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Species effects on nitrogen cycling: a test with perennial grasses

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Summary. To test for differing effects of plant species on nitrogen dynamics, we planted monocultures of five perennial grasses (*Agropyron repens*, *Agrostis scabra*, *Poa pratensis*, *Schizachyrium scoparium*, and *Andropogon gerardi*) on a series of soils ranging from sand to black soil. In situ net N mineralization was measured in the monocultures for three years. By the third year, initially identical soils under different species had diverged up to 10-fold in annual net mineralization. This divergence corresponded to differences in the tissue N concentrations, belowground lignin concentrations, and belowground biomasses of the species. These results demonstrate the potential for strong feedbacks between the species composition of vegetation and N cycling. If individual plant species can affect N mineralization and N availability, then competition for N may lead to positive or negative feedbacks between the processes controlling species composition and ecosystem processes such as N and C cycling. These feedbacks create the potential for alternative stable states for the vegetation-soil system given the same initial abiotic conditions.

Key words: Nitrogen cycling – Species effects – Grasses – Litter quality – N mineralization

The supply rates of limiting soil nutrients, such as nitrogen (N), have a major influence on primary productivity, species composition and species diversity in terrestrial ecosystems. The supply rate of N depends largely on N mineralization, the microbially mediated conversion of organic N to inorganic forms (NO_3^- and NH_4^+). N mineralization is regulated by both abiotic factors (e.g. soil moisture, temperature, and texture) and by the supply of above- and belowground litter (Jenny 1980), which can differ widely in decomposition rate, depending on the quality, quantity, and timing of inputs (Berendse et al. 1989; Melillo et al. 1982; Seastedt 1988). Thus,

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the nature of the vegetation in a system can have a large effect on that system's N supply rate, and N supply rates can in turn strongly influence vegetation composition. This feedback could create alternative stable states for the vegetation-soil system under abiotically identical conditions (Vitousek 1982; Pastor and Post 1986; DeAngelis et al. 1989). However, the multi-causal relationship between vegetation and soil development in natural ecosystems has obscured the differing roles of individual species in controlling soil N dynamics. Recent work suggests that differences among individual plant species could also cause significant differences in ecosystem-level processes (Pastor et al. 1984; Vitousek and Walker 1989; Berendse et al. 1989; Carney 1989). Others, though, have argued that most species differences "lose their identity" (Allen and Hoekstra 1989) at the ecosystem level, and that the study of ecosystem functions at the species-level may be counterproductive (O'Neill et al. 1986; Allen and Hoekstra 1989). Our experiments address these divergent viewpoints.

Demonstrating species-level effects on N cycling requires the field measurement of the N supply rates associated with different species grown under initially uniform conditions. To date, field observations of net N mineralization under different species have compared either clearly divergent species, such as N fixers versus non-N fixers (e.g. Vitousek et al. 1987), or long-established stands, such as different deciduous and/or coniferous forest communities (e.g. Flanagan and VanCleve 1983; Nadelhoffer et al. 1983; Pastor et al. 1984; Zak et al. 1986). Moreover, interpretation of observational studies can be confounded by uncontrolled differences in climate, soil parent material, or other factors. We addressed these two concerns through experiments in which monocultures of five perennial grass species were planted on a series of initially homogeneous soils ranging from an infertile sand to a fertile black soil. Using three years of in situ soil incubations, we determined the magnitude of species differences in: (1) annual net N mineralization; (2) the seasonal pattern of N availability; and (3) the relative supply of NH_4^+ versus NO_3^- . Related

studies observed the N-use efficiencies of the five species (Wedin 1990), the relationship of various plant traits to resource depletion (Tilman and Wedin 1990a), and the species' competitive interactions across the experimental soil N gradient (Wedin 1990; Tilman and Wedin 1990b).

Materials and methods

Our research was performed at the Cedar Creek Natural History Area (CCNHA), which is located on a glacial outwash sandplain in east-central Minnesota, USA. We studied the five most abundant grasses in the successional grasslands at CCNHA: *Agrostis scabra* Willd., *Agropyron repens* (L.) Beauv., *Poa pratensis* L., *Schizachyrium scoparium* (Michx.) Nash-Gould, and *Andropogon gerardi* Vitm. *Agrostis* is a native C_3 species common in early successional fields; *Agropyron* and *Poa* are Eurasian C_3 species common in early- and mid-successional grasslands, respectively; and *Schizachyrium* and *Andropogon* are C_4 bunchgrasses dominant in late-successional grasslands and undisturbed tallgrass prairie and oak savannah at CCNHA (Tilman 1988).

The 79 experimental monocultures (each 0.75×0.75 m), as well as plots used in related studies, were located in 9 large soil mixtures (each 3×12 m) which ranged in composition from all sand to all black soil. Each soil mixture was created by mixing black soil into the subsurface sand left after the removal of the upper 0.7 m of soil from a CCNHA old field with a bulldozer. The black soil (a Duelm sandy loam of the Hubbard-Isanti-Duelm association from outside CCNHA) averaged 72% sand, 4% clay and 24% silt, with 3% organic matter, 1100 mg/kg total N, and pH of 7.2. Subsurface sand averaged 93% sand, 3% clay and 4% silt, with 0.3% organic matter, 90 mg/kg total N, and pH of 6.6. All plots were mixed to an average depth of 23 cm with a rototiller and were separated by 25-cm deep sheet metal.

