whether linear or nonlinear) between N and/or P amendment rate and sequestration we used regression; where regression models were significant ( $P < 0.05$ ), polynomial terms (linear, quadratic or cubic) were included when they significantly improved  $r^2$  values (Wardle *et al.*, 2004). We used ANOVAs to determine whether the effects of N and P amendment were additive or nonadditive (i.e. whether the main effects of N and P could be used to 'predict' the combined effects of N and P amended together). To be as conservative as possible we first ran an ANOVA model where data from all experimental units were included. Given that our treatment design was not a full-factorial, this involved coding each experimental unit on the basis of whether it received N (0 if not or 1 if so) and/or P (0 or 1 as for N). This permitted us to run an unbalanced, full-factorial ANOVA, which demonstrated that N and P interacted nonadditively overall (N  $\times$  P effect:  $F_{1,33} = 6.36$ ,  $P < 0.05$ ); this finding was the same when we reran the

model without the lowest level of P addition (given its unusual behaviour: see later).

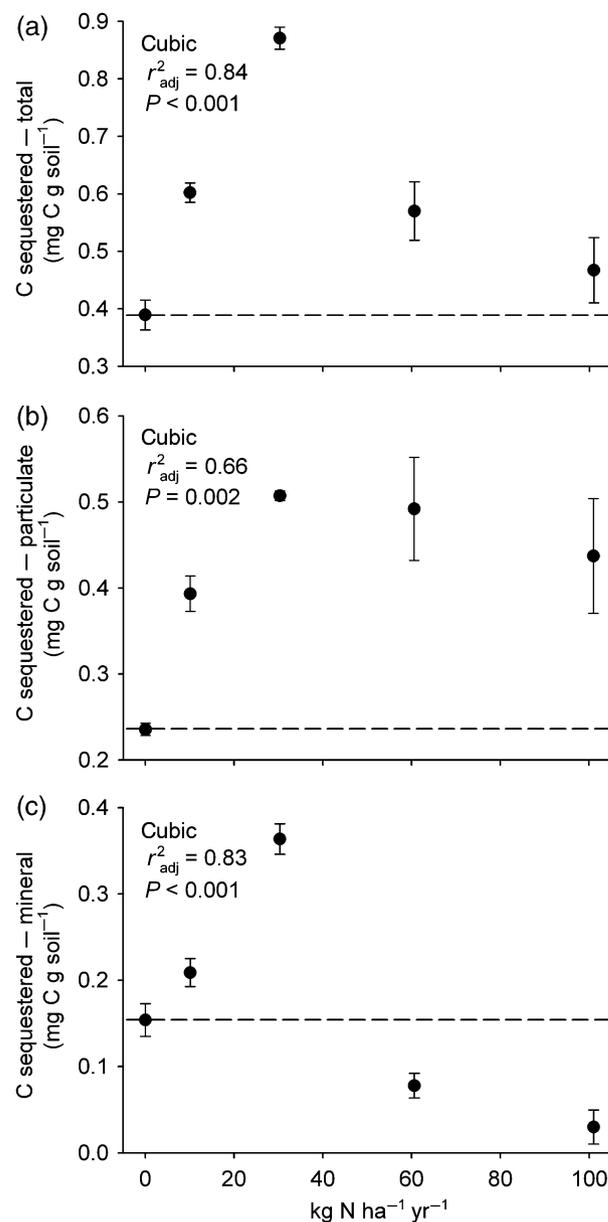
To investigate further the nonadditive responses we ran four, independent, full-factorial ANOVAs for each of the levels of N and P. For example, for the  $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  treatment and the  $2 \text{ kg P ha}^{-1} \text{ yr}^{-1}$  treatment we had experimental units that received water only, N only, P only and N and P together at  $10:2 \text{ kg N:P ha}^{-1} \text{ yr}^{-1}$ . Each ANOVA was therefore balanced. To corroborate the findings of these balanced ANOVAs, and to permit their graphical representation, we also calculated the expected SOC values for units that received N and P together and compared them with observed values. When the 95% confidence intervals of the 'observed minus expected' means did not cross zero, this was indicative of significant nonadditivity. To calculate expected values we used:  $C_{\text{expected}} = ((C_N - C_{\text{water}}) - (C_P - C_{\text{water}})) + C_{\text{water}}$ , where  $C_N$ ,  $C_P$  and  $C_{\text{water}}$  are the measured sizes of the pools (total, POM or mineral-associated SOC) under N, P and water only amendment, respectively. The N and P amendment rates used in calculating the expected values were the same as those rates when N and P were amended together. In all ANOVA models block was included as a noninteracting factor, and in calculating expected values for N and P amended mesocosms, we used SOC data for N, P and water only amended mesocosms within the same block.

