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Relationship between the structure of root systems and resource use for 11 North American grassland plants

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Abstract

Eleven Midwest North American grassland plant species differed in their construction, production, and placement of fine and coarse belowground biomass in the soil profile after having been grown in containers in the field for two and a half growing seasons. Based on the patterns of root system structure and resource utilization, the species we examined could be classified as 1) legumes, 2) high-nitrogen rhizomatous C_3 species, and 3) a separate gradient of differentiation from tall- to short-statured species (i.e. tallgrass to shortgrass species). Legumes depleted water evenly throughout the soil profile, with little capacity for acquisition of inorganic nitrogen throughout the 1m soil profile. The three rhizomatous species had shallow fine root distributions, a large relative investment in shallow rhizomes, and moisture and NO_3^- levels were low in shallow soils, but high at depth. Tallgrass species maintained a large standing root biomass of high-density, low-nitrogen fine roots, and acquire nitrogen and water from a large, deep volume of soil, in which inorganic nitrogen is present in low concentrations. Root systems of shortgrass species lacked coarse belowground biomass, had fine roots that were finer than those of the tallgrass species, and had a shallow root distribution. There was little support for functional dichotomies between the C_3 and C_4 species or between the grasses and forbs. For example, *Solidago rigida* (C_3 forb) and *Andropogon gerardii* (C_4 grass) were more similar to each other than to other C_3 forbs or C_4 grasses, respectively. Across all species and depths examined, there were strong relationships between the amount of fine root biomass present in a unit of volume of soil and the depletion of soil water and nitrogen, but there were no relationships with coarse belowground biomass. This reaffirms that differentiation of coarse and fine root biomass is as important as differentiating stems and leaves in evaluating plant allocation and ecosystem functioning.

Introduction

The functional attributes of root systems (e.g. specific root length (SRL), tissue density, tissue nitrogen (N) concentration, ratio of coarse and fine roots, placement of roots in the soil) are determinants of both ecosystem and plant community dynamics, including belowground resource acquisition, net primary production, and competitive interactions (Nedrow 1937; Parrish and Bazzaz 1976; Aerts et al. 1991; Nepstad et al. 1994; Jackson et al. 1999). Even though root systems clearly differ among species and ecosystems (Weaver 1968; Canadell et al. 1996; Jackson et al. 1996), the relationships of the functional attributes of root systems among species are not well-understood.

Early research on grassland root systems focussed on qualitative descriptions of root system structure in order to explain differences in the distribution and abundance of species and vegetation types (Waterman 1919). Severe droughts during the 1930's in North America led Weaver to initiate the first quantitative measurements of the depth distribution, biomass accumulation rates, longevities, and *in situ* decomposition rates of roots of various species across grassland

types (Weaver and Zink (1946a, 1946b); Weaver 1947; Weaver and Darland 1947). Though limited in the number of species examined, from tall to short-statured grasses, Weaver (1958b) showed that root longevity, root tensile strength, root diameter, depth of rooting, and maximum productivity decreases while tissue N increases. These studies were also among the first grassland studies to quantify plant-ecosystem relationships, such as relationships between root biomass and soil organic matter formation, soil resistance to erosion, and plant biomass recovery from drought (Weaver et al. 1935a; Albertson and Weaver 1944; Weaver and Bruner 1945; Weaver and Darland 1949).

Despite the work by Weaver and his contemporaries (Sperry 1935; Coupland 1950; Hopkins 1951; Albertson and Tomanek 1965) and over four decades of subsequent research, our understanding of root systems is still rudimentary. For example, the depth distributions of roots for different species, functional groups and ecosystems are only just beginning to be summarized and interpreted as to their role in plant-ecosystem resource exchange and associated ecosystem resource fluxes (e.g. Nepstad et al. (1994) and Canadell et al. (1996), Jackson et al. (1996)). For the most part, these syntheses have yet to differentiate among belowground biomass types (e.g. coarse belowground biomass and fine roots) that differ in their roles of acquisition and transport of resources.

Since Weaver's seminal work, our understanding of resource limitation and how it constrains grassland structure and function has changed as recent studies in North American tallgrass prairie and subhumid tropical savannas have emphasized nitrogen's role as a key limiting nutrient (Wedin 1995; Seastedt 1995; Hooper and Johnson 1999), a role largely under-appreciated by Weaver and contemporaries. Today, the relationships between different belowground biomass types and patterns of resource utilization and ecosystem nitrogen cycling are not well-quantified. More complete understanding of root systems requires understanding the relationships between root construction, total biomass and placement in the soil profile, and the consequences for water and nitrogen availability in the soil.