Prior to planting, four 20-cm deep cores were taken from each plot, pooled, and analyzed for total soil N (persulfate digestion method, see Tilman 1984). "Total soil N" or "total N" refer to these values of initial total soil N. Each monoculture was seeded with one of the five species in May, 1986 at approximately 3000 live seedlings per m^2 . There were 18 monoculture plots per species (2 plots in each of 9 soil mixtures), except that *Andropogon* had 9 plots (one per soil mixture), and two *Poa* monocultures are omitted because of poor seedling establishment. To insure that N was the only soil resource limiting growth, all plots received at least 1.5 cm/wk of water in April, May and October, and 2.5 cm/wk in June–September, and all plots were fertilized annually with all nutrients except N (P, K, Mg, S, Ca, Zn, Cu, B, Mn). The pH was adjusted, as needed, to 7.2 with finely ground lime. Complete methods are in Tilman and Wedin (1990a).

Net N mineralization was estimated monthly from June 1986 to November 1988, except when soils were frozen, with an in situ soil incubation using 1.9-cm diameter plastic tubes (PVC) sunk to a depth of 16 cm, and covered with caps to prevent leaching losses. Although such incubation tubes contain severed roots, which may immobilize N and bias estimates of net N mineralization, Binkley and Hart (1989) concluded that closed-top core incubations yielded results comparable to other in situ incubation techniques. Pre- and post-incubation soil samples were extracted with 2M KCl, shaken for 0.5 h, settled overnight at 4° C, and analyzed for $NH_4^+ - N$ and $NO_3^- - N$ with a Technicon II autoanalyzer. A subsample from each soil sample was used to determine moisture content. Net mineralization is the sum of final minus initial values for NH_4^+ and NO_3^- , and therefore refers to the sum of net in situ ammonification and nitrification.

Conversion of net N mineralization rates from mg of N per kg dry soil to $g N m^{-2}$ is based on the bulk density of the soil in each plot, which was measured in a subsample of plots and

estimated for the remainder ($B = 1.509 - 0.000103 \cdot N$, $R^2 = 0.98$, where B is bulk density and N is total soil N). The net N mineralization estimates presented here assume soil homogeneity to 23 cm, the depth of the mixed soil layer. The 1987 and 1988 annual net mineralization rates include the sum of six incubations (average length 34 days) as well as a five month over-winter incubation started the preceeding November. To allow comparison with 1987 and 1988 results, 1986 results, which did not include November to mid-June incubations, were divided by 0.56, which is the fraction, averaging over all species for both 1987 and 1988, of the annual mineralization occurring in mid-June through November.

We estimated nitrification as the percentage of annual net N mineralization converted to NO_3^- . Values for percent nitrification greater than 100% (4 out of 81 plots for 1988) were considered 100%. Because our in situ incubation method prevented competition between active plant roots and nitrifying bacteria, this method may overestimate actual nitrification rates (Pastor et al. 1984).

Biomass data and plant tissue samples were collected from adjacent monocultures used in a study of species' differences in productivity and N-use efficiency (Wedin 1990). The 10 plots (each 2.4×1.5 m, two replicates for each species) were larger than, but otherwise identical to, the 100% black soil plots used in the N mineralization study. Litter production was estimated in November 1988 by clipping 20×55 cm sections from areas in each plot where all litter from previous years had been removed by hand in July 1988. Any living (green) tissue present in the clipped samples was removed. Litter from each plot was ground with a UDY cyclone mill (0.25 mm mesh screen) and analyzed for total tissue C and N with a Carlo-Erba NA1500 Analyzer. Lignin is the percent mass remaining (minus ash) after dilute and concentrated (72% H_2SO_4) digestions (Van Soest 1963). Belowground biomass was sampled with 25-cm deep cores which were washed free of soil on a 1-mm sieve. The values of belowground biomass presented here are the maximum values from 10 sampling periods during 1988 (complete above- and belowground biomass and tissue N dynamics are in Wedin 1990). Tissue analyses for C, N, and lignin followed the procedures for litter except that belowground samples used for lignin analysis were collected in November 1989.

The relative contributions of species effects and initial total soil N in explaining the variance in annual net N mineralization were compared by fitting three different models to each year's results using the General Linear Models (GLM) routine of SAS (SAS 1988). Model 1 is a simple linear regression of N mineralization on total N. Model 2 allows for different intercepts among the species' responses to total N, but gives all species the same slope (the traditional Analysis of Covariance). A significant F test for the species effect in Model 2 indicates that Model 2 is a significant improvement over Model 1. Model 3 fits the response of N mineralization to total N with a separate intercept and slope for each species. The F test for the species X total N effects tests whether or not the individual species' regressions have significantly different slopes, i.e., whether Model 3 is a significant improvement over Model 2. If the species X total N effect is significant, the ability of the model incorporating species effects to explain the variance in N mineralization rates can be compared to the model with only total N by using the R^2 values for Model 3 and Model 1. If the species X total effect is not significant, the R^2 values of Model 2 and Model 1 are compared.