To determine whether sequestration responses were correlated with variation in plant and/or edaphic variables, we visually inspected scatter plots of SOC sequestration plotted against variables such as plant biomass. Where relationships were apparent, we regressed the SOC sequestration on the variable of interest to determine whether it significantly explained variation in the sequestration rates. In all analyses, 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  $P > 0.1$ . ANOVAs were performed in S-PLUS 7.0 (Insightful Corporation, Seattle, WA, USA) and regressions in SIGMA-PLOT (Systat Software Inc., Richmond, CA, USA).

## Results

The sequestration of fresh plant-C under an increasing gradient of N deposition was profoundly nonlinear, peaking at an intermediate deposition rate of  $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (Fig. 1). At this deposition rate, total C sequestration above the control was approximately sixfold greater than in soils receiving the highest level of



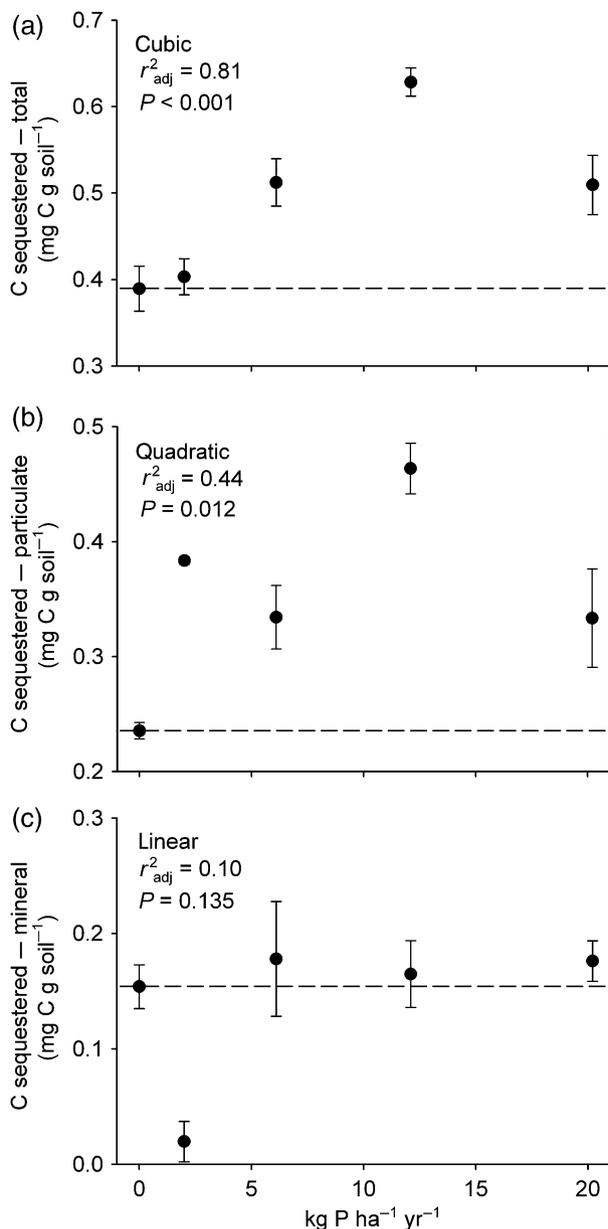
**Fig. 1** Sequestration of fresh plant-carbon (C) under increasing nitrogen (N) deposition. (a) Sequestration into total soil organic C (SOC) increases compared with the control and peaks at the  $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  deposition rate. (b) Sequestration into the particulate organic matter (POM) soil C pool follows a similar pattern to the total C up to the  $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  deposition rate but the decline in sequestration at higher deposition rates is much less pronounced. (c) Sequestration into the mineral-associated soil C pool mirrors the nonlinear pattern for total C but at the 60 and  $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  deposition rates sequestration is lower than for the control. Circles and bars are means  $\pm 1$  SE. For each plate, the polynomial terms (linear, quadratic or cubic), the variance in sequestration explained by N deposition rates ( $r^2_{\text{adj}}$ ), and the significance level of the regression, are indicated.

