Oikos 121: 1840–1848, 2012
doi: 10.1111/j.1600-0706.2011.20434.x

Subject Editor: Wim van der Putten. Accepted 19 December 2011

Microbial immobilization drives nitrogen cycling differences among plant species

Ramesh Laungani and Johannes M. H. Knops

R. Laungani (ramesh.laungani@doane.edu), Biology Dept, Doane College, 135 Lied Science Building, Crete, NE 68333, USA. – J. M. H. Knops, School of Biological Sciences, Univ. of Nebraska, 348 Manter Hall, Lincoln, NE 68588, USA.

In many terrestrial ecosystems nitrogen (N) limits productivity and plant community composition is influenced by N availability. However, vegetation is not only controlled by N; plant species may influence ecosystem N dynamics through positive or negative effects on N cycling. We examined four potential mechanisms of plant species effects on nitrogen (N) cycling. We found no species differences in gross ammonification suggesting there are no changes in the ecosystem N cycling rate between the soil organic matter pool (SOM) and the plant/microbial pool. We also found weak differences among plant species in gross nitrification, thus plant species only marginally change the relative sizes of the NH_4^+ and NO_3^- pools. Next, more than 90% of mineralized N was microbially immobilized, and microbial N immobilization was positively correlated with root biomass. Finally, while species differed in extractable soil NO_3^- concentration, these differences were not related to root biomass suggesting that microbial immobilization drives net N mineralization and soil NO_3^- levels. Our results indicate that plant species do not cause feedbacks on the N cycling rate among the three major ecosystem N pools over nine years. However, plant carbon (C) inputs to the soil control microbial N immobilization and thereby change N partitioning between the plant and microbial N pools. Furthermore our results suggest that the SOM pool can act as a strong bottleneck for N cycling in these systems.

In many terrestrial ecosystems nitrogen (N) limits productivity (Vitousek and Howarth 1991) and plant community composition is influenced by N availability (Tilman 1984). However, vegetation is not only controlled by N; plant species may influence ecosystem N dynamics through positive or negative effects on N cycling (Wedin and Tilman 1990, Van der Krift and Berendse 2001). Plant species have been shown to differentially influence N inputs and losses from ecosystems (Vitousek 1982, Laungani and Knops 2009b). This makes understanding plant species impacts on N cycling critical for predicting the ecosystem-level consequences of plant community alterations due to land use changes, global change and species introductions.

Nitrogen cycles within terrestrial ecosystems among three main pools (Fig. 1): 1) the soil organic matter (SOM) pool, 2) the live plant N pool, and 3) the soil microbial N pool. Organic N in SOM is mineralized by microbes in two steps; the first step converts SOM to NH_4^+ (gross ammonification) and the second microbially-mediated step converts NH_4^+ to NO_3^- (gross nitrification). Both these mineral N forms are then subsequently taken up by either the plants or the microbes (Fig. 1). However, these gross production rates may not correspond with the net mineral N fluxes to the plant community because of microbial N immobilization (Hart et al. 1994a). Microbial N immobilization is strongly influenced by carbon (C) availability in the soil (Hart et al. 1994a, Stark and Hart 1997, Booth et al. 2005) as the soil microbes are often C limited (Zak et al. 1994, Blumenthal et al. 2003). However, SOM carbon is highly recalcitrant and the soil microbes preferentially use labile forms of plant-derived C (Wardle 2002) such as leaf litter, root litter and root exudates (Zak et al. 1994, Knops et al. 2002). Taken together, plant driven influences on N cycling are likely to be indirect with plant species controlling the rate of C supply to the soil, which in turn controls the activity of the soil microbes, and subsequently both gross and net fluxes of N.

Studies have largely focused on four mechanisms by which plant species can impact ecosystem N dynamics. First, plant species are hypothesized to control the rate of ecosystem N cycling through differences in litter quality (Wedin and Tilman 1990, Van der Krift and Berendse 2001, Knops et al. 2010) and experimental studies comparing plant species have linked differences in litter quality to net N mineralization rates and the rate of ecosystem N cycling (Wedin and Tilman 1990, Van der Krift and Berendse 2001). While these studies can draw conclusions about plant species driven changes to the N cycle (i.e. relative fluxes of mineral N to the plant community as net ammonification and net nitrification measurements), and how much N is returned to the SOM pool via litterfall, conclusions about the rate at which N cycles among the three major pools are