Least Squares Means and pairwise comparisons ($P < 0.05$) generated by the Model 3 fit of the data from GLM (SAS 1988) were used to test for significant differences between species in annual net N mineralization. Least Squares Means are the estimated mean values of the dependent variable (N mineralization) for each species, with the covariate (total N) held at its mean value (ca 500 mg/kg). If the slopes of the individual species' responses to total N differ (i.e., a significant species X total N effect in Model 3), then the Least Squares Means comparisons are valid only for the middle of the total soil N gradient.

Mean values for annual net N mineralization were estimated using the regression equations of each species in each year. Means were determined for three values of total soil N: 200 mg/kg (ca

10% black soil and 90% sand), 500 mg/kg (ca 50% black soil), and 1000 mg/kg (ca 100% black soil). A 95% confidence interval for each estimated mean was generated using the Model 3 analysis from GLM.

Results

In 1986, the first year of the study, initial total soil N, alone, (Model 1) explained 40% of the variance in annual net N mineralization, while the model incorporating species' differences in mineralization (Model 2) explained 57% (R^2 values from Table 1). In 1987, initial total N (Model 1) explained 37% of the variance in N mineralization, but the model incorporating species' differences (Model 3) now explained 88%. In 1988, initial total N (Model 1) explained only 9% of the variance in N mineralization, whereas the model with both total N and species' differences (Model 3) explained 82% (Table 1).

The increased importance of species was caused by a divergence among species in annual net N mineralization (Table 2, Figs. 1 and 2). There was a significant species effect in the first year (Table 1), with *Poa* having significantly higher N mineralization than all other species except *Agropyron*, and *Andropogon* having significantly lower N mineralization than all other species except *Schizachyrium* (pairwise comparisons using Least Square Means; $P < 0.05$; see Materials and Methods). This is seen both in the slopes of the individual species' regressions (Table 2) and in the estimated mean values of annual net N mineralization (Fig. 2). The magnitude of the species effect was small compared to the total N effect, however (Table 1). In 1987 and 1988, N mineralization in *Agrostis* plots increased, but decreased for all other species. For both 1987 and 1988, all species pairs differed significantly in N mineralization except *Agropyron* versus *Poa*, and *Schizachyrium* versus *Andropogon* (pairwise comparisons using Least Square Means, $P < 0.05$). The divergence in N mineralization was greatest at higher total N (Fig. 2). By 1988, the regression of N mineralization and total N was significant only for *Agrostis* (Table 2; Fig. 1).

Seasonal patterns of net N mineralization were similar in 1987 and 1988, except for decreased mineralization for all species in the July incubation in 1988 (Fig. 3; 1987 data not shown). Only a small proportion of annual net mineralization took place in fall, winter, or early spring. However, the species did differ in their periods of peak mineralization (Fig. 3). *Agrostis* had peak N mineralization during August, while *Andropogon* and *Poa* had early June peaks. *Schizachyrium* and *Agropyron* did not have clear peak periods of mineralization, although *Schizachyrium* appeared to have higher mineralization in May–June and *Agropyron* in July–August in both 1987 and 1988 (Fig. 3).

The percentage of annual net N mineralization that was nitrified in 1988 (percent nitrification) differed significantly among species when tested with ANOVA ($F_{3,74} = 3.94$, $P = 0.0059$). *Agrostis* had the highest percent nitrification (97%), followed by *Agropyron* (93%),

Table 1. Effects of initial total soil N (Total N) and grass species (Species) on annual net N mineralization in 1986–1988. The F-tests for the two effects and their interactions, and the R^2 values for the various models are from GLM analyses; *** indicates $P \leq 0.001$, ** indicates $P \leq 0.01$, * indicates $P \leq 0.05$. See Materials and methods for descriptions of the various models and details of analyses

Model	Effect	F	df	R^2
1986				
1	Total N	48.41***	1, 74	0.395
2	Species	6.80***	4, 70	
	Total N	61.45***	1, 70	0.565
3	Species	0.15	4, 66	
	Total N	52.52***	1, 66	
	Species \times Total N	2.16	4, 66	0.625
1987				
1	Total N	44.74***	1, 77	0.367
2	Species	20.30***	4, 73	
	Total N	82.20***	1, 73	0.701
3	Species	2.89*	4, 69	
	Total N	132.99***	1, 69	
	Species \times Total N	25.06***	4, 69	0.878
1988				
1	Total N	7.47**	1, 77	0.088
2	Species	27.94***	4, 73	
	Total N	15.45***	1, 73	0.640
3	Species	1.02	4, 69	
	Total N	16.14***	1, 69	
	Species \times Total N	17.72***	4, 69	0.822

Table 2. Regressions for five grass species of annual net N mineralization ($\text{g N m}^{-2} \text{y}^{-1}$) versus initial total soil N (mg kg^{-1}). R^2 values are from independent fits of each species' regression, although the same coefficients are generated by the Model 3 analyses of Table 1. Slopes significantly different from zero are indicated *** ($P < 0.001$), ** ($P < 0.01$), or * ($P < 0.05$). The regressions for 1988 are shown in Fig. 1. See Materials and methods for details of analyses