N deposition (Fig. 1a). To determine if this enhanced C sequestration might constitute a long-term sink, the SOC was fractionated into distinct pools: a more rapidly cycling pool (POM) and a more stable, slower-cycling pool (mineral-associated C) (Schlesinger & Lichter, 2001). Under all deposition rates (10–100 kg N ha<sup>-1</sup> yr<sup>-1</sup>) sequestration into the POM pool was enhanced relative to the control (Fig. 1b) but only at the lower deposition rates (10 and 30 kg N ha<sup>-1</sup> yr<sup>-1</sup>) did we observe enhanced sequestration into the more stable, mineral-associated C pool (Fig. 1c). At the highest deposition rates (60 and 100 kg N ha<sup>-1</sup> yr<sup>-1</sup>) sequestration into the mineral-associated pool decreased relative to the control (Fig. 1c).

Under an increasing gradient of P deposition the sequestration of fresh plant-C into the total and POM soil C pools was also nonlinear (Fig. 2). Peak sequestration rates, as for N deposition, were not at the highest P amendment rate but rather at 12 kg P ha<sup>-1</sup> yr<sup>-1</sup> for the total and POM soil C (Fig. 2a and b). While the relationship for sequestration into the mineral-associated C pool under increasing P deposition was not significant, sequestration was markedly lower at the lowest P amendment rate (2 kg P ha<sup>-1</sup> yr<sup>-1</sup>) than under any other of the P amendment levels (Fig. 2c). For the mineral-associated fraction, the maximum sequestration under P amendment was twofold lower than the maximum sequestration rates observed with N amendment (compare Figs 1 and 2).

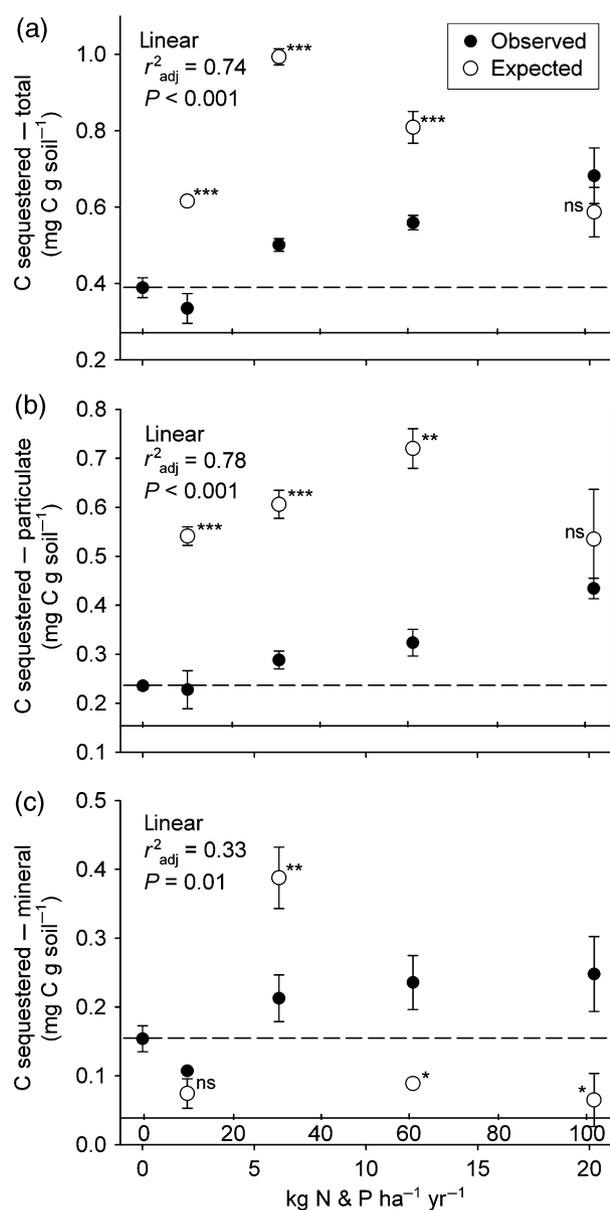
When we added P simultaneously with N we observed positive, statistically linear sequestration responses (Fig. 3) for all soil C pools. The marked nonlinearities observed under N or P deposition alone were absent (compare Figs 1 and 2 with 3). Notably, sequestration into the different C pools was either similar or lower under the lowest amendment rate (10:2 kg N:P ha<sup>-1</sup> yr<sup>-1</sup>) when compared with the control, whereas under all other deposition levels sequestration rates were greater than for the control mesocosms (Fig. 3).

