



Feedback on plant productivity can be constrained by SOM in N-limited grasslands

Ramesh Laungani*, Johannes M.H. Knops, Chad Brassil

School of Biological Sciences, University of Nebraska, 348 Manter Hall, Lincoln, NE 68588, USA

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ABSTRACT

In many terrestrial ecosystems plant productivity is limited by the availability of mineral nitrogen, which is produced by soil microbial transformations of organic N in soil organic matter (SOM-N). Mineral N availability results from two opposing processes, 1) gross mineral N production (gross ammonification/gross nitrification) and 2) microbial N immobilization. These processes can be influenced by the availability of plant-derived C (PDC) inputs to the microbes, SOM-N pool size, and the size of the microbial community (microbial biomass). We considered how changes in PDC inputs and SOM-N pool size together may alter microbial biomass, mineral N availability, and feedbacks on plant productivity. In areas dominated by one of six tallgrass prairie species along a natural gradient of PDC inputs and SOM-N pool size, we conducted a field survey of microbial biomass and gross ammonification. We also performed greenhouse manipulations of SOM-N pool size and PDC inputs on two species in our study area (*Poa pratensis* and *Schizachyrium scoparium*). Structural equation modeling of the field data showed that gross ammonification was both positively and directly related to microbial biomass and SOM-N pool size. Gross ammonification was positively and indirectly related to SOM-N pool size and belowground PDC inputs, via microbial biomass. In the short-term greenhouse study, PDC inputs and SOM-N pool size positively affected gross mineral N production, although only at high SOM-N pool size. If the patterns in the greenhouse can be applied to field conditions, this suggests that SOM-N pool size may constrain plant driven feedbacks on plant productivity by limiting gross mineral N production.

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1. Introduction

In many terrestrial ecosystems, plant productivity is strongly limited by the availability of mineral nitrogen (NH_4^+ and NO_3^-), which is primarily produced by soil microbial transformations of organic N in soil organic matter (SOM). Specifically, the availability of mineral N to the plant community is determined by the balance between gross mineral N production (gross ammonification and gross nitrification) and microbial N immobilization. As immobilization increases relative to gross production, the net availability of mineral N to the plant community decreases. Microbial N immobilization is strongly influenced by carbon (C) availability in the soil (Booth et al., 2005; Hart et al., 1994a; Stark and Hart, 1997) with higher C availability leading to microbial N limitation and subsequent increases in microbial N immobilization (Blumenthal et al., 2003; Zak et al., 1994). Generally, SOM carbon is highly

recalcitrant and the soil microbial community preferentially uses more labile, plant-derived forms of C (Wardle, 2002b) such as leaf litter, root litter, and root exudates (hereafter PDC) (Knops et al., 2002; Zak et al., 1994). In addition to numerous studies finding increases in microbial N immobilization with increases in PDC inputs to the soil microbial community (Blumenthal et al., 2003; Bowman et al., 2004), recent work has also shown that increases in PDC inputs can stimulate gross mineral N production (Dijkstra et al., 2009; Kuzyakov and Cheng, 2001). This suggests that PDC inputs may cause a feedback on plant productivity through changes in both microbial N immobilization and gross mineral N production (Ehrenfeld et al., 2005). In order to demonstrate such a feedback, (1) plant biomass must respond to changes in mineral N availability and (2) changes in PDC inputs to the soil must alter gross mineral N production and immobilization.

The availability of PDC inputs to the microbes is not the only factor determining gross mineral N production rates in soils. Booth et al. (2005) found SOM-N pool size was positively related to gross mineral N production rate, suggesting that SOM-N pool size exerts substrate-level control on gross mineral N production. In grasslands, SOM-N may exert a particularly strong influence over

* Corresponding author. Present address: Biology Department, Doane College, 135 Lied Science Building, Crete, NE 68333, USA. Tel.: +1 402 250 7873.

E-mail address: Ramesh.laungani@doane.edu (R. Laungani).

belowground N dynamics because more than 90% of total ecosystem N is in the SOM-N pool (Knops and Bradley, 2009). In addition, rates of gross mineral N production and microbial N immobilization have also been directly positively related to rates of microbial activity, as measured by microbial respiration rates (Bengtsson et al., 2003; Bowman et al., 2004) suggesting that changes in the size of the microbial community may also influence feedbacks on plant productivity.

Taken together, these results suggest that although PDC inputs to the soil may feedback on plant productivity through changes in gross mineral N production and microbial N immobilization, the strength of this feedback may be constrained by the size of SOM-N pool. Therefore we hypothesize that increases in PDC inputs could increase microbial biomass, causing increases in both gross mineral N production and microbial N immobilization (Hypothesis 1). These changes in mineral N production and immobilization could feedback on plant productivity by changing the net availability of mineral N to the plant. The increase in gross mineral N production and subsequent increase in microbial N immobilization may potentially be due microbial N limitation. This may occur in the presence of high levels of PDC availability (Blumenthal et al., 2003; Clein and Schimel, 1995) as the average C:N ratio of the soil microbial community is ~ 10 (Bengtsson et al., 2003; Wardle, 2002b), and in grasslands the average C:N ratio of PDC inputs of the dominant grasses (in the form of leaf/root litter) can range from 30–122 (Craine et al., 2002b, 2003). In addition, we hypothesize that the strength of this feedback will be constrained by the size of the SOM-N pool, as gross mineral N production rates will be strongly dependent on SOM-N pool size (Hypothesis 2). We expect this SOM-N effect on gross mineral N production rates as SOM-N is the substrate that is microbially broken down to produce mineral N in the soil.

We examined the interaction between plant productivity, SOM-N pool size, and changes in PDC inputs to the soil on microbial biomass and N cycling using a field survey and two controlled greenhouse experiments focusing on widespread perennial grass species. In both the field and the greenhouse studies, we utilized measurements of belowground plant biomass as a proxy for belowground PDC inputs to the soil (Kuzakov and Domanski, 2000). With the field survey, we measured microbial biomass and N cycling beneath 6 perennial grass species that differ in root biomass (Craine et al., 2002a) along a natural gradient of SOM-N pool size in a grassland in central Minnesota, USA. In the greenhouse, we focused on two of the most widespread species in our study area (*Poa pratensis* and *Schizachyrium scoparium*), in each case examining plant productivity responses to changes in mineral N availability, and how microbial biomass and gross and net N cycling rates respond to changes in SOM-N pool size and PDC inputs.