Associated with our lack of understanding of root system construction, root traits are generally excluded from functional classifications of species, almost certainly reducing the predictive power of ecosystem models (Woodward et al. 1997). Two general types of functional classifications have been used for grassland floras. First are classifications based on plant distributions, such as dichotomies between upland and lowland species (Weaver 1968), classifications of grassland species based on their moisture affinity (Curtis 1959), and divisions of core and satellite species (Collins et al. 1993). The second set of classifications are *a priori* classifications that classify species based on inherent traits that are measured independently of distribution (Weaver 1958b; Leishman and Westoby 1992; Kindscher and Wells 1995; Grime et al. 1997; Tilman et al. 1997; Sala et al. (1997, 1997)). Only Weaver's classification of grasses (tall, mixed, short) and forbs incorporated root traits to a significant degree. Weaver divided forbs into four root functional types based on depth, presence of a taproot, root length density per unit soil volume and placement of fine roots (Weaver 1958a).

In this paper, we 1) examine the relationship(s) of root system traits among a wide variety of prairie species, 2) quantify relationships between root biomass and nutrient use, and 3) classify species based on measured belowground traits. For the purposes of understanding the relationships of traits among species, we measured or derived 36 traits associated with the biomass, nutrient, and water dynamics of plants and include root and root system traits, whole-plant characteristics, associated soil water and N availability, and ecosystem N retention for 11 prairie species. Traits included root construction (SRL and its components tissue density and diameter), nutrient concentrations of tissues, biomass of different components (fine roots, coarse belowground biomass, crowns (or belowground bases of stems) and aboveground biomass) with belowground biomass separated by depth, water and nutrient availability at three depths, and whole system nutrient losses. In order to provide contrast in species traits, we chose species that are generally common in Midwest tallgrass communities but span a range of functional classifications (C_3 grass, C_4 grass, forb, legume; tallgrass-shortgrass; rhizomatous and non-rhizomatous species). Although not a focus of this paper, the experimental design included a moderate nitrogen fertilization treatment to determine if differences in N supply alter the relationships among traits, the relationship between biomass and N use, and/or cause differential responses among species.

Using Weaver's findings as a starting point along with accumulated evidence from other experiments (e.g. Tilman and Wedin (1991)), we hypothesized that plants with high density roots should have high

Table 1. Species used in this study, taxonomic reference, photosynthetic pathway, characteristic maximum shoot height (Short < 50 cm; Mid between 50–100 cm; Tall > 1 m), relative degree of rhizome formation, and functional group classifications of the species.

Species	Citation	Photosynthetic pathway	Shoot height	Rhizome	Forb-grass-legume
Achillea millefolium	L.	C_3	Short	Strong	Forb
Agropyron repens	(L.) Beauv.	C_3	Mid	Strong	C_3 Grass
Anemone cylindrica	Gray	C_3	Short	None	Forb
Andropogon gerardii	Vitman	C_4-NADME	Tall	Weak	C_4 Grass
Bouteloua gracilis	Willd. ex Kunth	C_4-NADPME	Short	Weak	C_4 Grass
Koeleria cristata	(L.) Pers.	C_3	Short	Weak	C_3 Grass
Lespedeza capitata	Michx.	C_3	Tall	None	Legume
Petalostemum villosum	(Vent.) Rydb.	C_3	Tall	None	Legume
Poa pratensis	L.	C_3	Short	Strong	C_3 Grass
Schizachyrium scoparium	(Michx.) Nash	C_4-NADME	Mid	Weak	C_4 Grass
Solidago rigida	*L.*	C_3	Tall	Weak	Forb

aboveground biomass, high belowground biomass produced throughout the first meter of soil depth, low tissue nutrient concentrations, reduce soil inorganic N concentrations in the soil to low levels, and high ecosystem N retention. Again, it should be noted that this hypothesis is only a starting point as we were examining a much wider variety of species and traits than had been measured before and most theories of plant functional trait relationships on which we could base hypotheses do not address the belowground traits we measured.