We assessed whether the observed sequestration rates under simultaneous N and P deposition could be statistically predicted from the sequestration responses observed when soils were amended with either N or P individually. In other words, we tested whether the observed sequestration rates under N and P codeposition were the additive consequence of amending soils with N and P in isolation. We tested this using both ANOVA and 95% confidence interval approaches (see 'Materials and methods') and in only one instance did the ANOVA results not match those for the confidence intervals. This instance was sequestration into the total C pool at the 100:20 kg N:P ha<sup>-1</sup> yr<sup>-1</sup> codeposition rate and the ANOVA results suggested additivity (N × P



**Fig. 2** Sequestration of fresh plant-carbon (C) under increasing phosphorous (P) deposition. (a) Sequestration into total soil organic C (SOC) increases compared with the control and peaks at the 12 kg P ha<sup>-1</sup> yr<sup>-1</sup> deposition rate. (b) Sequestration into the particulate organic matter (POM) soil C pool also peaks at the 12 kg P ha<sup>-1</sup> yr<sup>-1</sup> deposition rate but, in contrast to the response of the total SOC, sequestration at 2 kg P ha<sup>-1</sup> yr<sup>-1</sup> is considerably higher than under the control. (c) Sequestration into the mineral-associated soil C pool is statistically independent of the P deposition rate. However, it is clear that at the lowest P deposition rate (i.e. 2 kg P ha<sup>-1</sup> yr<sup>-1</sup>) sequestration is considerably lower than under any other of the deposition levels, including the control. Circles and bars are means ± 1 SE. For each plate, the polynomial terms (linear, quadratic or cubic), the variance in sequestration explained by N deposition rates ( $r^2_{\text{adj}}$ ), and the significance level of the regression are indicated.

effect:  $F_{1,6} = 2.74$ ,  $P = 0.15$ ). The inconsistency between the two statistical approaches may have arisen because whereas the ANOVA approach included block as a factor, the 95% confidence intervals did not take this factor into account. The observed sequestration responses for the total and POM soil C pools were lower than expected and, for the mineral-associated soil C pool, the differences between the observed and expected sequestration responses were dependent on the deposition level (Fig. 3). While effects were generally nonadditive, additivity was observed at the highest deposition rates for the total and POM soil C pools (Fig. 3a and b) and at the lowest deposition rate for the mineral-associated C pool (Fig. 3c).



To investigate potential mechanisms underlying the observed sequestration responses, we measured a suite of plant and edaphic variables in our mesocosms (Table 1) and assessed whether their responses to treatment correlated with the sequestration responses. For each soil C pool, only one of the measured variables significantly explained some of the variation in the sequestration responses. For sequestration into the total and POM soil C pools there was a positive, linear relationship with soil respiration rates (Fig. 4a and b, respectively); for mineral-associated C there was a positive, linear relationship with gravimetric soil moisture (Fig. 4c) and this relationship was driven by the N only amendments (Fig. 4). Notably, the positive plant biomass responses under the highest deposition levels of N, P and N:P (Table 1) were not associated with the greatest sequestration rates.

## Discussion

SOC responses to experimentally imposed gradients of availability in a single resource (e.g. atmospheric CO<sub>2</sub>; Gill *et al.*, 2002) are typically nonlinear. In our experiment, total C sequestration under both the N and P deposition gradients was best explained by cubic regressions (Figs 1 and 2). When these resources were amended simultaneously, however, the statistical relationship with total C sequestration was best explained by a linear fit (Fig. 3). It is not clear why the relationship between resource availability and total C sequestration was different when N and P were applied simultaneously (as opposed to singly) but the result highlights that effects of single global change factors on SOC are unlikely to predict their interactive effects (van Groen-