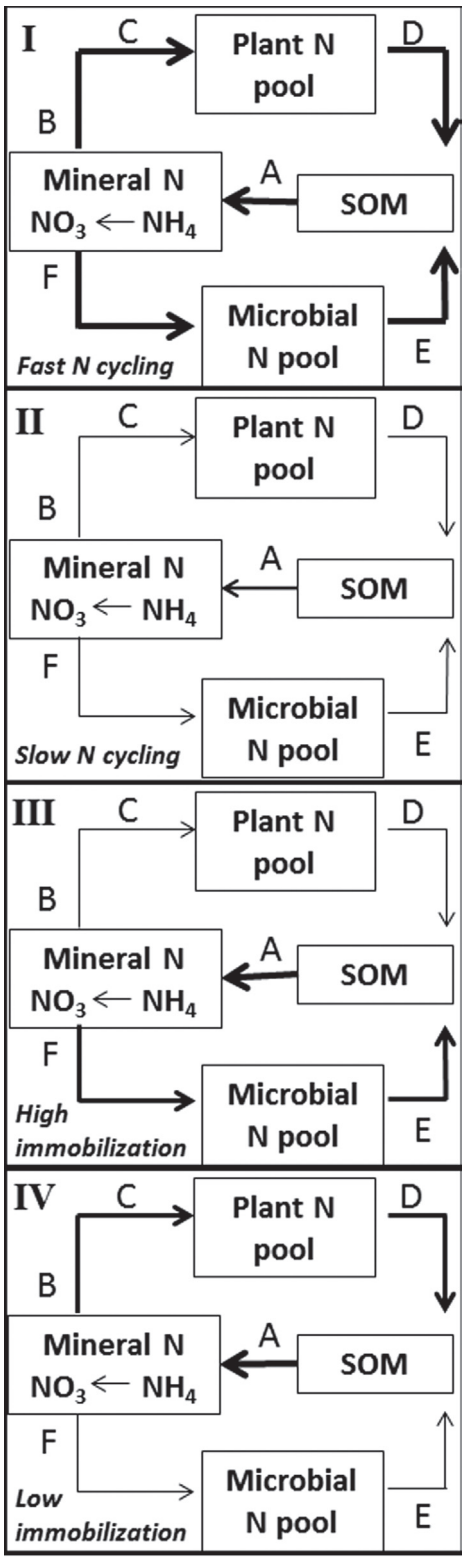

Figure 1. Conceptual diagram illustrating rate of ecosystem N cycling and partitioning of mineralized N from the soil organic matter (SOM) pool between plant biomass and microbial N pools. Arrow thickness represents magnitude of N flux (except for arrow in mineral N pool box which represents the process, not the rate, of nitrification). (I) High rate of ecosystem N cycling with high breakdown of SOM (arrow A). (II) Slow rate of ecosystem N cycling with low breakdown of SOM. (III) and (IV) Equivalent rates of ecosystem N cycling with equivalent breakdown of SOM, but different partitioning of mineral forms of N between net mineral N supply rate (arrow B) and microbial N immobilization (arrow F). (III) represents high rates of microbial N immobilization relative to net mineralization rates, while (IV) represents low rates of microbial N immobilization relative to net mineralization rates. Other letters represent other major fluxes in N cycle (arrow C: rate of plant mineral N uptake; arrow D: rate N returned to SOM in litterfall; arrow E: turnover rate of microbial biomass N returned to SOM pool).

more tenuous because measurements of net N mineralization do not indicate how much N is microbially immobilized and returned to SOM via microbial turnover. Changes in the gross ammonification rate will alter the flux of N to inorganic N pools, which in turn changes the plant and microbial N pools and will result in a feedback that alters the sizes of the major ecosystem N pools (Fig. 1, I and II). Therefore, in order to evaluate whether plant species alter the rate of N cycling among the three major ecosystem N pools, plant species must alter the rate at which the soil microbial community mineralizes SOM, gross ammonification (Fig. 2, no. 1).

Second, plant species can indirectly influence the size of the NH_4^+ and NO_3^- pools by influencing the activity of the ammonium oxidizing bacteria that produce NO_3^- in the soil (Mintie et al. 2003). If plant species are differentially impacting the activity of the ammonium oxidizing bacteria, we would predict that plant species should exhibit different gross nitrification rates (Hawkes et al. 2005) (Fig. 2, no. 2).

Third, plant species, through differences in C inputs to the soil, may influence the net supply of mineral N to the plant community (Bowman et al. 2004) by controlling microbial N immobilization through changes in microbial C limitation (Fig. 1, III). Changes in microbial N immobilization alter the partitioning of mineral N between the plant and microbial N pools (Fig. 1, III and IV). However, if plant species change this mineral N partitioning between the plant and microbial N pools, the size of the SOM pool associated with any given plant species does not change. This is because the total amount of N in organic matter that is returned to the SOM through both plant litter and microbial biomass turnover does not change and the rate at which SOM is mineralized does not change (Fig. 1, III and IV). This is in contrast to changes in the gross ammonification rate that would result in changes to the amount of N that is being returned to SOM and that is being microbially released from SOM (Fig. 1, I and II). If plant species alter the partitioning of mineral N between the plant and microbial N pools through their C inputs to the soil, we would expect a positive relationship between microbial N immobilization and either total root C or root quality (as measured by root C/N ratio), suggesting an indirect impact of plants on the plant available mineral N pool through changes in microbial N immobilization. Conversely, if plant C inputs to the soil do not affect partitioning of mineral N between plant and microbial N pools, we would expect no relationship between N immobilization and either total root C or root quality (Fig. 2, no. 3).

Fourth, species differences in soil N depletion (Tilman 1990) have been hypothesized to lead to changes

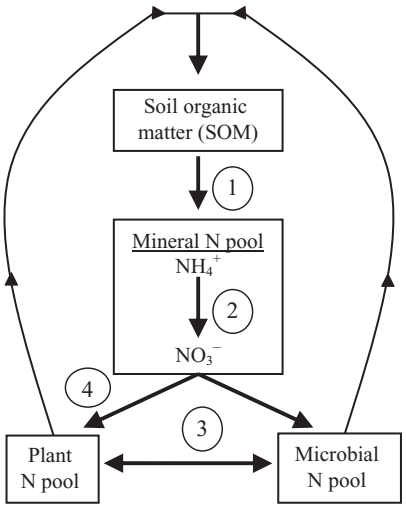

Figure 2. Hypothesized mechanisms of species impacts on N cycling. Numbers represent the four hypotheses being examined in this study. Flow diagram of N from soil organic matter to the soil microbial N pool and plant N pool through the mineral N pool. (1) species driven changes in the rate of ecosystem N cycling, specifically the conversion of organic to inorganic forms of N (SOM → mineral N); changes in the magnitude of this flux will alter the size of all ecosystem N pools; (2) changes in the relative abundance of the two forms of mineral N, specifically changes in the activity of the nitrifying community (e.g. gross nitrification rate); (3) changes in the partitioning of mineral N between the plant and microbial N pools; (4) species differences in mineral N depletion in the soil through plant uptake.