Species	Slope ($\times 10^{-4}$)	Y Intercept	R^2	n
1986				
<i>Agrostis</i>	48.56**	1.54	0.405	18
<i>Agropyron</i>	69.44***	1.22	0.656	16
<i>Poa</i>	76.77***	2.08	0.607	16
<i>Schizachyrium</i>	43.45***	1.26	0.416	18
<i>Andropogon</i>	13.22	1.52	0.106	8
1987				
<i>Agrostis</i>	107.22***	0.61	0.865	18
<i>Agropyron</i>	29.52***	2.49	0.569	18
<i>Poa</i>	49.78***	2.13	0.740	16
<i>Schizachyrium</i>	14.78**	1.10	0.422	18
<i>Andropogon</i>	15.89	1.28	0.372	9
1988				
<i>Agrostis</i>	93.58***	2.64	0.728	18
<i>Agropyron</i>	12.70	2.80	0.155	18
<i>Poa</i>	-1.95	3.02	0.002	16
<i>Schizachyrium</i>	-7.26	1.73	0.122	18
<i>Andropogon</i>	4.09	1.37	0.125	9

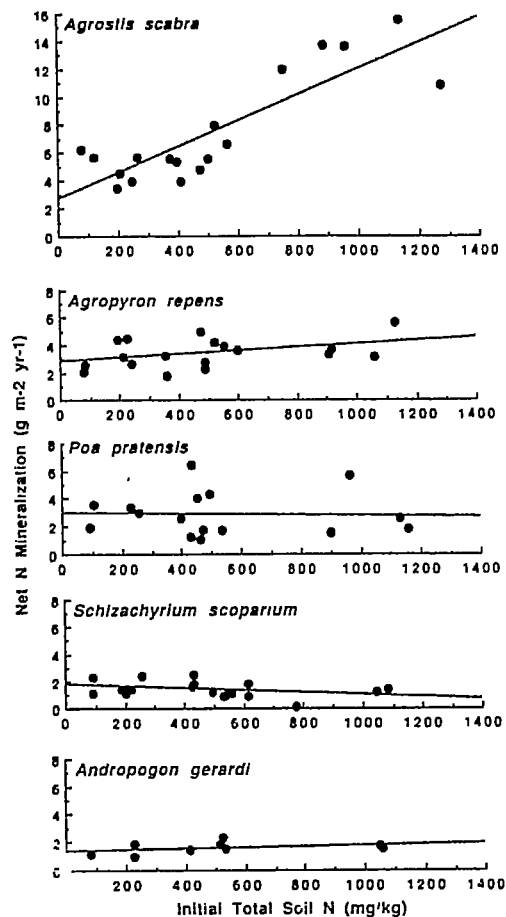


Fig. 1. Annual net N mineralization measured in situ in three-year old monocultures of five grass species versus initial total soil N. Coefficients for the regression lines are given in Table 2 (1988). See text for further explanation

Poa (85%), *Schizachyrium* (84%), and *Andropogon* (79%). Results for 1987 were similar. Plots with high net N mineralization always had high values of percent nitrification, while plots with low net N mineralization had both high (100%) and low (as low as 20%) values of percent nitrification (Fig. 4).

The litter and belowground tissue of the five species differed significantly in both lignin and N concentrations (Table 3). *Agrostis* had the highest N and lowest belowground lignin concentrations, followed by *Agropyron*, *Poa*, and then *Schizachyrium* and *Andropogon*, which had comparable values for N but differed significantly in lignin. The C:N ratios were inversely correlated with tissue N concentrations. Although there were significant differences among species in aboveground lignin, the magnitude of the differences was small and the rankings of the species did not follow the same order seen in the other tissue quality measures (Table 3). Our values for aboveground lignin concentration are low compared to other measurements on the two C_4 prairie species, *Andropogon* and *Schizachyrium* (W. Parton, Colorado State University, personal communication), perhaps because of the low numbers of reproductive stems, which tend to be highly lignified, in our plots.

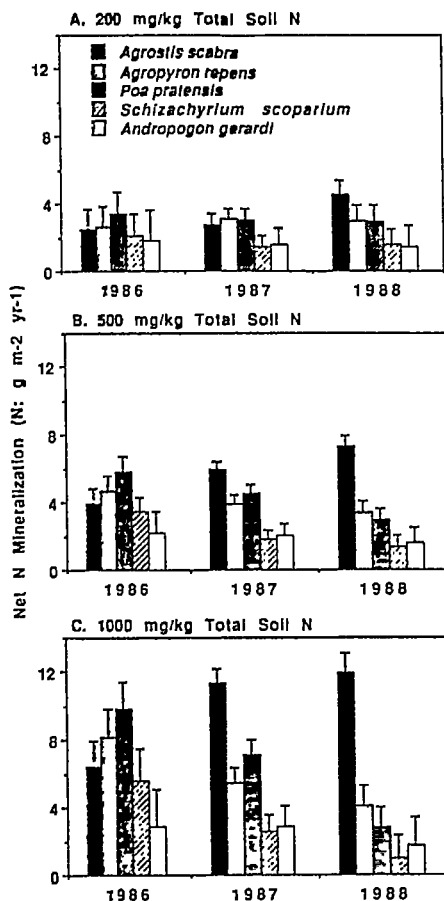


Fig. 2 A-C. Mean values (with 95% confidence intervals) for annual net N mineralization from 1986-1988 in monocultures of five grass species. Means were estimated for three values of total soil N using separate regressions for each species in each year (Table 2). See text for further explanation