2. Materials and methods

2.1. Field survey: microbial biomass and N cycling associated with 6 perennial grass species

The field survey was performed at Cedar Creek Ecosystem Science Reserve (hereafter Cedar Creek) in central Minnesota, USA (45° 24' N, 93° 12' W). Cedar Creek is located on an glacial outwash sand plain, and is strongly N limited with sandy soils (Grigal et al., 1974). For a description of the vegetation at Cedar Creek see Miles and Knops (2009).

We measured gross N cycling (see below for details) and soil microbial biomass (see below for details) associated with 6 grass species in 1 m² plots of the target species in natural “old field” communities at Cedar Creek. The species in this survey were *Bromus inermis* (C₃ n = 6 plots), *P. pratensis* (C₃ n = 6 plots), *Panicum*

virgatum (C₄ n = 3 plots), *S. scoparium* (C₄ n = 5 plots), *Sorghastrum nutans* (C₄, n = 5 plots), and *Stipa spartea* (C₃ n = 5 plots). These species are the dominant grasses that are found in old field communities at Cedar Creek (Inouye et al., 1987; Miles and Knops, 2009). As all species were not present in all fields, we used a total of 10 different old fields. The target species were dominant in the plots and contributed on average of $82.69\% \pm 2.65\%$ of the relative vascular plant cover. Only one replicate of each species was established in a field and each field had at least two species present. We measured microbial biomass (via substrate induced respiration), gross ammonification (¹⁵N isotope dilution), soil %C and %N, and above and belowground biomass in each experimental plot. These measurements were taken for all species in June and July of 2008 in order to capture any potential changes due to the activity of early and late season grass species. The growing season at Cedar Creek begins in mid-May and ends in late-August, with peak growth of C₃ grasses in June, and C₄ grasses in July. We measured root biomass C in each of these plots down to a depth of 10 cm at three points in each plot. Roots were washed and sorted into fine (diameter < 1 mm) and coarse (diameter > 1 mm) roots. Soil %C and %N were determined using combustion analysis with a Costech analytical ECS 4010. In this experiment the soil %C ranged from 0.20 to 1.03, while the soil %N ranged from 0.02 to 0.08. SOM-N and SOM-C are highly positively correlated ($r^2 = 0.941$) (Knops and Tilman, 2000) and we use SOM-N throughout the paper as an indicator for microbial substrate availability.

2.2. Greenhouse experiment I: mineral N availability effects on plant C

In the first of two greenhouse experiments we established *P. pratensis* and *S. scoparium* in soil with low SOM-N content for 11 weeks. These species were chosen because they are the two dominant grass species at Cedar Creek (Miles and Knops, 2009) and represent both early season C₃ (*Poa*) and late season C₄ (*Schizachyrium*) functional groups. The soil was collected from old fields at Cedar Creek (0–10 cm) which differed in SOM content (Knops and Tilman, 2000). Once the soil from each field was collected it was homogenized in a large cement mixer before being used in the experiment. The %C and %N for the homogenized soil in our study were 0.49 ± 0.01 and 0.045 ± 0.001 respectively. Grasses were allowed to establish for six weeks in pots that were 13 cm wide and 11.5 cm deep. After this initial six week establishment period, we created three levels of mineral N availability (high, control, and low). ‘High’ was created with the addition of NH₄NO₃ solution at 6.5 g N m⁻² yr⁻¹ (high N), ‘control’ had no addition (only water), and ‘low’ was established with the addition of sucrose solution at 2000 g C m⁻² yr⁻¹. The mineral N level in the unamended soil was 2.7 mg N kg⁻¹ soil⁻¹. Amendments were applied weekly for 5 weeks. The NH₄NO₃ application rate is representative of the net N mineralization rate in the old fields at Cedar Creek (Pastor et al., 1987). The C application rate was chosen following Blumenthal et al. (2003), which in that study significantly reduced soil mineral N availability relative to unamended plots by more than 50%. In a small set of soil samples (n = 5), this rate of C addition reduced mineral N concentrations in the soil by 7 fold as compared to unamended control samples. Due to the relatively short duration of the C amendment (5 weeks) we only used sucrose additions as our source of C, as opposed to a combination of sucrose and sawdust which is often used in longer term amendments (Blumenthal et al., 2003). Monocultures were grown at 26.7 °C, watered daily, and on average received 900 μmol m⁻²s⁻¹ of light during the experiment (light measurements taken at mid-day). After the 5 week application process all biomass in the pot was

harvested, sorted between above and belowground, dried and weighed.

2.3. Greenhouse experiment II: SOM pool size and plant C effects on microbial biomass and N cycling

Secondly, we established an experiment to examine how changes in SOM-N and belowground plant biomass affect soil microbial biomass and N cycling. We established monocultures of *S. scoparium* and *P. pratensis* seeded at two different densities in two soils that differed in SOM-N content (“high” and “low”). The two seeding densities were utilized to manipulate belowground plant biomass and therefore belowground plant C inputs. Bare soil controls at each level of SOM-N were also included in this experiment to examine the impact of plants on soil microbial biomass and N cycling ($n = 7$ for each SOM-N level). We established 8 monocultures of each planted treatment combination. The %C for the soils with high and low SOM pools in the current study were 0.77 ± 0.01 and 0.49 ± 0.01 respectively. The %N for the soils with high and low SOM pools in the current study were 0.058 ± 0.001 and 0.045 ± 0.001 respectively. The high SOM soil and the low SOM soil were homogenized separately as in the first greenhouse experiment. These monocultures were established in pots that were 13 cm wide and 11.5 cm deep and were grown for 8 weeks starting in mid-July. Monocultures were grown at 26.7°C , watered daily, and on average received $900 \mu\text{mol m}^{-2}\text{s}^{-1}$ of light during the experiment (light measurements taken at mid-day). Measurements of plant biomass, microbial biomass, and gross N cycling were taken 8 weeks after establishment. Typically after about 8–11 weeks plants in this size pot become root bound (Laungani *pers. observation*), and therefore to ensure that this was not going to impact our plants we chose the shorter duration period of 8 weeks.

2.4. Gross N cycling measurements

For the field survey we sampled soil at a depth of 0–10 cm in June and July of 2008, and at each time we composited three soil cores (5 cm core) from each of the 30 experimental field plots. These samples were immediately sieved (2 mm). For each plot in the field survey, we measured rates of gross N ammonification using ^{15}N pool dilution (Hart et al., 1994a,b) in the lab with an incubation period of 24 h at 22°C . Approximately 250 g (fw) of the sieved soil was amended with 10 ml of 0.329 mM 99% atom solution of $(^{15}\text{NH}_4)_2\text{SO}_4$ which brought the soil moisture level to 5.5% on average (gravimetric moisture content). Soils were amended in a resealable plastic bag and homogenized by hand to ensure even distribution of the solution throughout the soil.