in ecosystem N cycling through species differences in plant capture of mineral N through uptake. Differences in plant N uptake among species may cause differences in productivity and therefore the amount of organic N in plant litter that is returned to the SOM pool. Some studies have found that available soil NO_3^- levels negatively correlate with root biomass, supporting a plant uptake based mechanism driving the observed soil N levels (Tilman 1990, Tilman and Wedin 1991, Fargione and Tilman 2006). However, plant litter is not the only organic N returned to the SOM, species differences in root biomass may also lead to increased microbial N limitation and in turn higher microbial N immobilization. Microbially immobilized N is ultimately returned to the SOM pool via microbial turnover and may be counterbalanced by plant organic N return via litterfall. Thus, to determine if species differences in soil N depletion lead to N cycling changes, we have to examine not only soil mineral N levels and net nitrification but also microbial N immobilization and determine if plant uptake or microbial N immobilization determine the mineral soil N levels. If microbial immobilization is driving species differences in available soil N levels we predict a positive relationship between root C and microbial N immobilization, a negative relationship between root C and net nitrification, and a non-significant relationship between root C and available soil N level as root C is acting as a source of C for the soil microbial community. This would again suggest that plants, and specifically plant C inputs to the soil, are indirectly impacting mineral N pools through changes in microbial N immobilization. Alternatively, if plant uptake directly drives the available soil N level, we would predict a negative relationship between root C and available soil N level (Tilman 1990, Tilman and Wedin 1991, Fargione and Tilman 2006) (Fig. 2, no. 4).

In order to evaluate how plant species influence ecosystem N dynamics we measured all relevant fluxes, i.e. gross ammonification, gross nitrification, microbial NH_4^+ immobilization, microbial NO_3^- immobilization, net ammonification, and net nitrification. We also measured levels of soil NO_3^- associated with these species (R*), and belowground C input quantity (root biomass C) and quality (root C/N ratio) (Laungani and Knops 2009a, b). The suite of measurements used in our study allowed us to concurrently address all four hypothesized mechanisms by which species may influence N cycling in a detailed and systematic manner. We used seven co-occurring old field species that differ widely in growth form, allocation patterns, and litter production and quality (Laungani and Knops 2009a, b). By using seven divergent species, in a common garden study with the same initial soil, we maximized differences among species, eliminated soil variability, and maximized the potential to detect plant species impacts on N dynamics.

Methods

Study site and species

This study was conducted at the Cedar Creek Ecosystem Science Reserve in central Minnesota, where N is the primary resource limiting plant productivity (Tilman 1984). We compared seven species that co-occur; two dominant grasses; the C_3 *Poa pratensis* and the C_4 *Schizachyrium scoparium*, and two forbs that can locally attain high abundances, the symbiotic N-fixing legume *Lespedeza capitata* and the forb *Solidago canadensis*. We also compared *Pinus strobus*, which is rapidly invading grasslands and two historically dominant oak species (*Quercus ellipsoidalis* and *Q. macrocarpa*) that occur at low abundance throughout Cedar Creek (Inouye et al. 1994).

Experimental monocultures of seven grassland and forest species and one bare soil treatment were established in late 2000. There were six replicates of each species treatment (except *Solidago* and *Lespedeza* which only had four replicates each). At Cedar Creek, soils are sandy and derived from glacial outwash (Grigal et al. 1974). We maintained monoculture status of the plots by periodic hand weeding throughout the nine growing seasons. Experimental monocultures consisted of large plastic pots (60 cm in diameter and 50 cm deep) that were dug into the ground so that the top of the pot was flush with the soil surface. Each pot was filled with locally collected field soil, with the lower 40 cm being filled with sub-surface soil and the top 10 cm being filled with topsoil. Grasses and forbs were seeded at the time of initial setup, *Quercus* acorns were planted in the fall of 2000 and one year old *Pinus* seedlings were planted in the early spring 2001. For further details on experimental mesocosms see Laungani and Knops (2009b).

Plant sampling

Belowground biomass was sampled in 2006 at three points within a 10 cm by 60 cm area through the center of the pot at three depths (0–10 cm, 10–25 cm and 25–50 cm) using a 5-cm diameter core. Root in-growth cores were used as an index of annual root growth. These 5-cm root in-growth cores were placed at a depth of 20 cm in May 2006 and harvested in late August 2006. Roots and root ingrowth cores were dried to a constant weight and analyzed for C and N using combustion analysis. Plant biomass was multiplied by measured C concentrations to determine plant C mass. Root biomass was considered to be in steady state by 2006 (Reich et al. 2006). Other work conducted at the same site showed that after 3–4 years biomass yields were also at steady state and sampling of both above and belowground biomass was carried out after five years (Reich et al. 2006). Although we did not directly measure root exudation in this experiment, recent work suggests that root biomass can act as a proxy for belowground plant C inputs including quantity of root exudates (Kuzyakov and Domanski 2000).

Nitrogen cycling measurements

In June 2009 we measured seven response variables in each of our replicate monocultures: gross ammonification, gross NH_4^+ consumption, net ammonification, NH_4^+ immobilization, gross nitrification, NO_3^- immobilization, and net nitrification. We sampled soil from 0–10 cm from each replicate monoculture. We composited and sieved (2 mm) three soil cores (5 cm diameter) from each of the 44 experimental mesocosms. Approximately 250 g (fresh weight) of the sieved soil was amended with 10 ml of 0.147 mM 99% atom solution of $(^{15}NH_4)_2SO_4$ or 0.301 mM 99% atom solution of $K^{15}NO_3$ for gross ammonification or nitrification samples respectively. Soils were amended in re-sealable bags and homogenized by hand for even distribution of the solution.

To determine ^{15}N enrichment levels in pre- and post-incubation soil, N from 2 M KCl soil extracts was concentrated by diffusion on to acidified discs following Stark and Hart (1996), and analyzed for $^{15}N/^{14}N$ by a stable isotope mass spectrometer. Gross N cycling rates were calculated using time zero (taken 5–10 min after ^{15}N addition) and 24 h pool measurements following Eq. 1 (Yamamuro 1988, Takahashi 2001): where m_N is the gross ammonification or nitrification rate (mg N kg^{-1} soil day), $[Pool]_0$ the NH_4-N or NO_3-N concentration at time 0 (mg N kg^{-1} soil), $[Pool]_1$ NH_4-N or NO_3-N concentration at 24 h (mg N kg^{-1} soil), and APE is the atom percent excess over background (atom% ^{15}N–0.37 atom% ^{15}N). Mineral N pools were measured by KCl extraction in 2 M KCl (Hart et al. 1994b). 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 (Yamamuro 1988, Takahashi 2001), as compared to the more commonly used calculation of Hart et al. (1994b). Inorganic N measurements from our 2 M KCl soil extractions were analyzed colorimetrically for NH_4^+ and NO_3^- concentration.