Belowground biomass differed thirty-fold among the species. *Agrostis* had only 40 g/m² belowground biomass in the black soil plots. *Agropyron* and *Poa* had approximately 500 g/m², and *Schizachyrium* and *Andropogon* had approximately 1200 g/m² (Table 3). These differences in belowground standing crop correspond to large differences in belowground productivity among the species (Wedin 1990). Differences among the species in aboveground litter production were much smaller (Table 3) and not significant, although increased replication might have detected significant differences. The method we used may underestimate aboveground litter production for the three C_3 species if there were significant losses, prior to November, of litter produced early in the growing season.

Discussion

Species differences in net N mineralization and litter quality

The dramatic divergence of net N mineralization rates in initially identical soils under five perennial grass spe-

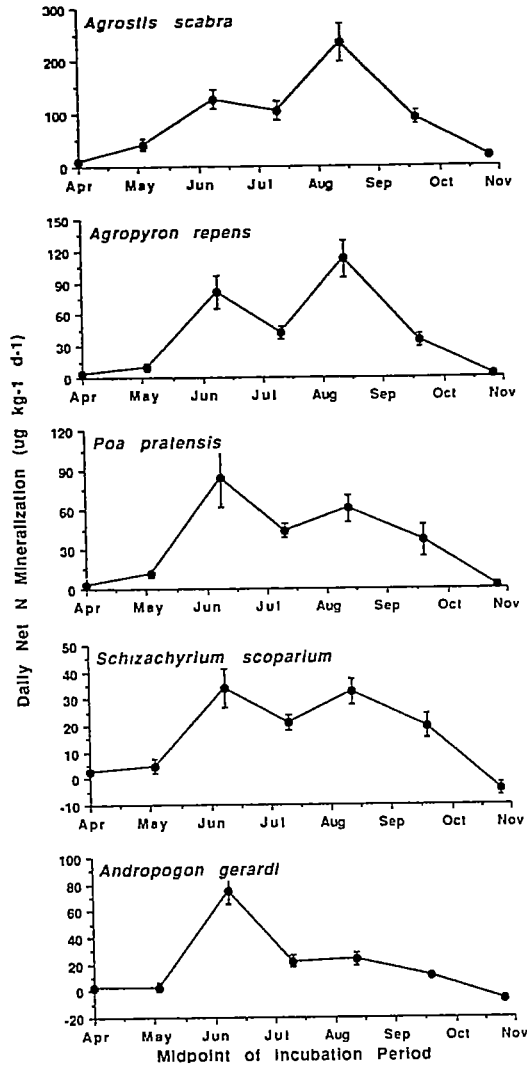


Fig. 3. Daily net N mineralization rates (micrograms of N per kilogram of soil per day) in three-year old monocultures of five grass species. Each point is the mean (and standard error) of a species' monocultures from across the soil N gradient, and is graphed at the midpoint of the incubation period. The values for April 1 represent over-winter incubations started the previous November. Large horizontal-axis marks indicate the first of each month

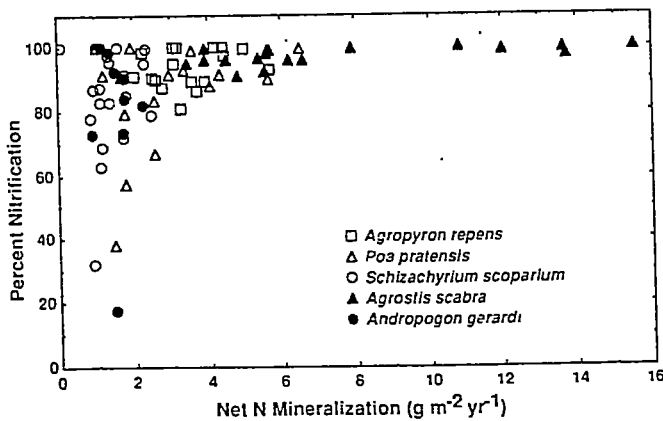


Fig. 4. Percent nitrification [(ratio of annual net nitrification to annual net mineralization) $\times 100$, maximum = 100%] versus annual net N mineralization measured in three-year old monocultures of five species

Table 3. Various litter quality and quantity measures for five species growing in three-year old monocultures on 100% black soil plots. Aboveground data are for the current year's senescent tissue (litter). Belowground data are for living and dead roots and rhizomes. N and Lignin Concentrations are given as percentages ($\text{g/g} \times 100$). Data are means and standard errors, $n=2$ for each species. Means with the same letter did not differ significantly (PLSD contrasts, $P<0.05$) when analyzed by ANOVA. Belowground biomass results were log-transformed prior to analysis. See text for further explanation