For the second greenhouse experiment, soil from the entire pot was sampled and we measured rates of gross ammonification and gross nitrification. The protocol for determination of rates of gross N cycling was identical to that of the field study with the exception of the concentrations of the ^{15}N solutions applied to the soil. Soil was amended with 10 ml of 0.138 mM 99% atom solution of $(^{15}\text{NH}_4)_2\text{SO}_4$ or 0.283 mM 99% atom solution of K^{15}NO_3 for gross ammonification or nitrification, respectively.

Following Stark and Hart (1996) we determined ^{15}N enrichment levels in the amended soil by extracting samples in 2M KCl immediately (~ 5 min) after tracer addition and again at 24 h. Nitrogen from soil extracts was concentrated by diffusion on to paper discs and were subsequently analyzed for $^{15}\text{N}/^{14}\text{N}$ by a stable isotope mass spectrometer at the University of California Davis Stable Isotope Facility (Europa Integra). Gross process rates of N cycling were calculated using time 0 and time 24 h pool measurements following [Eq. (1)] (Takahashi, 2001; Yamamuro, 1988) where m_{N} is the gross N mineralization or nitrification rate

($\text{mg N kg}^{-1} \text{ soil}^{-1} \text{ day}^{-1}$), $[\text{Pool}]_0$ the NH_4^+-N or NO_3^--N concentration at time 0 ($\text{mg N kg}^{-1} \text{ soil}^{-1}$), $[\text{Pool}]_{24}$ NH_4^+-N or NO_3^--N concentration at 24 h ($\text{mg N kg}^{-1} \text{ soil}^{-1}$), and APE the atom percent excess over background ($\text{atom}\% ^{15}\text{N}-0.37 \text{ atom}\% ^{15}\text{N}$) at time 0 and 24 h respectively. Yamamuro (1988) includes the effect of N pool concentration on N immobilization, which in soils like ours where N immobilization is large, gives a more accurate estimate of gross N dynamics (Takahashi, 2001; Yamamuro, 1988), as compared to the more commonly used calculation of Hart et al. (1994b). Microbial N consumption rates were calculated as the difference between gross N production and the difference between final and initial pools for both NH_4^+ and NO_3^- . Immobilization of NH_4^+ was calculated as the difference between NH_4^+ consumption and gross nitrification (Booth et al., 2005). We calculated NO_3^- immobilization as the difference between gross nitrification and net nitrification, because in our sandy, well drained soils denitrification does not represent a significant flux of N (Zak and Grigal, 1991).

$$m_{\text{N}} = \frac{\left[\text{Pool}_{24} - \left(\text{Pool}_0 \frac{\text{APE}_{24} \times \text{Pool}_{24}}{\text{APE}_0 \times \text{Pool}_0} \right) \right] \times -\text{LN} \left(\frac{\text{APE}_{24} \times \text{Pool}_{24}}{\text{APE}_0 \times \text{Pool}_0} \right)}{1 - \frac{\text{APE}_{24} \times \text{Pool}_{24}}{\text{APE}_0 \times \text{Pool}_0}} \quad (1)$$

2.5. Microbial biomass measurements

Soil microbial biomass was determined using substrate induced respiration (Anderson and Domsch, 1978). We used rates of CO_2 flux from soil samples amended with glucose as an index of microbial biomass (Anderson and Domsch, 1978; Horwath and Paul, 1994; Johnson et al., 1996). Soil CO_2 flux measurements were taken using a Li-COR 6400 Soil CO_2 flux chamber (Li-COR Lincoln, NE). Other studies have used measurements of soil CO_2 flux to assess microbial biomass and have been shown to be a strong predictor of gross N cycling (Bengtsson et al., 2003). For the field experiment in June and July of 2008 we sampled soil at a depth of 0–10 cm. We composited five soil cores (5 cm wide core) from each of the 30 experimental plots. These samples were immediately sieved (2 mm) and placed into a resealable bag. Gravimetric moisture content of each sample was determined by drying a small (≈ 15 g) sample from each bag for 24 h at 105°C . The remaining soil was refrigerated at 4°C overnight in resealable bags so as to maintain soil moisture levels in each sample. Optimal soil glucose amendment level for substrate-induced respiration was determined by amending 450 g (d.w.) soil along a gradient of glucose solutions for 1 h. Concentrations of glucose used were 0, 1, 3, 6, 8, and 10 mg g^{-1} soil following Johnson et al. (1996). CO_2 flux rates was greatest for soil samples amended with 6 mg glucose g^{-1} soil, plateauing at all higher concentrations for a 1 h incubation time (Laungani, unpublished data), allowing us to use this measurement as an index of microbial biomass. Once moisture content of each sample was determined, 10 ml of the optimized glucose solution (6 $\text{mg glucose g soil}^{-1}$) was added to 580 g (d.w.) soil and then water was added to the soil to bring the sample up to 55% WFPS using the determined moisture content. Each sample was gently homogenized in the bag by hand to ensure even distribution of the water and glucose solution. Soil was packed to a bulk density of 1.45 g cm^{-3} in a 4" PVC drain cap (10.16 cm \times 4.5 cm). Soil was packed to this value of bulk density because this is representative of average bulk density values that occur in these fields at Cedar Creek (Laungani and Knops, 2009; Wedin and Tilman, 1990). Soil was packed into the PVC drain cap so that the surface of the soil was not any higher than the top of the drain cap. The soil was incubated for

1 h at 22 °C before a single soil CO₂ flux measurement was recorded.

For the second greenhouse experiment, soil from the entire pot was sampled. The method to determine microbial biomass was the same as in the field study with the exception of the size of the incubation container used (10.16 cm wide by 1.27 cm deep) and the amount of soil (113 g soil d.w).