$$m_N = \frac{\left[Pool_1 - \left(Pool_0 \frac{APE_1 \times Pool_1}{APE_0 \times Pool_0}\right)\right] \times -LN\left(\frac{APE_1 \times Pool_1}{APE_0 \times Pool_0}\right)}{1 - \frac{APE_1 \times Pool_1}{APE_0 \times Pool_0}} \quad (1)$$

Net ammonification and net nitrification were calculated as the difference between $[Pool]_1$ and $[Pool]_0$ for NH_4^+ and NO_3^-, respectively. Microbial N consumption rates were calculated as the difference between gross N production and net rates, for both NH_4^+ and NO_3^-. Immobilization of NH_4^+ was calculated as the difference between NH_4^+ consumption and gross nitrification (Davidson et al. 1991, Booth et al. 2005). Immobilization of NH_4^+ was calculated this way because in soils NH_4^+ has three fates: 1) become available to the plant community for uptake, 2) be immobilized into microbial biomass, and 3) oxidized into NO_3^- making it unclear whether consumption of NH_4^+ is the result of either NO_3^- production or microbial immobilization of NH_4^+. Therefore we consider NH_4^+ consumption to be the sum of all NH_4^+ consumptive processes (NH_4^+ immobilization and gross nitrification). We can calculate NO_3^- immobilization as the difference between gross nitrification and net nitrification, because in our well aerated sandy soils denitrification is not a significant N flux (Zak and Grigal 1991). In addition, we calculated NH_4^+ immobilization, net ammonification, NO_3^- immobilization, and net nitrification as a percentage of gross ammonification in order to determine the relative change in the microbial N pools and plant available N pools.

Additionally, in September 2007, May, June and July of 2008, we also measured gross ammonification rates from these same monocultures following the same methods. Also in, May, June and July of 2008 we measured plant-available soil NO_3^- levels (R*) through 0.01 M KCl extraction of N following Tilman and Wedin (1991). A representative R* value for each species was calculated as the average soil NO_3^- level across May, June and July 2008. Inorganic N measurements from our 0.01 M KCl soil extractions were analyzed colorimetrically for NO_3^- concentration.

Statistical analysis

Species differences in the measured response variables were analyzed with a MANOVA with species as the fixed factor. The response variables included in the MANOVA were gross ammonification, NH_4^+ consumption, NH_4^+ immobilization, net ammonification, gross nitrification, NO_3^- immobilization and net nitrification. If there was a significant species effect from the MANOVA, post hoc Tukey tests were conducted in order to examine specific differences among species. As root biomass was assumed to be in steady state in our mesocosms by 2006, we used the 2006 root biomass data in our analysis. Backwards elimination multiple regression analysis was conducted to understand the relationship between root C, soil N and NH_4^+ dynamics and to determine the relationship between root C, extractable NH_4^+ pool, NO_3^- dynamics and measurements of R*. All analyses were conducted in SPSS ver. 17 (2008).

Results

From the MANOVA, we found no significant differences among species in gross ammonification ($F_{7,42} = 0.938$, $p > 0.05$), net ammonification ($F_{7,42} = 1.149$, $p > 0.05$), or NH_4^+ immobilization ($F_{7,42} = 0.743$, $p > 0.05$) with the bare soil treatment not significantly differing from any plant species ($F_{7,42} = 4.996$, $p > 0.05$) (Table 1). There was also no effect of species on gross NH_4^+ dynamics when all sampling periods were analyzed in a repeated measures analysis ($F_{7,42} = 4.996$, $p > 0.05$). Thus plant species did not, over the nine years of this experiment, change the rate of N cycling. Species also did not differ in the amount of NH_4^+ immobilized or the amount of NH_4^+ made available to the plant community as a percentage of gross N mineralized ($F_{7,42} = 1.13$, $p > 0.05$; Table 2). Net ammonification was negative during our incubation as microbial NH_4^+ consumption was larger than gross ammonification. The results did not change when rates were calculated per unit soil N.

Species significantly affected gross nitrification ($F_{7,42} = 2.66$, $p < 0.05$; Table 1). However, when the bare soil control was removed from the analysis, species had only a marginally significant effect ($F_{6,36} = 1.31$, $p = 0.065$). Thus, plant species only marginally changed the activity of the nitrifying community. Species had a significant but weak impact on NO_3^- immobilization as well. These weak species effects on gross nitrification and NO_3^- immobilization translated into significant but weak differences in net nitrification among species with and without the inclusion of bare soil in the analysis ($F_{7,42} = 5.33$, $p < 0.001$; Table 1).

We did not find a significant relationship between belowground root quantity or quality and gross ammonification or NH_4^+ immobilization. However, a backwards multiple regression analysis showed that root biomass C and root ingrowth C/N ratio were positively related to gross nitrification, NO_3^- immobilization, and negatively related to net nitrification (Table 3A, B). The initial pool of NH_4^+ was not related to NO_3^- dynamics.

In testing for species effects on available soil NO_3^- level, we found significant differences among species in soil NO_3^- levels ($F_{7,36} = 33.7$, $p < 0.001$; Fig. 3). However there was a significant relationship between root C and NO_3 immobilization ($F_{1,36} = 26.86$, $p < 0.0001$, corr = 0.659; Table 3) and a negative relationship between root C and net nitrification ($F_{1,36} = 19.33$, $p < 0.0001$, corr = −0.597; Table 3).