We quantified relationships between root biomass (both fine roots and coarse belowground biomass) and plant-mediated soil resource availability (both water and nitrogen). Although analogous relationships have long been known for leaves and light, it is still unknown whether similar standard relationships exist for root biomass and nitrogen or water availability. Even though differences in nitrogen availability may be due directly to uptake by roots or indirectly through plant effects on microbial decomposition, we hypothesized that there would be negative relationships between root biomass and both soil inorganic N and water availability. As fine roots should have greater specific acquisition rates than coarse belowground biomass and also affect microbial decomposition greater than coarse belowground biomass, we hypothesized that there should be stronger relationships between fine root biomass and soil resources than for coarse belowground biomass. Although it is possible that deep roots are more important for water uptake than nutrient uptake, we hypothesized that there would be no differences in the relationships between biomass and resource availability at different depths in the soil profile.

Lastly, we examine the functional classifications that are derived from data on root systems and resource utilization. We then examine the congruency between these classifications and those based on current *a priori* classifications (e.g. photosynthetic pathway, grasses vs. forbs) or distributional data.

Methods

Experimental design

A total of 66 monocultures were grown in the field for two and a half growing seasons. Treatments were applied in a factorial design: 4 functional groups, 3 species per functional group (1 functional group only had 2 species), two nitrogen levels per species, and 3 replicates per treatment combination (4 replicates perished). Treatment combinations were arranged randomly in two rows.

The species included 3 C_3 grasses, 3 C_4 grasses, 3 C_3 forbs, and 2 legumes (Table 1). These species are known to vary in their degree of rhizome development and maximum shoot height (Table 1). Ten of the eleven species are common in the sand prairies and oak savannas found at the study site, the Cedar Creek Natural History Area (CCNHA) in east-central Minnesota, USA. *Bouteloua gracilis* does not occur naturally at CCNHA, and is generally found in mixed- and shortgrass prairie to the west, though it is found in Minnesota and is used extensively in experiments at CCNHA. Two of the C_3 grasses, *Agropyron repens*

and *Poa pratensis* are non-native, but widely naturalized in area grasslands.

Growth conditions

Monocultures were contained in polyvinylchloride tubes, 20 cm in diameter and 96 cm high. Each monoculture had minirhizotron tubes installed horizontally through the container at 12 cm, 40 cm, and 76 cm depth. The tubes were filled with homogenized soil that was obtained from the top 10 cm of soil from an abandoned agricultural field at CCNHA that had previously had the top 10–15 cm of soil removed. Soils in the field were sandy (94% sand, 6% silt plus clay), with low soil carbon (0.45% C in 0–20cm horizon). After filling the container with soil, the soil was supersaturated with water (a.k.a. tamping), which minimizes variation in bulk density with depth. We had structured the soils in this general manner to minimize differences in bulk density and nutrient content that may influence patterns of belowground biomass distribution with depth. The bottom of each container was fitted with a plastic cap through which was placed a plastic bushing filled with polyester batting to facilitate drainage.

Monocultures were seeded in early June of 1997 at the rate equivalent to 12 g seed m^{-2} and watered frequently during the first 6 weeks of the first growing season. These monocultures were placed in 1 m deep trenches in a field at CCNHA during the summers to allow access to minirhizotron tubes. In winter, the trenches surrounding the monocultures were filled to the soil surface of the monocultures with soil to ameliorate harsh temperatures. Half of the monocultures for each species (3) were amended with a total of the equivalent of 6.7 g N m^{-2} in the form of NH_4NO_3 during the 1998 and 1999 growing season, applied three times in 1998 and two times in 1999.

Measurements

Aboveground biomass

Beginning July 19, 1999, all aboveground biomass was clipped and sorted into dead and live fractions. The live fractions included any leaf, stem or reproductive biomass that was at least partially green.

Soil moisture and extractable nitrogen

Within three days after the aboveground biomass was clipped, each container was cut into three sections. Since root biomass should be concentrated at the top of the containers, we produced three strata, 0–24, 24–56, and 56–96 cm in depth to more closely mimic the expected pattern of root biomass with depth. After sectioning, we removed a 2.5 cm diameter, 20 cm deep core from the top of each strata. Soils were kept at 5 °C for no more than 24 hours before processing. 0.01 M KCl soil extractable NO_3^- and NH_4^+ and gravimetric soil moisture were determined according to Wedin and Tilman (1993).

Root biomass

After the soil core was removed from the sections, the root mass was washed free of soil under running water over a 1.3 mm screen. Root samples were stored at 5 °C for no more than 48 hours before being separated into coarse, fine, and crown biomass fractions. For non-grasses and those rhizomatous grasses that did not have true crowns, the "crown" category included the bases of stems, considered to be the interface between aboveground and belowground parts, and included no more than 1 cm of aboveground material and 1 cm of belowground biomass. All non-crown root and rhizome segments greater than 1 mm in diameter were considered coarse belowground biomass and those roots less than 1 mm in diameter were considered fine roots.