2.6. Statistical analysis

We analyzed the field survey data using structural equation modeling in AMOS (SPSS, 2008), to examine direct and indirect effects of plant C inputs and SOM-N pool size on gross ammonification rates. Based on our hypotheses our model examined a number of relationships: 1) the direct effect of changes in plant C and SOM-N pool size on microbial biomass; 2) the direct effect of changes in SOM-N pool size on gross ammonification; 3) the direct effect of microbial biomass on gross ammonification; and 4) the indirect effect of changes in plant C and SOM-N pool size on gross ammonification via microbial biomass. The direct effect of SOM-N or plant C inputs on microbial biomass are represented by the standardized path coefficients from the analysis between the factor and the response variable (Fig. 1). The indirect effect of SOM-N on gross ammonification was calculated as the product of the standardized path coefficient from SOM-N to microbial biomass and the standardized path coefficient from microbial biomass to gross ammonification. The total effect of SOM-N on gross ammonification was calculated as the sum of the indirect effects on gross ammonification via microbial biomass and the direct effects on gross ammonification. The indirect effect of plant C inputs on gross ammonification was calculated as the product of the standardized path coefficient from plant C inputs to microbial biomass and the standardized path coefficient from microbial biomass to gross ammonification. For plant C inputs the total effect on gross ammonification was equal to the indirect effect of plant C inputs on gross ammonification, as there was no direct relationship between plant C inputs and gross ammonification (Fig. 1 gray arrow). Goodness of fit of our model compared to the saturated model was assessed using both a Chi-square test and root mean square error of

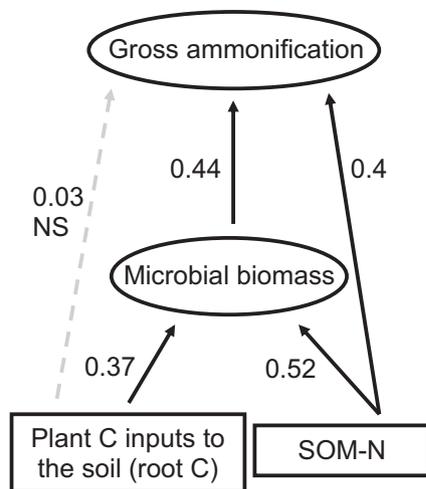


Fig. 1. Results from a structured equation model of the controls over gross ammonification in a field survey. Boxes represent pools that were examined in the model (plant root C inputs and SOM-N pool) with microbial biomass and gross ammonification rate as response variables (ovals). Solid black arrows represent significant relationships between boxed factors. Gray arrow represents a non-significant direct relationship between plant C inputs and gross ammonification. Numbers beside arrows represent standardized path coefficients.

approximation (RMSEA). For the greenhouse experiment that altered mineral N availability (*Greenhouse I*) we used a 2-way ANOVA to examine effects of species and mineral N availability on total plant biomass and root biomass. Within species we used one way ANOVAs with *post-hoc* Tukey tests to examine specific differences in total biomass and root biomass due to changes in mineral N availability. In the second greenhouse study (*Greenhouse II*), manipulating plant density and SOM-N pool size, the data were analyzed using a three factor MANOVA with species, SOM-N pool size, and density as the main factors. In order to determine whether the *P. pratensis* and *S. scoparium* were having different effects on microbial biomass and our measures of N cycling, bare soil was initially excluded from the MANOVA as density and species would have been confounded with the inclusion of the bare soil plots in the analysis. We found that species and density did not significantly affect our response variables, and so in subsequent analyses both plant species were combined. Because density did not have a significant effect, we combined both densities and examined the effect of plants on microbial biomass and N cycling by comparing planted and unplanted treatments at each level of SOM-N. We used a two factor MANOVA with SOM-N pool size and plant presence as the main factors and this analysis included all 78 replicates.

3. Results

3.1. Field survey: microbial biomass and N cycling associated with 6 perennial grass species

We found that both SOM-N pool size and belowground plant C biomass were positively related to microbial biomass, and that microbial biomass was positively related to gross ammonification rates (Fig. 1). There was also a positive relationship between SOM-N and gross ammonification (Fig. 1). In addition, soil C content was positively related to microbial biomass and gross ammonification, reflecting covariance between soil C and N concentrations. Overall, our structured equation model (reduced model) fit the data just as well as the saturated model ($\chi^2 = 4.294$; $p > 0.05$; RMSEA < 0.001 ; $p > 0.05$). Using the standardized path coefficients, our model showed that the direct effect of microbial biomass on gross ammonification was 0.442. It also showed that the total effect of SOM-N pool size on gross ammonification was 0.723. The direct effect of SOM-N on gross ammonification was 0.492 (Fig. 1). The direct effect of SOM-N pool size on microbial biomass was 0.522 (Fig. 1). The indirect effect of SOM-N pool size on gross ammonification via microbial biomass was 0.231. The total effect of plant C inputs on gross ammonification was 0.162 (indirect effect only). The direct effect of plant C inputs on microbial biomass was 0.366 (Fig. 1). Our model showed that plant belowground biomass and SOM-N explained 41% of the variation in microbial biomass. Microbial biomass and SOM-N explained 66% of the variation in gross ammonification. Root biomass among species ranged from 112 g m⁻² to 1054 g m⁻² across all fields.

3.2. Greenhouse experiment I: mineral N availability effects on plant C

After 11 weeks, mineral N availability ($F_{2,30} = 129.7$; $p < 0.001$) and species ($F_{1,30} = 46.9$; $p < 0.001$) had a significant effect on total plant biomass (Fig. 2). There was no treatment \times species interaction ($p > 0.05$). The average total biomass production in the unamended treatment for these two species was 488 ± 38 g biomass m⁻², which was about 75% of the standing biomass in the field (632 ± 104 g biomass m⁻² as measured in our field survey). Compared to the unamended control, both species produced about 55% less total biomass in the low mineral N availability treatment (Fig. 2). In the high mineral N treatment, *S. scoparium*

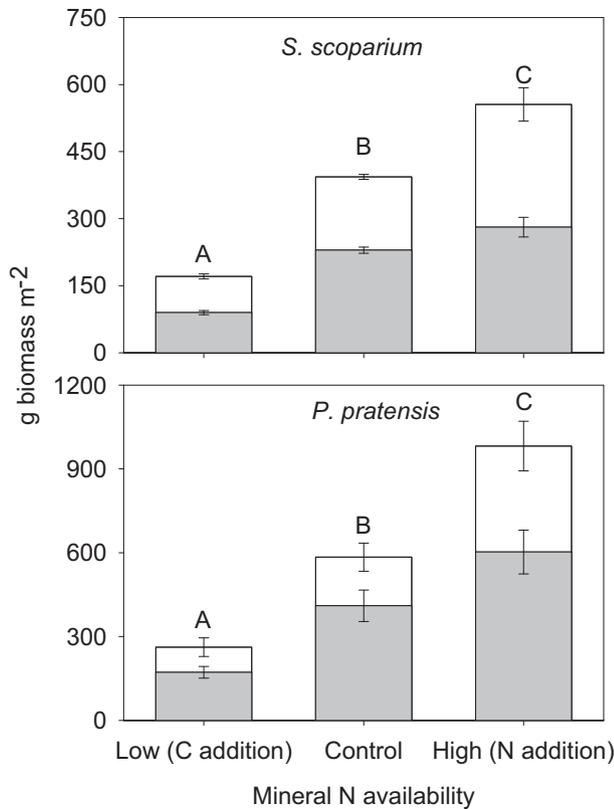


Fig. 2. Mineral N availability effects on plant biomass in a greenhouse experiment. Mineral N availability on x-axis and biomass (g m^{-2}) on the y-axis. Total bar height represents total biomass. Gray bars represent root biomass and white bars represent leaf biomass (g m^{-2}). Letters represent significant differences in total biomass among treatments from *post-hoc* Tukey tests ($p < 0.05$). Error bars represent ± 1 standard error of total biomass (upper) and of root biomass (lower).

produced 41% and *P. pratensis* 68% more total biomass than the unamended control (Fig. 2).