	<i>Agrostis scabra</i>	<i>Agropyron repens</i>	<i>Poa pratensis</i>	<i>Schiz. scoparium</i>	<i>Andro. gerardi</i>
Belowground N Concentration	1.81 (0.11)a	0.98 (0.01)b	0.70 (0.05)c	0.49 (0.02)d	0.51 (0.03)cd
Belowground C:N Ratio	23.6 (0.9)a	46.0 (0.3)b	69.2 (5.0)c	101.5 (6.0)d	85.8 (6.5)cd
Belowground Lignin Conc.	5.20 (0.45)a	8.39 (0.82)b	10.13 (0.34)c	11.28 (0.01)c	13.68 (0.16)d
Belowground Biomass (g/m^2)	45.3 (9.5)a	502.8 (13.2)b	536.8 (8.4)b	1164.6 (63.9)c	1233.4 (36.9)c
Aboveground N Concentration	1.01 (0.01)a	0.87 (0.01)b	0.71 (0.03)c	0.40 (0.01)d	0.33 (0.02)d
Aboveground C:N Ratio	43.8 (0.8)a	55.7 (2.3)a	63.7 (1.8)a	109.1 (0.5)b	122.1 (10.5)b
Aboveground Lignin Conc.	5.10 (0.11)b	6.10 (0.19)a	3.98 (0.09)c	5.76 (0.05)a	5.08 (0.09)b
Aboveground Litter Production ($\text{g m}^{-2} \text{ y}^{-1}$)	196.4 (52.2)a	162.2 (14.6)a	163.0 (40.5)a	280.0 (13.4)a	313.7 (34.5)a

cies demonstrates the potential for strong interactions between the species composition of vegetation and nitrogen cycling. Black soil (high total soil N) plots with *Schizachyrium* or *Andropogon* had mineralization rates of 1 to 2 $\text{g N m}^{-2} \text{ y}^{-1}$, whereas *Agrostis* plots had mineralization rates of approximately 12 $\text{g N m}^{-2} \text{ y}^{-1}$ in the third year of the study (Fig. 2C). Similar divergences occurred on medium and low total N soils (Fig. 2A-B). Despite the 10-fold range in initial total soil N, less than 10% of the variance in net N mineralization was explained by differences in total N by the third year. The differences among these perennial grasses in net N mineralization are comparable to or greater than differences in in situ net mineralization between different forest types (e.g. Pastor et al. 1984) or grasslands and croplands (e.g. Schimel 1986).

Although our in situ incubation technique, like all such techniques, may affect soil processes in unintended ways, such in situ incubations provide a good index of net N mineralization rates (see recent reviews by Binkley and Hart 1989; Vitousek et al. 1989). If our estimates of net N mineralization are accurate, they should, when added to other components of N supply, equal or exceed the amount of N required annually for above- and belowground plant production. This was true for *Agrostis*, *Agropyron* and *Poa*, but in situ annual net N mineralization, combined with the estimated atmospheric N deposition for CCNHA ($0.7 \text{ g N m}^{-2} \text{ y}^{-1}$) was approximate-

Table 4. Correlation coefficients between the various litter quality and quantity parameters in Table 3 and estimated annual net N mineralization for 100% black soil plots (Part C, Fig. 2). The various measures are ranked in order of correlation (absolute value) with N mineralization. Also presented are the correlations between the best correlate, Belowground Tissue N Concentration, and the other parameters. Correlations marked * are significant at $P \leq 0.05$ for $n = 5$

Litter Quality Parameter	Net N Mineralization	Belowground Tissue N
Below N	+0.992*	+1.000
Below C:N	-0.895*	-0.930*
Below Lignin	-0.890*	-0.933*
Below Biomass	-0.862	-0.897*
Below Lignin:N	-0.838	-0.893*
Above N	+0.817	+0.873
Above C:N	-0.735	-0.794
Above Lignin:N	-0.664	-0.712
Litter Prod.	-0.419	-0.497
Above Lignin	-0.490	+0.240

ly $1.0 \text{ g N m}^{-2} \text{ y}^{-1}$ less than the estimated annual N requirements of *Schizachyrium* and *Andropogon* growing on high total N soils (Wedin 1990). Thus, we may have underestimated either net N mineralization, N supply from atmospheric deposition, or N fixation. Also, in extrapolating our results from 16-cm deep cores to a 23-cm depth, we may have overestimated species effects on N mineralization at greater depth, where such effects are presumably smaller. Although these estimates of net N mineralization may differ from the actual rates, we nonetheless feel they reflect real and significant differences among species.

Various researchers have found that litter N content, C:N ratio (Flanagan and Van Cleve 1983; Taylor et al. 1989), lignin content (Meentemeyer 1978; Berg and McLaugherty 1989), and lignin:N ratio (Melillo et al. 1982; Pastor and Post 1986) can predict the decomposition rates and the potentials of various litter types to immobilize N prior to N mineralization. In our study, differences in net N mineralization rates among monocultures of these five species were highly correlated with differences in belowground litter quality (Table 4). Differences in belowground biomass among these five species were much greater than differences in aboveground litter production (Table 3). Together, the large differences among species in both the quality and quantity of belowground litter appear to be driving the divergence in N mineralization. However, because of the high correlations among litter quality parameters (Table 4), and because we compared only five species, these results cannot indicate which parameters are most important in controlling N mineralization (e.g. belowground N versus lignin). A more complete understanding of belowground litter and N dynamics in grasslands will require the study of additional processes, including the turnover rates of different size classes of roots, the quantities of C and N in root exudates, and the ability of plant roots to translocate N prior to senescence.