The most striking result we found was that across species more than 90% of the mineralized N was immobilized (Fig. 4). Moreover, 72.5% (± 3.7) of the mineralized NH_4^+ was immobilized as NH_4^+. We also found that 45.5% (± 4.8) of the mineralized N was immobilized as NO_3^- (Table 2). The dominance of microbial N immobilization in our system resulted in an overall net decline in the availability of NH_4^+ and in a very small net flux of NO_3^- (Fig. 4). Among species there was an average decline of 18.1% (± 3.3) in available NH_4^+ as a percentage of mineralized N, while only 11.2% (± 3.9) of mineralized N became available to the plants as NO_3^-. The average flux of NH_4^+ immobilized across species was significantly larger than the flux of NO_3^- that was microbially immobilized, and the net ammonification and nitrification rates (Fig. 3). In addition, the flux of NO_3^- immobilized was significantly larger than the net flux of NH_4^+ and NO_3^- (Fig. 4).

Discussion

We examined four mechanisms by which plant species can influence ecosystem N dynamics. We found that species did not differ in the rate of gross ammonification, and moreover that species did not differ from the bare soil control. This result suggests that plant species, over the nine years of this experiment, do not alter the rate at which N cycles in the ecosystem. Other studies have found similar results when examining species effects on gross ammonification in the short term (Hawkes et al. 2005). Differences in gross ammonification have been found among species that had been established at least 50 years (Booth et al. 2003, Mack and D'Antonio 2003) by comparing different vegetation types established in close proximity (McKinley and Blair 2008). Furthermore, since gross ammonification is largely controlled by the size of the SOM pool (Booth et al. 2005), a pool that contains up to 98% of ecosystem N in our system (Knops and Bradley 2009), our results suggest that plant species may only directly cause feedbacks (positive or negative) on the rate of ecosystem N cycling through long-term changes in the SOM pool. A study by Mack and D'Antonio (2003), examining the impact of exotic

Table 1. Species differences in gross N cycling response variables. All rates are shown as mg N kg^{-1} soil day^{-1}. Values represent averages for each species. Values in parentheses are standard error of the mean. Different letters represent significant differences between the species from post hoc Tukey tests ($p < 0.05$). BS (bare soil); Lc (*Lespedeza capitata*); Ps (*Pinus strobus*); Pp (*Poa pratensis*); Qe (*Quercus ellipsoidalis*); Qm (*Q. macrocarpa*); Ss (*Schizachyrium scoparium*); Sc (*Solidago canadensis*).

Species	Gross ammonification $F_{7,42} = 1.16$ $p = 0.350$	NH_4^+ consumption $F_{7,42} = 1.10$ $p = 0.382$	NH_4^+ immobilization $F_{7,42} = 0.765$ $p = 0.620$	Net ammonification $F_{7,42} = 1.48$ $p = 0.206$	Gross nitrification $F_{7,42} = 2.66$ $p = 0.026$	NO_3^- immobilization $F_{7,42} = 4.60$ $p = 0.001$	Net nitrification $F_{7,42} = 5.33$ $p = 0.000$
BS	1.51 (0.50)	1.75 (0.54)	1.28 (0.44)	−0.24 (0.14)	0.47 (0.12)^{a}	0.14 (0.14)^{ab}	0.33 (0.09)^{bc}
Lc	1.38 (0.15)	1.75 (0.22)	0.98 (0.11)	−0.37 (0.09)	0.77 (0.13)^{ab}	0.57 (0.12)^{ab}	0.20 (0.09)^{abc}
Ps	1.84 (0.29)	1.99 (0.27)	1.42 (0.24)	−0.15 (0.14)	0.57 (0.07)^{ab}	0.49 (0.15)^{ab}	0.08 (0.11)^{abc}
Pp	2.45 (0.32)	2.53 (0.29)	1.78 (0.26)	−0.08 (0.10)	0.76 (0.12)^{ab}	0.33 (0.09)^{a}	0.43 (0.09)^{c}
Qe	2.03 (0.17)	2.41 (0.17)	1.10 (0.42)	−0.39 (0.13)	1.32 (0.34)^{b}	1.49 (0.44)^{b}	−0.18 (0.13)^{a}
Qm	2.02 (0.16)	2.33 (0.13)	1.49 (0.12)	−0.31 (0.10)	0.84 (0.09)^{ab}	0.49 (0.10)^{ab}	0.36 (0.08)^{bc}
Ss	2.14 (0.37)	2.60 (0.33)	1.74 (0.34)	−0.46 (0.10)	0.86 (0.02)^{ab}	0.93 (0.08)^{ab}	−0.07 (0.07)^{ab}
Sc	2.07 (0.25)	2.15 (0.29)	1.49 (0.30)	−0.09 (0.11)	0.66 (0.04)^{ab}	0.40 (0.11)^{ab}	0.27 (0.11)^{abc}

Table 2. Species effects on proportional flux of mineralized N into microbially immobilized N pools and plant available N pool using gross N cycling rates in mg N kg^{-1} soil day^{-1}. Values calculated as a percentage of gross ammonification rate. Different letters represent significant differences between the species from post hoc Tukey tests ($p < 0.05$). For species abbreviations refer to Table 1.

Species	% of mineralized N immobilized as NH_4^+ $F_{7,42} = 1.13$ $p = 0.364$	% of mineralized N plant available as NH_4^+ $F_{7,42} = 1.13$ $p = 0.367$	% of mineralized N immobilized as NO_3^- $F_{7,42} = 2.27$ $p = 0.051$	% of mineralized N plant available as NO_3^- $F_{7,42} = 3.45$ $p = 0.007$
BS	83.25 (9.65)	−19.22 (6.64)	−3.79 (18.79)	39.80 (15.89)[b]
Lc	71.27 (4.42)	−26.30 (3.92)	40.61 (5.6)	14.36 (5.28)[ab]
Ps	77.88 (5.60)	−10.66 (7.78)	25.19 (8.76)	7.88 (8.67)[ab]
Pp	71.63 (5.11)	− 4.90 (3.99)	14.94 (4.47)	18.33 (3.42)[ab]
Qe	50.06 (20.52)	−21.06 (6.84)	79.53 (29.39)	−8.57 (7.68)[a]
Qm	73.96 (4.43)	−17.22 (6.27)	26.12 (6.73)	17.17 (3.07)[ab]
Ss	82.14 (4.91)	−38.11 (22.7)	67.72 (31.08)	−11.76 (11.21)[a]
Sc	69.81 (10.37)	−3.58 (6.05)	20.56 (5.91)	13.30 (6.03)[ab]

grass introduction into Hawaiian forests, showed how plant driven changes to SOM over the long term (~50 years) resulted in significant changes in gross N cycling when comparing invaded sites as compared to uninvaded sites. Taken together successful invaders may alter N cycling over the long term through changes in the SOM pool, and these changes, depending on the particular traits of the invader, may either negatively or positively impact the success of native species (Laungani and Knops 2009a, b).