Our two way ANOVA showed a significant impact of mineral N availability ($F_{2,30} = 67.3$; $p < 0.001$) and species ($F_{1,30} = 52.3$; $p < 0.001$) on root biomass as well (Fig. 2). There was no significant treatment \times species interaction ($p > 0.05$). For *P. pratensis*, the average unamended treatment produced $410 \pm 56 \text{ g m}^{-2}$ of root biomass, which was comparable to the field ($367 \pm 42 \text{ g m}^{-2}$). *S. scoparium* produced $230 \pm 7 \text{ g m}^{-2}$ of root biomass which was approximately 50% of the field ($460 \pm 122 \text{ g m}^{-2}$). Both species produced more than 3 times the root biomass when fertilized as compared to the low mineral N availability treatment. Within species analysis showed that root biomass significantly differed across all treatments for *S. scoparium* ($F_{2,15} = 52.495$; $p < 0.001$). Although mineral N availability affected root biomass production in *P. pratensis* ($F_{2,15} = 20.889$; $p < 0.001$) only the low mineral N treatment significantly differed from the other treatments ($p < 0.001$), but the control and fertilized treatments did not differ from each other ($p > 0.05$).

3.3. Greenhouse experiment II: SOM pool size and plant C effects on microbial biomass and N cycling

Over 8 weeks we found, that both SOM-N pool size ($F_{1,56} = 41.9$; $p < 0.001$) and initial seeding density ($F_{1,56} = 111.0$; $p < 0.001$) resulted in significant differences in root biomass, and that species significantly differed in root biomass production ($F_{1,56} = 28.7$; $p < 0.001$) (Tables 1 and 2). *S. scoparium* produced significantly more roots than *P. pratensis* by 44%, soils with high SOM-N increased root production

Table 1

MANOVA results from an experimental test of the effects of species, initial seeding density, and SOM-N pool size on root biomass in the greenhouse (Greenhouse Experiment II). Significant effects ($p < 0.05$) are in bold.

	F-value	Sig.
Species	$F_{1,56} = 28.6$	<0.001
Density	$F_{1,56} = 41.9$	<0.001
SOM-N pool size	$F_{1,56} = 111.0$	<0.001
SOM-N pool size \times Density	$F_{1,56} = 0.722$	0.399
Species \times SOM-N pool size \times Density	$F_{3,56} = 0.900$	0.447

Model: Root Biomass = Intercept + Species + SOM-N pool size + Density + SOM-N pool size \times Density + Species \times SOM-N pool size \times Density.

more than low SOM-N soils by 44%, and higher initial seeding density had twice as much root biomass than low initial seeding density. The root biomass in the monocultures at the end of the 8 week period ranged from 27 g m^{-2} to 211 g m^{-2} .

In contrast to the field survey, when we examined the impact of species, SOM-N pool size, and plant density (excluding bare soil; see methods) on microbial biomass and N cycling, there was a significant SOM-N pool size \times density interaction ($F_{1,56} = 6.2$; $p = 0.016$) (Fig. 3). This interaction however was weak given that when we included the bare soil treatment in the analysis (without species as a factor; see methods), only SOM-N pool size significantly increased soil CO_2 flux ($F_{1,73} = 160.4$; $p < 0.001$) (Fig. 3). Compared to the average soil microbial CO_2 flux across these species in the field ($0.86 \pm 0.19 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), the greenhouse monocultures had a similar soil microbial CO_2 flux ($0.87 \pm 0.10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).

When we compared planted to unplanted treatments across SOM-N levels in a MANOVA there was a significant SOM-N pool size \times planted interaction ($F_{6,68} = 2.65$; $p < 0.05$). However when we subsequently examined the ANOVAs of each single response variable, only net ammonification showed a significant SOM-N pool size \times planted interaction ($p < 0.03$; Table 3), all other response variables showed no significant SOM-N pool size \times planted interaction. When we examined the effects of plant presence and SOM-N pool size on net ammonification alone we found a significant difference between planted and unplanted with high SOM-N pool size ($p < 0.01$), but not between planted and unplanted with low SOM-N pool size ($p > 0.05$) (Fig. 4c), which would explain the observed interaction. With the exception of net ammonification (which showed the significant SOM-N pool size \times planted interaction; Table 3), the main effect of SOM-N pool size in our single response variable ANOVAs was significant for all other N cycling measurements (Table 3), while the presence of plants significantly increased gross ammonification (Fig. 4a), gross nitrification (Fig. 4d), and NO_3^- immobilization (Fig. 4e) relative to the unplanted treatment (Table 3).

4. Discussion

In both a field survey and a short-term greenhouse study we examined how microbial biomass and gross N cycling respond to changes in SOM-N pool size and PDC inputs to the soil and how these changes may feedback on plant productivity. Overall our data from both experiments suggest that PDC inputs exert a secondary control

Table 2

Treatment effects on root biomass in the greenhouse (Greenhouse Experiment II). Numbers in parenthesis represent standard error values.

	Root biomass (g/m^2)	
Species	<i>P. pratensis</i> : 69.3 (5.86)	<i>S. scoparium</i> : 99.8 (8.60)
Initial seeding density	Low: 55.9 (3.66)	High: 113.2 (7.55)
SOM-N pool size	Low: 68.7 (7.05)	High: 100.4 (7.56)

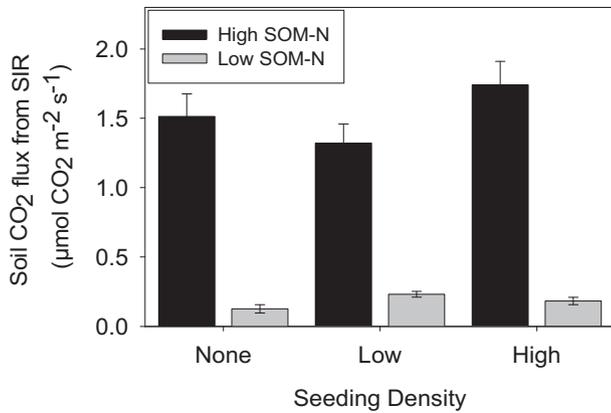


Fig. 3. The effects of SOM-N pool size and plant density on soil CO₂ flux (µmol CO₂ m⁻² s⁻¹) in Greenhouse Experiment II. Error bars represent ±1 standard error.

on rates N cycling relative to SOM-N pool size. Furthermore our data suggest a potential feedback on plant productivity (*sensu* Ehrenfeld et al., 2005) that may be constrained by the size of the SOM-N pool.