The decreasing correlation of net N mineralization with soil total N for *Agropyron*, *Poa*, *Schizachyrium*, and *Andropogon* over the three years was probably caused by increased N immobilization. Although gross N mineralization presumably increased along the total soil N gradient, the larger quantities of low quality litter, particularly roots, produced in the high total N soils provide the potential for greater N immobilization. Aboveground litter and roots from *Schizachyrium* and *Andropogon*, the two species with the highest belowground biomass, the lowest litter quality, and the lowest net N mineralization rates, decompose slowly and immobilize significant amounts of N over a two year period (Pastor et al. 1987b; Seastedt 1988). In the longterm, net mineralization rates may increase in monocultures of these four species as litter which has been immobilizing N during the first three years begins to release N. Such an increase seems reasonable when mineralization rates reported here are compared to a net N mineralization rate of approximately $4.5 \text{ g m}^{-2} \text{ y}^{-1}$ measured in a Cedar Creek field with total soil N of 800 mg kg^{-1} dominated by *Schizachyrium* and *Poa* (Pastor et al. 1987a).

Seasonal patterns of N mineralization

The seasonal patterns of net mineralization for the five species correspond to differences in phenology, which are in turn related to differences in photosynthetic pathway. *Agrostis*, a C_3 species, begins growth in early spring, senesces during seed production in mid-summer (mid- to late-July), and produces new leaves again starting in late August. The monthly net N mineralization rate for *Agrostis* was over twice as high during this period of dormancy in August as in any other period (Fig. 3). Because of its mid-season senescence, *Agrostis* makes large quantities of litter available to decomposers when soil temperatures are high and plant demands on soil moisture and N are minimal. Therefore, the phenology of *Agrostis* apparently interacts with its high litter quality (Table 3) to cause rapid decomposition and high net mineralization rates at mid-growing season. The asynchrony of plant uptake and N mineralization in the *Agrostis* plots resulted in ten-fold greater concentrations of extractable soil NO_3^- in August compared to the rest of the year ($F_{6,84} = 12.233$, $P = 0.0001$, ANOVA testing the effect of date on 2M KCl extractable soil NO_3^- in *Agrostis* plots on soils ranging from 0–600 mg N kg^{-1} soil). Similar patterns of N cycling occur in California annual grasslands (Jackson et al. 1988) and wheat fields (Buyanovsky et al. 1987).

In contrast, *Andropogon*, a warm-season, C_4 grass, had a peak mineralization rate in early June, and a low mineralization rate in August. *Andropogon* begins aboveground growth in mid-May at CCNHA, and has maximum aboveground biomass in late July and early August when *Agrostis* is senescing. The spring peak in mineralization in *Andropogon* monocultures was not matched, however, by an increase in extractable soil NO_3^- . Soils under *Andropogon*, which is a superior competitor (Til-

man and Wedin 1990b) and a dominant species in native grasslands (Tilman 1988) on nitrogen-poor sandy soils in Minnesota, had low extractable soil NO_3^- concentrations throughout the growing season, with no significant differences between dates ($F_{6,42} = 2.076$, $P = 0.0766$, ANOVA testing the effect of date on 2M KCl extractable soil NO_3^- in plots ranging from 0–600 mg N kg^{-1} soil). This does not support the generalization that infertile habitats are characterized by periodic pulses in soil nutrient concentrations and that nutrient uptake by species adapted to infertile habitats is dominated by high rates of uptake during such pulses (Chapin 1980; Campbell and Grime 1989).

Species effects on nitrification

In addition to affecting the annual rates or seasonal patterns of N mineralization, individual plant species may affect the relative availabilities of NH_4^+ and NO_3^- . Rice (1984) hypothesized that late successional plant species, particularly bunchgrasses such as *Schizachyrium* and *Andropogon*, have an allelopathic inhibition on nitrifying bacteria. This would cause a shift from NO_3^- dominated systems early in succession to NH_4^+ dominated systems late in succession. This conclusion, however, has been frequently questioned (Robertson and Vitousek 1981; Robertson 1982; Nadelhoffer et al. 1983; Vitousek et al. 1989).

The five grass species studied here differed significantly in the percentage of annual net N mineralization that was nitrified to NO_3^- , with a lower percentage for the late successional grasses. Although values of percent nitrification were greater than 75% for all species, the ranking of the species is consistent with Rice's (1984) hypothesis. However, the parallel rankings of the species for percent nitrification and net N mineralization (Fig. 4) support an alternative explanation: that nitrification rates and the sizes of nitrifier populations are limited by the supply of NH_4^+ , i.e. net N mineralization (Robertson and Vitousek 1981).