Our second hypothesis examined whether plant species can alter the activity of the nitrifying community in the soil. We found weak support for this hypothesis with only bare soil and *Quercus ellipsoidalis* being significantly different from each other in the rate of gross nitrification. In contrast to the weak direct species effect on gross nitrification, we found a strong relationship between root C and NO_3^- dynamics and root in-growth C/N and NO_3^- dynamics. This suggests that plant traits, such as quantity and quality of inputs to the soil, may potentially directly influence the activity of the nitrifying community, although we did not find species differences in our study. Our results differ from other studies that have found significant differences among species in gross nitrification rates in the short term (Booth et al. 2003, Hawkes et al. 2005) and this may imply that the nitrifying community may respond to species specific inputs such as polyphenols (Castells et al. 2004) in addition to species differences in C inputs into the soil. These alternative mechanisms through which plant species can alter the functioning of the soil microbial community (which we did not evaluate in this study) may play an important role in determining invasive success of some plants (Klironomos 2002, Callaway et al. 2004).

Third, we found that, across these divergent species, immobilization by the soil microbes consistently was the fate of mineral N in the soil. We found that across species the vast majority of the gross mineralized N was immobilized by the soil microbial community and the net

Table 3. Backwards elimination regression for NO_3^- transformations. (A) Gross nitrification, NO_3^- immobilization, and net NO_3^- flux were regressed with plant root C (g C m^{-2}) and NH_4^+ pool (mg N kg^{-1} soil) as predictors. (B) Gross nitrification, NO_3^- immobilization, and net NO_3^- flux were regressed with root in-growth C/N ratio and NH_4^+ pool (mg N kg^{-1} soil) as predictors. All three factors were not included in a single analysis due to high colinnearity between root C and root in-growth C/N ratio. Non-significant factors ($p > 0.05$) were eliminated from the analysis. Standardized correlation coefficients shown for significant factors. p-values and R2 for overall regression presented for model with significant factors.

Factors	NO_3^- transformation: Gross nitrification	NO_3^- immobilization	Net nitrification
(A)			
Root C (g C m^{-2})	0.521***	0.659***	−0.597***
NH_4 pool (mg N kg^{-1} soil)	NS	NS	NS
F; Overall p-value; R^2	$F_{1,36} = 6.62$ $p < 0.005$ $R^2 = 0.272$	$F_{1,36} = 26.86$ $p < 0.0001$ $R^2 = 0.434$	$F_{1,36} = 19.33$ $p < 0.0001$ $R^2 = 0.356$
(B)			
Root in-growth C:N ratio	0.424***	0.529***	−0.471***
NH_4^+ pool (mg N kg^{-1} soil)	NS	NS	NS
F; Overall p-value; R^2	$F_{1,36} = 7.67$ $p < 0.01$ $R^2 = 0.180$	$F_{1,36} = 13.59$ $p < 0.001$ $R^2 = 0.280$	$F_{1,36} = 9.97$ $p < 0.004$ $R^2 = 0.222$

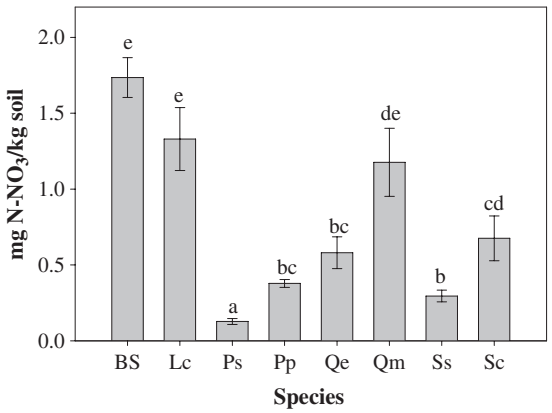

Figure 3. Species differences in soil NO_3^- levels (R*) averaged across May, June and July 2008 sampling periods. All values are shown as mg NO_3-N kg^{-1} soil. Letters represent significant differences between species from post hoc Tukey tests ($p < 0.05$). Error bars represent ± 1 standard error. BS (bare soil); Lc (*Lespedeza capitata*); Ps (*Pinus strobus*); Pp (*Poa pratensis*); Qe (*Quercus ellipsoidalis*); Qm (*Q. macrocarpa*); Ss (*Schizachyrium scoparium*); Sc (*Solidago canadensis*).

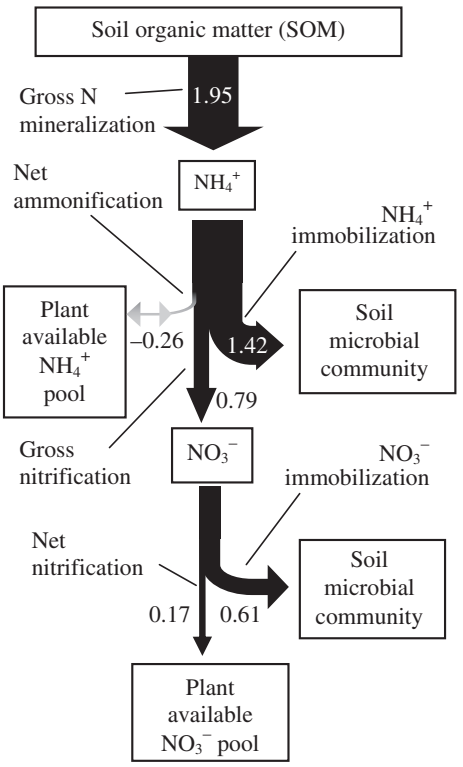

Figure 4. Flow diagram of N from soil organic matter to the soil microbial N pool and plant available NH_4^+ and NO_3^- pools. Arrow size represents magnitude of flux relative to gross N mineralization (SOM → NH_4^+). Flow of NH_4^+ into the plant available NH_4^+ pool is represented by double sided gray arrow because in our system there was net immobilization of NH_4^+, hence this flux may or may not be present in other systems. Values represent average N transformation rates across all species in mg N kg^{-1} soil day. Values showed similar results when calculated in mg N kg^{-1} soil N day^{-1}.