4.1. Effects of PDC inputs on microbial biomass, N cycling, and potential feedbacks on plant productivity (Hypothesis 1)

Our data support our hypothesis that concurrent changes in gross mineral N production and immobilization with the addition of PDC inputs could result in a negative feedback on plant productivity (*sensu* Ehrenfeld et al., 2005). In the greenhouse (Greenhouse I) the reduction in plant productivity with C addition and highlights that plant productivity is sensitive to increases in microbial N immobilization that can reduce the availability of mineral N to the plant community (Bowman et al., 2004). The positive relationship between plant C inputs and microbial biomass and the positive relationship between microbial biomass and gross ammonification observed in our field study, suggest that plants indirectly control gross mineral N production through changes in microbial biomass. However this mechanism was not confirmed in the greenhouse as neither presence of a plant nor the level of plant C inputs to the soil changed the size of the microbial community. The lack of microbial response may be explained by the fact that on an area basis *Poa* and *Schizachyrium* had four times as much root biomass in the field survey as compared to the greenhouse study. There was only a slight overlap in the ranges of root biomass between the two experiments with the high end of the root biomass range in this greenhouse study overlapping the low end of the range of root biomass in the field survey. It may also be partly explained by the significantly lower soil CO₂ flux in the greenhouse with more than a 30% decline in soil CO₂ flux on average in the greenhouse as compared to the field survey. In addition there are other factors that can impact microbial biomass and N cycling rates including soil moisture and temperature, with microbial biomass going down with declining temperatures and soil moisture

(Wardle, 2002a). While our data also indicate that changes in belowground plant C inputs may only exert secondary control on short-term gross mineral N production relative to SOM-N pool size, other work has shown that plants can impact SOM breakdown by creating spatial heterogeneity in the distribution of microbial activity through changes in soil structure, soil aggregate size, and availability of SOM for microbial breakdown (Blanco-Canqui and Lal, 2004; Dormaar, 1990). Although our short-time greenhouse ¹⁵N pool dilution experiments utilized disturbed soil and therefore may not necessarily reflect actual microbial activity and N transformation rates in the field, our findings could facilitate experimental manipulations which help to explain mechanisms that cause variation in N transformation rates among soils that vary in the size of the SOM-N pool.

In regard to N cycling, our greenhouse study (Greenhouse II) showed that plant presence significantly increased gross ammonification, gross nitrification, NO₃⁻ immobilization, decreased net ammonification, and trended towards increased NH₄⁺ immobilization. Our results agree with carbon enrichment studies that show that changes in plant C inputs to the soil can stimulate the breakdown of SOM (the 'priming effect') (Körner and Arnone, 1992; Kuzyakov and Cheng, 2001; Kuzyakov and Domanski, 2000; Paterson, 2003) and that these changes can lead to increases in gross mineral N production (Dijkstra et al., 2009). Increases in SOM breakdown with the addition of plant-derived C has been cited as mechanism by which plant C may cause a feedback on plant productivity through increased mineral N availability and plant N uptake (Drake et al., 2011; Langley et al., 2009; Phillips et al., 2011). However our results suggest that the magnitude of the priming effect and the subsequent availability of mineral N to the plants may be influenced by the SOM-N pool size (Fig. 4e), with microbes in low SOM-N soils ultimately becoming substrate limited regardless of plant C inputs to the soil (Norby et al., 2010). Furthermore, our results at high SOM-N pool size may suggest that PDC inputs caused microbial N limitation and in turn increased SOM-N mineralization as compared to unplanted soils. Microbial N limitation in response to plant C is also supported by the significant increase in the NO₃⁻ immobilization rate, the trend of increased NH₄⁺ immobilization, and the significantly lower net ammonification rate with the presence of a plant. This microbially immobilized N may become available to the plant community through turnover of the microbial biomass (Clein and Schimel, 1995; Schmidt et al., 2007). As such PDC inputs to the soil may cause a longer-term feedback on productivity by shifting N from the SOM-N pool, which has a long turnover rate, to the more rapidly cycling microbial biomass N pool (Schmidt et al., 2007), although our study could not address this longer term dynamic.

4.2. Effect of SOM-N pool size on microbial biomass, N cycling, and potential feedbacks on plant productivity (Hypothesis 2)

Our data from both the field and the greenhouse suggest that the size of the SOM-N pool can constrain the magnitude of PDC driven changes to microbial biomass and N cycling via substrate

Table 3
MANOVA results from an experimental test of the effects of SOM pool size and plant presence on nitrogen cycling measurements in the greenhouse. Significant effects ($p < 0.05$) are in bold. F -value and model R^2 reflect the model fit for each N cycling measurement.

	Gross ammonification	NH ₄ ⁺ immobilization	Net ammonification	Gross nitrification	NO ₃ ⁻ immobilization	Net nitrification
	$F_{3,73} = 99.8$; $R^2 = 0.804$	$F_{3,73} = 11.7$; $R^2 = 0.325$	$F_{3,73} = 23.3$; $R^2 = 0.489$	$F_{3,73} = 83.5$; $R^2 = 0.774$	$F_{3,73} = 43.8$; $R^2 = 0.643$	$F_{3,73} = 14.3$; $R^2 = 0.370$
SOM-N pool size	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Planted	0.007	0.537	0.014	<0.001	0.029	0.124
SOM-N pool size × Planted	0.105	0.346	0.028	0.650	0.050	0.684

All data LN transformed to increase normality.