Fig. 3 Sequestration of fresh plant-carbon (C) under increasing nitrogen (N) and phosphorous (P) deposition. (a) Sequestration into total soil organic C (SOC), (b) particulate organic matter (POM) soil C and (c) mineral-associated soil C increases linearly (from a statistical perspective) with increasing codeposition rates of N and P. Contrast with the nonlinear patterns shown in Figs 1 and 2. Filled circles and bars are means  $\pm$  1 SE for the observed values. For each plate, shown are the polynomial terms (linear, quadratic or cubic), the variance in sequestration explained by N deposition rates ( $r^2_{adj}$ ), and the significance level of the regression for these observed values. Open circles and bars are means  $\pm$  1 SE for 'expected' sequestration given the sequestration responses observed under N or P deposition alone (see 'Materials and methods'). Observed values that differ significantly from the expected values are marked with \*, \*\* or \*\*\* ( $P < 0.05$ , 0.01 and 0.001, respectively), indicating that observations were statistically nonadditive. Conversely, 'ns' ( $P > 0.05$ ) indicates that observed values did not differ significantly from the expected, additive effects of N and P deposition alone.

**Table 1** Soil and plant variable responses under increasing nitrogen (N) and phosphorous (P) deposition

N level (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	P level (kg P ha <sup>-1</sup> yr <sup>-1</sup> )	Foliar biomass (g)	Root biomass (g)	Plant biomass (g)	Microbial biomass (µg C g soil <sup>-1</sup> h <sup>-1</sup> )	Soil respiration (µg C g soil <sup>-1</sup> day <sup>-1</sup> )	Soil moisture (%)	pH
0	0	16.1 (1.54)	19.4 (1.80)	35.5 (0.52)	2.9 (0.20)	3.0 (0.34)	13.2 (0.56)	5.3 (0.03)
10	0	17.3 (0.28)	12.5 (3.85)	29.7 (3.97)	3.2 (0.33)	3.0 (0.40)	13.5 (0.20)	5.2 (0.08)
30	0	20.4 (1.07)	4.6 (0.86)	25.0 (1.90)	3.9 (0.30)	3.9 (0.22)	13.7 (0.63)	5.1 (0.09)
60	0	20.5 (1.49)	22.0 (6.45)	42.5 (5.74)	3.3 (0.40)	3.4 (0.40)	12.6 (0.65)	5.0 (0.05)
100	0	22.6 (1.80)	19.4 (1.32)	42.1 (0.53)	2.8 (0.33)	2.8 (0.38)	11.8 (0.47)	4.8 (0.02)
0	2	24.4 (2.24)	18.7 (6.21)	43.1 (6.54)	3.4 (0.21)	3.3 (0.50)	13.1 (0.49)	5.3 (0.02)
0	6	22.6 (3.91)	11.2 (3.93)	33.8 (7.40)	3.1 (0.17)	2.8 (0.21)	14.1 (0.50)	5.2 (0.08)
0	12	26.7 (1.80)	11.5 (0.84)	38.2 (1.53)	3.9 (0.15)	4.0 (0.29)	13.6 (0.31)	5.3 (0.03)
0	20	21.1 (6.65)	19.2 (3.55)	40.3 (9.86)	3.1 (0.25)	3.1 (0.27)	13.3 (0.26)	5.3 (0.05)
10	2	22.5 (2.79)	6.8 (1.96)	29.3 (3.42)	3.5 (0.67)	2.4 (0.45)	13.3 (0.72)	5.2 (0.03)
30	6	24.2 (2.43)	10.9 (2.68)	35.1 (3.01)	3.5 (0.66)	2.7 (0.47)	14.2 (0.85)	5.0 (0.10)
60	12	26.7 (5.49)	23.7 (3.23)	50.4 (7.34)	3.0 (0.21)	2.6 (0.15)	12.4 (0.48)	4.9 (0.09)
100	20	28.5 (2.50)	25.6 (0.97)	54.2 (3.12)	3.3 (0.17)	3.1 (0.06)	12.9 (1.17)	4.7 (0.07)

Values shown are means ( $\pm 1$  SE). pH values were converted to mol H<sup>+</sup> L<sup>-1</sup> before mean and standard error calculations and then back calculated. For this reason, values for pH are means ( $-1$  SE).

gen *et al.*, 2006). Indeed, for total C sequestration we generally found that the expected sequestration rates (based on the effects of N and P in isolation) were significantly higher than the observed rates under simultaneous N and P deposition (Fig. 3). These results highlight the importance of using both multifactor and multilevel approaches to predict soil C sequestration responses to multiple global change factors, such as N and P deposition. Indeed, we anticipated that there would be a positive, saturating sequestration response under increasing N, P and N:P deposition, but under none of these gradients did we observe such a relationship for total C sequestration (although we did for the POM C with N amendment, and the mineral-associated C sequestration with N:P amendment).