N mineralization flux was relatively small (Fig. 4, Table 1). This is consistent with other studies that have found that the soil microbial community can act as a sink for mineralized N in both grassland (Davidson et al. 1990) and forest (Davidson et al. 1992) ecosystems. We also found that most of the N that was immobilized by the soil microbes was in the form of NH_4^+ suggesting that heterotrophic bacteria were superior competitors for NH_4^+ as compared to the nitrifying community (Sias and Ingraham 1979, Rice and Tiedje 1989, Chen and Stark 2000). This is exemplified by the net decline in the size of the NH_4^+ pool during the incubation across all treatments including the bare soil control. This heterotrophic microbial competitive advantage for NH_4^+ has also been found in a number of laboratory studies (Sias and Ingraham 1979, Rice and Tiedje 1989, Chen and Stark 2000). Substrate limitation of the nitrifiers may explain the low rates of gross nitrification relative to gross ammonification and NH_4^+ immobilization that we observed (Fig. 4). Furthermore, the strong relationships between microbial N immobilization and belowground plant C inputs also supports that, in the short-term, plant species indirectly alter ecosystem N dynamics through changes in the partitioning of mineralized N between the microbial N pool and the plant available mineral N pool (Bowman et al. 2004). This may explain species differences in net N mineralization rates observed in other short-term studies (Wedin and Tilman 1990, Van der Krift and Berendse 2001).

Our fourth hypotheses examined species differences in levels of available soil NO_3^-. While we found species differences in soil NO_3^- levels (Fig. 3), the positive relationship between root C and NO_3^- immobilization (Table 3) and the negative relationship between root C and net nitrification supports the hypothesis that microbial N immobilization responding to plant C inputs may be driving supply rates of NO_3^- to the plant community and observed levels of available soil NO_3^-. Furthermore, if it is assumed that 50% of C uptake by the soil microbial community is respired off as CO_2 and that the soil microbial community typically has a C/N ratio of 10 (Wardle 2002), the soil microbial community may become N limited when breaking down substrates (i.e. root litter) that have C/N ratios that are above 20 (Bengtsson et al. 2003). This potential microbial N limitation is highlighted by the positive relationship between root in-growth C/N ratio (which had an average C/N ratio of 58) and NO_3^- immobilization (Table 3). Therefore research on plant competitive ability must focus on those traits that interact with the soil microbial community such as litter quality and quantity, root turnover rates, and timing of C inputs to the soil, in addition to those that are linked to plant N capture.

In conclusion, our study demonstrates that plant species, over nine years, do not significantly alter the rate of N cycling as evidenced by the lack of species differences in measurements of gross and net N cycling rates. Plant species also do not alter the relative abundance of the two mineral N forms, as plant species largely did not differ in gross nitrification rates. While our data suggest that plant species do not directly influence mineral N availability through uptake (Tilman and Wedin 1991, Fargione and Tilman 2006), plant species can indirectly influence mineral N availability through changes in microbial N immobilization. This is driven by species differences in quantity and quality of C inputs to the soil. This indirect influence of plant species on N cycling does not result in a feedback, i.e. the SOM pool does not change, but does alter the mineral N partitioning between the plant available N pool and the microbial N pool by controlling the relative N versus C limitation of the microbes. While this study did not address the possibility of plants bypassing mineralization and nitrification through the use of organic N, it is still unclear how significant of a flux this can be in temperate systems (Jones et al. 2005). Therefore our conclusions should only be applied to systems where plant use of organic N is low and the microbial community is strongly C limited, such as temperate grasslands. However, by measuring gross N cycling rates, as opposed to the commonly measured net N mineralization rates (Wedin and Tilman 1990, Van der Krift and Berendse 2001), we can gain a more complete understanding of plant species effects N cycling because we can quantify how plant species can indirectly alter N immobilization through changes in the functioning of the soil microbial community. In total, plants indirectly influence N cycling because plant species control the supply of C to the soil microbes. Differences in this C supply in turn determine the strength of microbial

N limitation and immobilization, which subsequently drives net fluxes of mineral N to the plant.

Acknowledgements – We thank Chad Brassil, Stan Harpole, Sarah Hobbie, Cathleen McFadden and Dave Wedin for comments and Wendy Bengston, Jenny Goth, Troy Mielke, Kally Worm and the Cedar Creek interns for analytical and field help and Cathleen McFadden at the Univ. of Nebraska-Lincoln Ecosystem Analysis Laboratory. This research was supported by the National Science Foundation and the Univ. of Nebraska.

References

Bengtsson, G. et al. 2003. Gross nitrogen mineralization-, immobilization- and nitrification rates as a function of soil C/N ratio and microbial activity. – Soil Biol. Biochem. 35: 143–154.

Blumenthal, D. M. et al. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. – Ecol. Appl. 13: 605–615.

Booth, M. S. et al. 2003. Inorganic N turnover and availability in annual- and perennial-dominated soils in a northern Utah shrub-steppe ecosystem. – Biogeochemistry 66: 311–330.

Booth, M. S. et al. 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. – Ecol. Monogr. 75: 139–157.

Bowman, W. D. et al. 2004. Litter effects of two co-occurring alpine species on plant growth, microbial activity and immobilization of nitrogen. – Oikos 104: 336–344.

Callaway, R. et al. 2004. Soil biota and exotic plant invasion. – Nature 427: 731–733.