Root traits

After washing, representative subsamples of fine roots were removed from each monoculture for determining specific root length (SRL), root tissue density, and average diameter. We used a digital scanner to simultaneously provide data on average root diameter and root volume. Each subsample was suspended in 1 cm of water in a 10 × 15 cm clear acrylic tray and then scanned at 600 dots per inch (0.04 mm resolution) with a Hewlett Packard Scanjet 4c and WinRhizo root analysis program (Regent Instruments, Quebec, Canada). After scanning, each subsample was drained and placed into a small paper envelope, dried for 72 hours at 65 °C and weighed.

WinRhizo 4.0 (Régent Instruments, Quebec) was used to analyze scanned images. This program traces roots present on an image and quantifies total root length and the diameter of each segment of length. The threshold was set at Automatic and Adaptive. Lagarde's method for pale roots was used in the analyses with normal sensitivity and no filter. The analysis process creates a data file that includes the total amount of root length and root volume present in each size class (e.g. 0.10 – 0.15 mm) for each subsample scanned. Root data were summarized into 40 size

classes, 0.05 mm each. The average diameter of a given unit of length of the fine roots was calculated from the resultant data set. Tissue density was calculated as the ratio of the subsample's mass and the total root volume of the subsample. SRL was calculated as the ratio of the subsample's total root length to the mass of the subsample.

Tissue carbon and nitrogen

All biomass was dried at 50 °C until constant mass, generally seven days, and then weighed. Each biomass fraction type (fine, coarse, crown, and shoot) was composited over the three depths, if applicable, and then ground in a cyclone mill (Udy Corp., Ft. Collins, CO). Carbon and nitrogen concentrations were determined with a Leco CN2000 analyzer (Leco Corp, St. Joseph, MI).

Calculations

Belowground biomass was scaled to g m^{-2} for the entire 96 cm depth. This required multiplying the measured mass of each section by the appropriate constant to take into account the root biomass present in the 2.5 cm core that was removed earlier. In addition to calculating the amount of biomass in a belowground biomass fraction per unit ground area, fine root and coarse belowground biomass per unit soil volume were calculated. This allows standardized comparisons of the amount of root biomass per unit volume among depths since each stratum had a different volume. The fraction of fine root biomass in each of the three depths relative to the total fine root biomass was calculated as well as the relative amount of coarse belowground biomass in each of the three depths. We also calculated the amount of biomass in a given fraction across all depths relative to the total biomass. Total biomass N was calculated as the sum of the total N for each fraction (g biomass m^{-2} * %N). Since no measurement of crown tissue N was determined, we used a standard tissue N value that was derived from the average of coarse belowground tissue N concentrations for all species (0.85% N).

Data analysis

All statistical analyses were performed using JMP 3.0 (SAS Institute, Cary, NC, USA). To determine the relative influence of the identity of the 11 species and the N treatment on functional parameters, we used the multiple linear regression protocol to regress each of the 33 functional parameters on the following explanatory variables: a categorical representation of the 11 species, a categorical representation of the N treatment (elevated vs. ambient) and an interaction term between species identity and N treatment. To determine the differences between species in functional traits, we performed an ANOVA for each of 33 different functional traits. Differences among species were determined with a Tukey-Kramer HSD test.

We used a multiple linear regression model to determine if there were relationships between root biomass and soil moisture content. This model tested relationships between both fine root and coarse belowground biomass density in the soil and soil moisture. The model also tests for separate relationships for each root biomass type under elevated and ambient N (interaction terms between the root biomass type and the N treatment), and separate relationships for each root biomass type for each of the three depth categories (interaction terms between the root biomass type and a categorical coding of the three strata). A similar model was used to examine the relationship of root biomass and inorganic soil N concentrations.

To linearize certain relationships in the above models, we applied a log transformation to the inorganic N concentrations, the fine root biomass density, and the coarse belowground biomass density. A few of the inorganic N measurements were close to the detection limit of our methodology and were measured to have N concentrations that were lower than our blank standards. Hence, the reported values are negative. Instead of removing these values from the data set or artificially setting them to a positive number (which would bias relationships), we added a constant to all values to make each value positive prior to log transformation. As some of the root biomass measurements for a stratum were zero, a constant was also added to all fine root and coarse belowground biomass values before log-transformation.