We hypothesized that N deposition would increase the rate of plant-C sequestration, with the majority of the C being sequestered in POM soil C fractions because N deposition has been shown to accelerate the turnover of this pool while decelerating the turnover of mineral-associated soil C fractions (Neff *et al.*, 2002). Our results were only consistent with this hypothesis at the two highest N deposition rates (the highest being equivalent to that used in the study by Neff *et al.*, 2002), where we found that sequestration increased in the POM fraction, and decreased in the mineral fraction, relative to the controls. Given that our study was not run long enough to resolve differences in pre-experimental SOC turnover (see 'Materials and methods'), we cannot reliably estimate turnover times for the different soil C fractions. However, it is notable that at N deposition rates of 30 kg N ha<sup>-1</sup> yr<sup>-1</sup> or less, sequestration into the mineral-

associated C fraction was greater than under the controls (Fig. 1), suggesting that the turnover rates of this fraction might actually increase when N deposition rates are lower. If this is true, then we will need to question whether SOC responses to N deposition are generally predictable from experimental manipulations where this factor is applied at a single, high deposition rate.

We expected that P deposition would decrease sequestration into all soil C pools because previous studies have shown that P amendments can induce a net loss of soil C (Mack *et al.*, 2004; Cleveland & Townsend, 2006). This expectation was not supported by our data: total C sequestration rates were greater than control rates when the experimental mesocosms were exposed to P deposition rates of 6 kg P ha<sup>-1</sup> yr<sup>-1</sup> or greater (Fig. 2). Under every level of P deposition sequestration into the POM C fraction was greater than under the control (Fig. 2). However, sequestration into the mineral-associated C fraction was not markedly greater than control rates for any level of P deposition, suggesting that P deposition may impair the long-term sink potential of soils. To test this possibility will require work on a greater number of systems, of differing background fertility, with a gradient of P deposition. For example, in the studies of both Mack *et al.* (2004) and Cleveland & Townsend (2006), P was added at a higher rate (50 kg ha<sup>-1</sup> yr<sup>-1</sup>) than in our study and in the case of Cleveland & Townsend (2006), total P in their low-fertility site was approximately 4.5 times higher than at our site (Cleveland *et al.*, 2006).

A number of studies (Ågren & Bosatta, 2002; Kirschbaum, 2004; Eliasson *et al.*, 2005; Knorr *et al.*, 2005) have

highlighted the importance of considering SOC as a conglomeration of multiple fractions of C cycling at different rates. Our results support this hypothesis as we found that the different C fractions did not respond uniformly to N or P amendment. For example, under high rates of N deposition sequestration into the POM fraction was greater than under the control, but sequestration into the mineral-associated fraction was less than under the control (Fig. 1). Under P deposition sequestration into the POM pool was always positive, whereas sequestration into the mineral-associated fraction either did not differ from the control, or was lower (Fig. 2). Only under codeposition of N and P did

sequestration into the two fractions respond in an approximately similar manner (Fig. 3). We cannot explain why the fractions should respond in an approximately similar manner under codeposition, and not under deposition of N or P alone. It may be that the microbes associated with these pools of C are differentially limited by N and P. Whatever the mechanism, our results highlight that the sequestration response of the total SOC will not necessarily be indicative of the change in the long-term sink strength of a soil. A focus on SOC fractions that vary in turnover rates seems important to understand SOC sink responses to nutrient amendments.