Castells, E. et al. 2004. Are phenolic compounds released from the Mediterranean shrub *Cistus albidus* responsible for changes in N cycling in siliceous and calcareous soils? – New Phytol. 162: 187–195.

Chen, J. and Stark, J. M. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. – Soil Biol. Biochem. 32: 47–57.

Davidson, E. A. et al. 1990. Microbial-production and consumption of nitrate in an annual grassland. – Ecology 71: 1968–1975.

Davidson, E. A. et al. 1991. Measuring gross nitrogen mineralization, immobilization and nitrification by N-15 isotopic pool dilution in intact soil cores. – J. Soil Sci. 42: 335–349.

Davidson, E. A. et al. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. – Ecology 73: 1148–1156.

Fargione, J. and Tilman, D. 2006. Plant species traits and capacity for resource reduction predict yield and abundance under competition in nitrogen-limited grassland. – Funct. Ecol. 20: 533–540.

Grigal, D. F. et al. 1974. Soils of the Cedar Creek Natural History Area. – Univ. of Minnesota Agric. Exp. Stn, St. Paul, MN.

Hart, S. C. et al. 1994a. Dynamics of gross nitrogen transformations in an old-growth forest – the carbon connection. – Ecology 75: 880–891.

Hart, S. C. et al. 1994b. Nitrogen mineralization, immobilization and nitrification. – In: Weaver, R. et al. (eds), Methods of soil analysis part 2 – microbiological and biochemical properties. Soil Sci. Soc. Am., pp. 985–1018.

Hawkes, C. V. et al. 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. – Ecol. Lett. 8: 976–985.

Inouye, R. S. et al. 1994. Old field succession on a Minnesota sand plain – effects of deer and other factors on invasion by trees. – Bull. Torr. Bot. Club 121: 266–276.

Jones, D. L. et al. 2005. Dissolved organic nitrogen uptake by plants – an important N uptake pathway? – Soil Biol. Biochem. 37: 413–423.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. – Nature 417: 67–70.

Knops, J. M. H. and Bradley, K. L. 2009. Soil carbon and nitrogen accumulation and vertical distribution across a 74-year chronosequence. – Soil Sci. Soc. Am. J. 73: 2096–2104.

Knops, J. M. H. et al. 2002. Mechanisms of plant species impacts on ecosystem nitrogen cycling. – Ecol. Lett. 5: 454–466.

Knops, J. M. H. et al. 2010. The role of litter quality feedbacks in terrestrial nitrogen and phosphorus cycling. – Open Ecol. J. 3: 14–25.

Kuzyakov, Y. and Domanski, G. 2000. Carbon input by plants into the soil. Review. – J. Plant Nutr. Soil Sci. Z. Pflanzenernahrung Bodenkunde 163: 421–431.

Laungani, R. and Knops, J. M. H. 2009a. The impact of co-occurring tree and grassland species on carbon sequestration and potential biofuel production. – Global Change Biol. Bioenergy 1: 392–403.

Laungani, R. and Knops, J. M. H. 2009b. Species-driven changes in nitrogen cycling can provide a mechanism for plant invasions. – Proc. Natl Acad. Sci. USA 106: 12400–12405.

Mack, M. C. and D'Antonio, C. M. 2003. Exotic grasses alter controls over soil nitrogen dynamics in a Hawaiian woodland. – Ecol. Appl. 13: 154–166.

McKinley, D. C. and Blair, J. M. 2008. Woody plant encroachment by *Juniperus virginiana* in a mesic native grassland promotes rapid carbon and nitrogen accrual. – Ecosystems 11: 454–468.

Mintie, A. T. et al. 2003. Ammonia-oxidizing bacteria along meadow-to-forest transects in the oregon cascade mountains. – Appl. Environ. Microbiol. 69: 3129–3136.

Reich, P. B. et al. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. – Nature 440: 922–925.

Rice, C. W. and Tiedje, J. M. 1989. Regulation of nitrate assimilation by ammonium in soils and in isolated soil microorganisms. – Soil Biol. Biochem. 21: 597–602.

Sias, S. R. and Ingraham, J. L. 1979. Isolation and analysis of mutants of *Pseudomonas aeruginosa* unable to assimilate nitrate. – Arch. Microbiol. 122: 263–270.

Stark, J. M. and Hart, S. C. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. – Soil Sci. Soc. Am. J. 60: 1846–1855.

Stark, J. M. and 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 Sci. Soc. Am. J. 65: 244–246.

Tilman, D. 1984. Plant dominance along an experimental nutrient gradient. – Ecology 65: 1445–1453.

Tilman, D. 1990. Mechanisms of plant competition for nutrients the elements of a predictive theory of competition. – In: Grace, J. B. and Tilman, D. (eds), Perspectives on plant competition. Academic Press, pp. 117–141.

Tilman, D. and Wedin, D. A. 1991. Plant traits and resource reduction for five grasses growing on a nitrogen gradient. – Ecology 72: 685–700.

Van der Krift, T. A. J. and Berendse, F. 2001. The effect of plant species on soil nitrogen mineralization. – J. Ecol. 89: 555–561.

Vitousek, P. M. 1982. Nutrient cycling and nutrient use efficiency. – Am. Nat. 119: 553–572.

Vitousek, P. M. and Howarth, R. W. 1991. Nitrogen limitation on land and in the sea – how can it occur. – Biogeochemistry 13: 87–115.
Wardle, D. A. 2002. Plant species control of soil biota and processes. – In: Wardle, D. A (ed.), Communities and ecosystems: linking the aboveground and belowground components. Princeton Univ. Press, pp. 56–103.
Wedin, D. A. and 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 NH_4–^{15}N tracer technique. – Jap. J. Soil Sci. Plant Nutr. 59: 538–548.
Zak, D. R. and Grigal, D. F. 1991. Nitrogen mineralization, nitrification and denitrification in upland and wetland ecosystems. – Oecologia 88: 189–196.
Zak, D. R. et al. 1994. Plant-production and soil-microorganisms in late-successional ecosystems – a continental-scale study. – Ecology 75: 2333–2347.