Rice and Panchoy (1972) used the low concentrations of extractable soil NO_3^- in tallgrass prairie as evidence that the dominant bunchgrasses inhibited nitrification. However, because instantaneous measurements of NH_4^+ and NO_3^- pool sizes reflect both N supply rates and plant uptake, conclusions cannot be drawn about N mineralization or nitrification rates based on such measurements (Robertson and Vitousek 1981; Nadelhoffer et al. 1983; Binkley and Hart 1989). Significant differences in soil NO_3^- concentration among our five species were highly negatively correlated with large differences in belowground biomass (Tilman and Wedin 1990a). The increase in root biomass during succession in grasslands (e.g. Monk 1966; Gleeson and Tilman 1990) and the high root biomass of native bunch and tussock grasslands (Walter 1979) are well documented. Therefore, we suggest that high rates of plant uptake combined with low rates of net N mineralization are the most straightforward explanation of the low soil NO_3^- concentrations found in native grasslands.

The feedback between plant competition and nitrogen cycling

If competition for N is a major process structuring vegetation, the litter feedback effect of an individual plant species on N dynamics could be an important element of its competitive ability. In related pairwise competition experiments with various combinations of these five grasses, *Schizachyrium* and *Andropogon* completely displaced the other three species within three years on low total soil N plots (Wedin 1990; Tilman and Wedin 1990b). However, competitive displacement did not occur within three years for *Agropyron* versus *Poa*, or *Schizachyrium* versus *Andropogon*. The apparent mechanism behind these competitive displacements was the ability of a superior N competitor to reduce soil solution concentrations of NH_4^+ and NO_3^- , (estimated by 0.01 M KCl extracts: Tilman and Wedin 1990a) to a lower level than its competitor. The plant traits correlated with this ability were low tissue N concentrations and a high allocation of biomass to roots, the same traits linked to a high competitive ability for N by experimental (McGraw and Chapin 1989) and theoretical (Tilman 1990) studies. Therefore, the species that are superior N competitors are also the species that cause low N supply rates through their litter feedback effects on net N mineralization (Pastor et al. 1984; Tilman 1988). *Agrostis*, which had the highest mineralization rate, was the poorest N competitor, while *Andropogon* and *Schizachyrium*, which had low mineralization rates, had high competitive abilities for N. *Poa* and *Agropyron* were intermediate in both traits. Thus, the effects of *Andropogon* and *Schizachyrium* on N mineralization provide a positive feedback effect that reinforces their superior competitive ability for N.

Positive feedbacks may also potentially reinforce the dominance of plants with higher tissue N concentrations, short-lived tissues, and high allocation to stems and leaves (Vitousek 1982). These traits, which are associated with a high competitive ability for light (Tilman 1988), are characteristic of species that dominate productive habitats (Grime 1979; Tilman 1988). They also lead to higher litter quality and higher net N mineralization rates (Pastor et al. 1984). Both *Agropyron* and *Poa* displace *Schizachyrium* and *Andropogon* following N addition to grasslands at Cedar Creek, presumably because of a superior competitive ability for light (Tilman 1987). If the higher mineralization rates we observed under these species (Fig. 2) lead to increased productivity and greater light limitation, these effects on N cycling could cause multiple stable equilibria in CCNHA grasslands.

Negative feedbacks between plant competition and N cycling require that a species produce litter that leads to a N supply rate for which it is not a good competitor. Examples include N-fixing plants in low-N primary successions, such as species of *Myrica*, *Alnus*, or *Dryas* (Vitousek and Walker 1987). These species have high litter quality, and increase N supply rates (Vitousek et al. 1987), but although good competitors under low N supply rates, they are usually displaced under more productive conditions. Thus, both positive and negative feed-

back effects between competitive ability and nutrient cycling may exist in nature. Such species-specific feedback effects could magnify founder effects, creating localized soil heterogeneity that contributes to the maintenance of local species diversity.

The early successional species *Agrostis* was a poor competitor on both low-N and high-N soils (Tilman and Wedin 1990b), yet persists in disturbed fields by virtue of its rapid growth rate and high rate of production of well-dispersed seeds (Tilman and Wedin 1990a). "Ruderals" (Grime 1979), such as *Agrostis*, may in general have high litter quality and lead to increased net N mineralization rates (Covington 1981; Vitousek and Walker 1987). Although these effects on N supply rates are probably short-lived (less than a decade), they may affect species composition later in succession. They may also lead to significant losses of N from already low-N systems during the first stages of secondary succession (Vitousek et al. 1989).

In summary, our results demonstrate that there can be distinct species effects on N cycling, even among species as superficially similar as five perennial grasses. The species effects in this study appeared to be primarily on net N mineralization, and, to a lesser degree, on the seasonal pattern of N supply and rates of nitrification. Such species effects, when combined with differences in competitive ability, could provide positive feedbacks that might lead to multiple stable equilibria in plant communities. The importance of such feedbacks in grasslands at CCNHA still remains to be demonstrated experimentally however. The five grass species studied here can be placed in three functional groups, based on N and lignin concentration and biomass allocation patterns, for which we can predict both their competitive interactions and their effects on N mineralization rates. Clearly, though, placing all five species in the functional group "perennial grasses" is not sufficient. Predicting the effects of other plant species on N cycling will require a better understanding of species' differences in physiological and morphological traits, such as N use and biomass allocation, and a better understanding of the trade-offs and constraints that determine these traits.

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