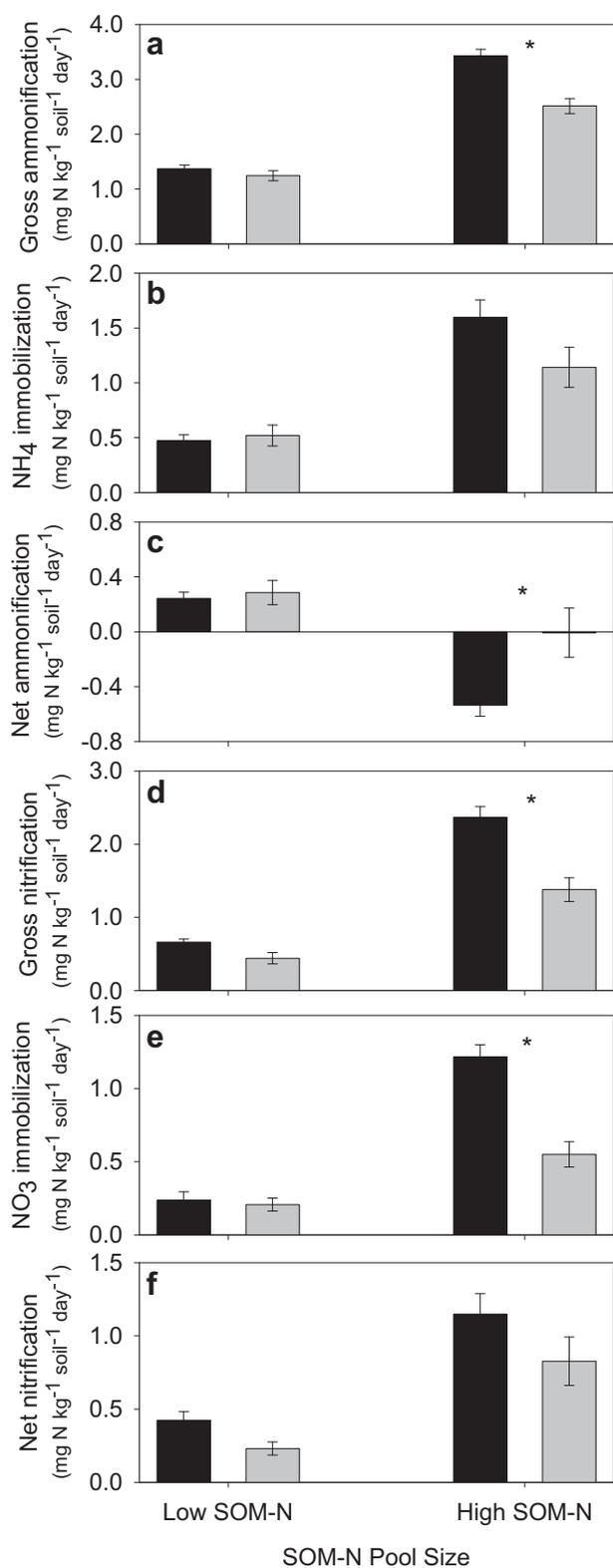


Fig. 4. The effects of SOM-N pool size and plant presence on a) gross ammonification; b) NH₄ immobilization; c) net ammonification; d) gross nitrification; e) NO₃ immobilization; f) net nitrification in Greenhouse Experiment II. Black and gray bars represent planted and unplanted treatments respectively. Asterisks represent significant differences ($p < 0.05$) between planted and unplanted treatments and error bars represent ± 1 standard error. All rates are in mg N kg⁻¹ soil⁻¹ day⁻¹. Note that scales on y-axis is different for each panel.

limitation. Although we found that PDC inputs to the soil were positively related to microbial biomass in the field, we also found that SOM-N pool size had a larger direct effect on microbial biomass (Fig. 1). In addition, there was a direct positive relationship between SOM-N pool size and gross ammonification, suggesting substrate-level control of gross ammonification by SOM-N pool size. Moreover, as the total effect of SOM-N pool size on gross ammonification was more than four times as large as the effect of plant C inputs, our field results may suggest that PDC inputs only exert secondary control on N cycling relative to SOM-N pool size (Fig. 1).

In our greenhouse study (Greenhouse II), this secondary impact of PDC inputs relative to SOM-N pool size was supported by 1) the increase in microbial biomass with higher SOM-N content (Fig. 3), 2) the lack of an effect of PDC inputs on microbial biomass, and 3) the impact of PDC presence/absence on gross mineral N production only at high SOM-N content (Fig. 4a,d). The plant effect at high SOM-N only suggests that any plant driven feedbacks on plant productivity may be constrained by SOM-N pool size. This could be important, as studies at our field site have shown an increase in SOM-N content of the soil along a successional series of old fields (Inouye et al., 1987; Knops and Tilman, 2000), suggesting that if our greenhouse results can be applied to the field that feedbacks on plant productivity may only operate once a large SOM-N pool has accumulated.

In conclusion our results support the hypothesis that plant C inputs can feedback on plant performance through changes in gross mineral N production rates, but the strength of this feedback is largely dependent on SOM-N pool size. However, it is clear that changes in SOM-N pool size and those processes that alter SOM-N pool size (*i.e.* litter decomposition and humus formation) may be important drivers of plant productivity in N limited systems dominated by grasses (Mack and D'Antonio, 2003). Overall plant driven feedbacks on plant productivity through changes in microbial community structure and N cycling may be constrained by SOM availability.

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References

- Anderson, J., Domsch, K., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology & Biochemistry* 10, 215–221.
- Bengtsson, G., Bengtson, P., Mansson, K.F., 2003. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology & Biochemistry* 35, 143–154.
- Blanco-Canqui, H., Lal, R., 2004. Mechanisms of carbon Sequestration in soil Aggregates. *Critical Reviews in Plant Sciences* 23, 481–504.
- Blumenthal, D.M., Jordan, N.R., Russelle, M.P., 2003. Soil carbon addition controls weeds and facilitates prairie restoration. *Ecological Applications* 13, 605–615.
- Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecological Monographs* 75, 139–157.
- Bowman, W.D., Steltzer, H., Rosenstiel, T.N., Cleveland, C.C., Meier, C.L., 2004. Litter effects of two co-occurring alpine species on plant growth, microbial activity and immobilization of nitrogen. *Oikos* 104, 336–344.
- Clein, J.S., Schimel, J.P., 1995. Nitrogen turnover and availability during succession from Alder to Poplar in Alaskan Taiga forests. *Soil Biology & Biochemistry* 27, 743–752.
- Craine, J.M., Tilman, D., Wedin, D., Reich, P., Tjoelker, M., Knops, J., 2002a. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. *Functional Ecology* 16, 563–574.