To understand why we observed the different sequestration responses under N, P and N:P deposition, we measured a suite of plant and edaphic variables and found that neither total plant, foliar nor root biomass explained any significant variation in sequestration rates. Although seemingly counterintuitive, these findings appear consistent with N and P fertilization studies in noncropped systems where, despite positive plant biomass responses, negative, positive and negligible effects on SOC stocks have been observed (Neff *et al.*, 2002; Mack *et al.*, 2004; Waldrop *et al.*, 2004). Plant productivity may not correlate positively with SOC stocks in these systems because the majority of plant-C in noncropped systems is likely to enter mineral soils via roots (van Groenigen *et al.*, 2006). More specifically, there is now substantial evidence that the dominant input of root-C to soil is in the form of labile-C compounds (i.e. rhizodeposition) (van Hees *et al.*, 2005; Högberg & Read, 2006; Boddy *et al.*, 2007). These inputs

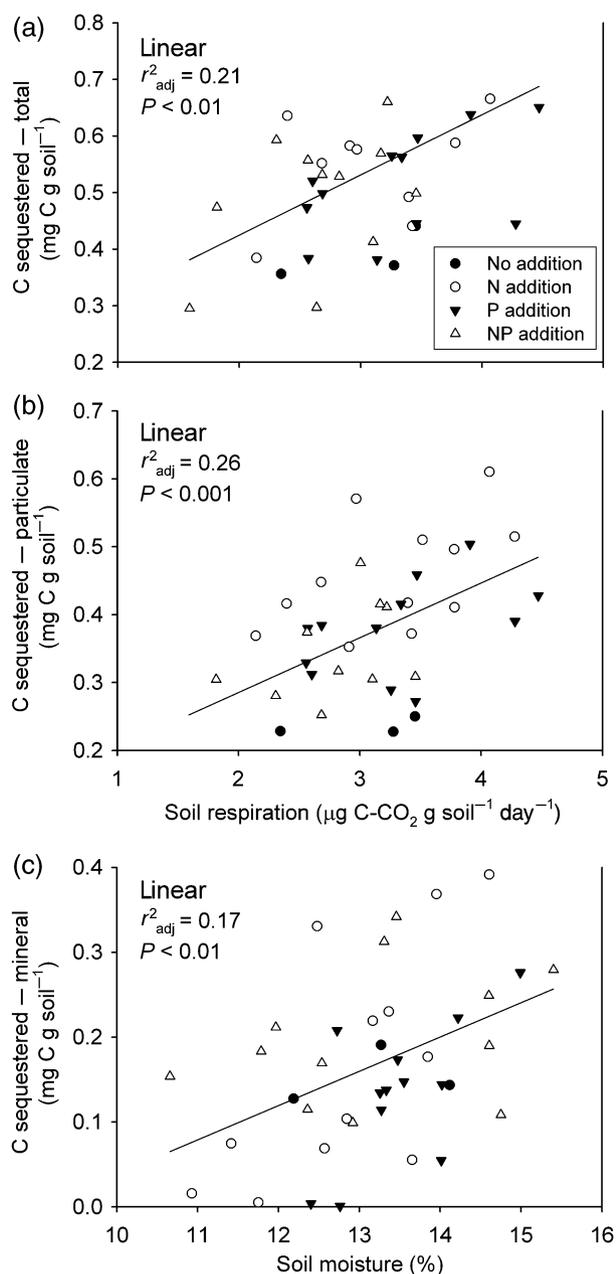


Fig. 4 Relationships between sequestration of fresh plant-carbon (C) under nitrogen (N), phosphorous (P) and N:P deposition and other measured factors. Soil respiration significantly explained >20% of the variation in sequestration into the total soil organic C (SOC) (a) and particulate organic matter (POM) soil C (b). For the mineral-associated soil C, gravimetric soil moisture significantly explained 17% of the variation in sequestration (c). For each of the three soil pools, only one of the measured plant and soil variables (i.e. those shown) explained a significant proportion of the variation. Each point represents an individual soil monolith and different symbols represent whether that mesocosm was exposed to water, N only, P only or simultaneous N and P amendment. For each plate, the polynomial terms (linear, quadratic or cubic), the variance in sequestration explained ( $r^2_{adj}$ ), and the significance level of the regression are indicated. Notably, the relationship between mineral-associated C sequestration and soil moisture appeared to be driven by the N only treatments: for these monoliths gravimetric moisture explained 36% of the variation ( $P = 0.01$ ) in a linear regression, while the same relationships for the monoliths receiving P only and N:P were not significant ( $P > 0.05$ ).

may be more closely related to the fate of SOC in mineral soils than plant biomass *per se*. For example, Dijkstra & Cheng (2007) showed that rates of new SOC formation explained much more of the variation in loss of older soil C than total or root plant biomass. Measurement of plant physiological variables in future work may help elucidate linkages between microbial and plant responses to environmental variation to understand better the consequences for soil C sequestration (e.g. Sampson *et al.*, 2007).