- Craine, J.M., Wedin, D.A., Chapin, F.S., Reich, P.B., 2002b. Relationship between the structure of root systems and resource use for 11 North American grassland plants. *Plant Ecology* 165, 85–100.
- Craine, J.M., Wedin, D.A., Chapin, F.S., Reich, P.B., 2003. The dependence of root system properties on root system biomass of 10 North American grassland species. *Plant and Soil* 250, 39–47.
- Dijkstra, F.A., Bader, N.E., Johnson, D.W., Cheng, W.X., 2009. Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability? *Soil Biology & Biochemistry* 41, 1080–1087.
- Dormaar, J.F., 1990. Effect of active roots on the decomposition of soil organic materials. *Biology and Fertility of Soils* 10, 121–126.
- Drake, J.E., Gallet-Budynek, A., Hofmockel, K.S., Bernhardt, E.S., Billings, S.A., Jackson, R.B., Johnsen, K.S., Lichter, J., McCarthy, H.R., McCormack, M.L., Moore, D.J.P., Oren, R., Palmroth, S., Phillips, R.P., Pippin, J.S., Pritchard, S.G., Treseder, K.K., Schlesinger, W.H., DeLucia, E.H., Finzi, A.C., 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO₂. *Ecology Letters* 14, 349–357.
- Ehrenfeld, J.G., Ravit, B., Elgersma, K., 2005. Feedback in the plant-soil system. *Annual Review of Environment and Resources* 30, 75–115.
- Grigal, D.F., Chamberlain, L.M., Finney, H.R., Wroblewski, D.W., Gross, E.R., 1974. *Soils of the Cedar Creek Natural History Area*. University of Minnesota Agriculture Experiment Station, St. Paul.
- Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A., 1994a. Dynamics of gross nitrogen transformations in an old-growth forest – the carbon connection. *Ecology* 75, 880–891.
- Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K., 1994b. Nitrogen mineralization, immobilization, and nitrification. In: Weaver, R., Angle, J., Bottomley, P. (Eds.), *Methods of Soil Analysis Part 2-Microbiological and Biochemical Properties*. Soil Science Society of America, Madison, pp. 985–1018.
- Horwath, W.R., Paul, E.A., 1994. Microbial biomass. In: Weaver, R., Angle, J., Bottomley, P. (Eds.), *Methods of Soil Analysis Part 2-Microbiological and Biochemical Properties*. Soil Science Society of America, Madison, pp. 753–775.
- Inouye, R.S., Huntley, N.J., Tilman, D., Tester, J.R., Stillwell, M., Zinnel, K.C., 1987. Old-field succession on a Minnesota sand plain. *Ecology* 68, 12–26.
- Johnson, C.K., Vigil, M.F., Doxtader, K.G., Beard, W.E., 1996. Measuring bacterial and fungal substrate-induced respiration in dry soils. *Soil Biology & Biochemistry* 28, 427–432.
- Knops, J.M.H., Bradley, K.L., 2009. Soil carbon and nitrogen accumulation and vertical distribution across a 74-year chronosequence. *Soil Science Society of America Journal* 73, 2096–2104.
- Knops, J.M.H., Bradley, K.L., Wedin, D.A., 2002. Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecology Letters* 5, 454–466.
- Knops, J.M.H., Tilman, D., 2000. Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. *Ecology* 81, 88–98.
- Körner, C., Arnone, J.A., 1992. Responses to elevated carbon dioxide in artificial tropical ecosystems. *Science* 257, 1672–1675.
- Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biology & Biochemistry* 33, 1915–1925.
- Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. *Review. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* 163, 421–431.
- Langley, J.A., McKinley, D.C., Wolf, A.A., Hungate, B.A., Drake, B.G., Megonigal, J.P., 2009. Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO₂. *Soil Biology & Biochemistry* 41, 54–60.
- Laungani, R., Knops, J.M.H., 2009. Species-driven changes in nitrogen cycling can provide a mechanism for plant invasions. *Proceedings of the National Academy of Sciences of the United States of America* 106, 12400–12405.
- Mack, M.C., D'Antonio, C.M., 2003. Exotic grasses alter controls over soil nitrogen dynamics in a Hawaiian woodland. *Ecological Applications* 13, 154–166.
- Miles, E.A., Knops, J.M.H., 2009. Shifting dominance from native C-4 to non-native C-3 grasses: relationships to community diversity. *Oikos* 118, 1844–1853.
- Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E., McMurtrie, R.E., 2010. CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences of the United States of America* 107, 19368–19373.
- Pastor, J., Stillwell, M.A., Tilman, D., 1987. Nitrogen mineralization and nitrification in four Minnesota old fields. *Oecologia* 71, 481–485.
- Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. *European Journal of Soil Science* 54, 741–750.
- Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO₂ fumigation. *Ecology Letters* 14, 187–194.
- Schmidt, S.K., Costello, E.K., Nemergut, D.R., Cleveland, C.C., Reed, S.C., Weintraub, M.N., Meyer, A.F., Martin, A.M., 2007. Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* 88, 1379–1385.
- SPSS, 2008. *SPSS Statistics*, 17.0.0 ed. SPSS Inc, Chicago.
- Stark, J.M., Hart, S.C., 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. *Soil Science Society of America Journal* 60, 1846–1855.
- Stark, J.M., Hart, S.C., 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* 385, 61–64.
- Takahashi, S., 2001. Comparison of gross nitrogen mineralization rates by zero-order models. *Soil Science Society of America Journal* 65, 244–246.
- Wardle, D.A., 2002a. Global change phenomena in an aboveground-belowground context. In: Wardle, D.A. (Ed.), *Communities and Ecosystems: Linking the Aboveground and Belowground Components*. Princeton University Press, Princeton, NJ, pp. 239–294.
- Wardle, D.A., 2002b. Plant species control of soil biota and processes. In: Wardle, D.A. (Ed.), *Communities and Ecosystems: Linking the Aboveground and Belowground Components*. Princeton University Press, Princeton, NJ, pp. 56–103.
- Wedin, D.A., Tilman, D., 1990. Species effects on nitrogen cycling: a test with perennial grasses. *Oecologia* 84, 433–441.
- Yamamuro, S., 1988. A theoretical approach to the fate of nitrogen in paddy field by using an NH₄-15N tracer technique. *Japanese Journal of Soil Science and Plant Nutrition* 59, 538–548.
- Zak, D.R., Grigal, D.F., 1991. Nitrogen mineralization, nitrification and denitrification in upland and wetland ecosystems. *Oecologia* 88, 189–196.
- Zak, D.R., Tilman, D., Parmenter, R.R., Rice, C.W., Fisher, F.M., Vose, J., Milchunas, D., Martin, C.W., 1994. Plant-production and soil-microorganisms in late-successional ecosystems – a continental-scale study. *Ecology* 75, 2333–2347.