In our study, we found that soil respiration potential, which provides an estimate of microbially available C, was the only variable to significantly explain variation in total and POM C sequestration rates (Fig. 4). This relationship would be expected as an increase in labile, root-C inputs to soil is likely to increase microbially available C. There is an increasing appreciation for the ecological significance of these belowground inputs (e.g. Pollierer *et al.*, 2007), and the expectation that they are processed differently within soils to aboveground residue inputs is explicit in much of the theory relating soil decomposer food web structure to function (e.g. Hendrix *et al.*, 1986). The absence of a positive correlation between plant biomass and SOC sequestration in our study, and the fact total SOC contents do not necessarily correlate positively with plant biomass in global change experiments conducted in natural and seminatural ecosystems (Neff *et al.*, 2002; Mack *et al.*, 2004; Waldrop *et al.*, 2004; Carney *et al.*, 2007), implies that 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 root-C. What seems certain is that the current understanding of SOC responses to N and P fertilization in cropped systems (McLauchlan, 2006) is not adequate for predicting SOC responses to N and P deposition in natural and seminatural ecosystems.

There are mechanistic questions our study leaves unanswered. We cannot explain why the only variable that correlated significantly with variation in mineral-associated C sequestration was gravimetric soil moisture (Fig. 4). Further, an estimate of rhizodeposition rates would have facilitated a more comprehensive evaluation of whether variation in labile, root-C input rates under the different treatments could explain the POM C sequestration responses. Given that mycorrhizae may play a central role in the turnover of SOC fractions (Manning *et al.*, 2006; Osler & Sommerkorn, 2007), their assessment would also have been valuable. Also, we cannot know whether, when we added N and P simultaneously, the fact that we added P at 20% of the N deposition rate played a significant role in determining the observed SOC responses. Similarly, the counter-ion

added with  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  may have played a role in determining treatment responses. For example, just as did Cleveland & Townsend (2006), we used K as the counter-ion to  $\text{PO}_4^{3-}$  when making P additions and the addition of such non-N nutrients may stimulate  $\text{N}_2$  fixation (van Groenigen *et al.*, 2006) and so confound results of reactive N deposition. Lastly, while oxidized N dominates N deposition in the United States (Holland *et al.*, 2005), we simulated N deposition using  $\text{NH}_4^+$  (a reduced source). While reduced N is the dominant input in other parts of the world, and a significant input in the United States in areas local to intensive, livestock practices such as pig farming, research is required to assess whether SOC responses to reactive N deposition are dependent on whether the N is oxidized or reduced.

What our research does show is that we do not know enough about C sequestration dynamics in nonagricultural systems to explain the magnitude and partitioning of fresh plant-C sequestration in SOC pools of differing sink strengths. Future research in this area should be a priority and must resolve not only how fresh-plant C sequestration responds across gradients of N and P deposition, but also how the present stocks of SOC will respond. Indeed, we only examined the sequestration of fresh plant-C and losses of C from the large background of pre-existing SOC could offset any gains in sequestration of fresh plant-C (Bellamy *et al.*, 2005; Schulze & Freibauer, 2005; Dijkstra & Cheng, 2007). Our results do suggest, however, that the global increase in N and P deposition may provide a counterbalance to other global changes, such as elevated temperature and atmospheric  $\text{CO}_2$ , that are expected to decrease soil sequestration of fresh plant-C inputs (Heath *et al.*, 2005; Knorr *et al.*, 2005).

Our results and those of others (Mack *et al.*, 2004; Cleveland & Townsend, 2006) suggest that P availability, in addition to N availability, may play a critical role in determining SOC sink strengths. The profound nonlinearities we observed, both for total sequestration responses and the partitioning of C into SOC pools with different sink potentials, suggests that the rate of N and P deposition to ecosystems will be the critical determinant of whether these nutrients enhance or decrease the long-term sequestration of fresh plant-C inputs to soils. These nonlinearities, and the generally nonadditive interactions between N and P amendment on sequestration rates, emphasize the need for studies that assess responses to multifactor and multilevel resource manipulations if we want to predict accurately soil C sequestration responses to elevated N and P deposition.

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