To determine the relationships among functional traits across species, we performed a principal components analysis (PCA) on 31 functional traits for which data existed for each monoculture (see below), using a correlational matrix structure. Although some pairwise relationships were not linear, the PCA can qualitatively address the nature of relationships using untransformed data. The first three axes of the PCA were the most biologically interpretable and are reported here. The scores on each axis were also included in a model similar to the one used to test the

relative influence of species identity and N treatment on functional parameters. To test for differences among species in the species' scores on each of the first three axes, the data for each axis were subjected to a Tukey-Kramer HSD test.

Coarse belowground biomass C and N content were not collected for all individual monocultures since some species did not produce coarse belowground biomass. Aboveground plant N, belowground plant N, and total plant N are derived mathematically from other parameters that were included in the PCA. To understand the relationships between these traits and the other suite of traits, we performed additional pairwise correlations of the PCA axes with C:N of coarse belowground biomass, aboveground plant N, belowground plant N, and total plant N. These correlations test whether the parameter is associated with the set of traits corresponding to a given axis.

Results

Differences among species

There were large differences among species in nearly all of the functional traits (Table 3). Species differences accounted for most of the variation in functional traits (Table 2) and N fertilization little. Shoot biomass ranged from 55 g m^{-2} (*Poa pratensis*) to 442 g m^{-2} (*Solidago rigida*), fine root biomass ranged from 51 g m^{-2} (*Anemone cylindrica*) to 1892 g m^{-2} (*Andropogon gerardii*), and crown biomass ranged from 12 g m^{-2} (*A. cylindrica*) to 461 g m^{-2} (*S. rigida*) (Table 3). Some species had no coarse belowground biomass (e.g. *Schizachyrium scoparium*), while species such as *Agropyron repens* had large amounts of coarse belowground biomass (721 g m^{-2}) (Table 3). The relative amounts of each fraction as well as the placement of root biomass in the soil profile also differed among species (Table 3). Total plant N varied from 3.3 g N m^{-2} to 16.5 g N m^{-2} (*A. cylindrica, L. capitata*) with half of the species accumulating the equivalent of over 13 g N m^{-2} (Table 3). Among species, there was greater variation in biomass than total biomass N, suggesting that dilution of N (i.e. differences in N use efficiency) is more important in determining differences in production than differences in total N uptake.

Species had strong effects on the soil environment. Soil moisture content ranged from 2% (*S. rigida*, all depths) to 12% (*P. pratensis*, 56–96 cm) (Table 3). Ranges were similar among the three depths. Inorganic nitrogen concentrations in the soil solution ranged from near zero (0.0 and 0.02 mg kg^{-1} soil for *S. rigida* at 0–24 cm and 56–96 cm respectively) to 2.5 and 4.2 mg kg^{-1} soil (*Lespedeza capitata at* 0–24 cm, Poa pratensis at 56–96 cm, respectively).

Root biomass and resource availability

Across all species and all soil depths, soil moisture content decreased with increasing fine root biomass (Figure 1, Table 4), presumably because fine roots reduced soil moisture. The relationship between fine root biomass and soil moisture did not differ between nitrogen treatments and was the same across all three depth strata. There was no relationship between coarse belowground biomass and soil moisture content. The significant interaction between coarse belowground biomass and nitrogen treatment probably reflected species-specific responses to nitrogen fertilization and was difficult to interpret as a general pattern.

A similar negative linear relationship was seen for log-transformed inorganic soil nitrogen concentrations and log-transformed fine root biomass (Figure 2, Table 4), indicating that fine root biomass was important in lowering inorganic nitrogen concentrations in soil solution. Most of this pattern was due to differences in extractable NO_3^- rather than NH_4^+ (data not shown). As with soil moisture, coarse belowground biomass was not a significant predictor of inorganic N concentrations in the soil solution. The relationship between fine root biomass and inorganic N concentrations in the soil solution was the same for both nitrogen treatments and the same across all three depth strata. In all, these results show that fine root biomass reduces both inorganic N and moisture for both grasses and forbs and that these relationships are similar throughout a soil profile.

Principal components analysis

In the principal components analysis of 31 traits, the first axis (Axis 1) accounted for 35% of the explainable variation (3.1% expected by chance alone) (Table 5). The 11 species are distributed across Axis 1 (Figures 3a, 3b). When individual species scores for Axis 1 were used as the response variable in regression analyses, species identity explained most of the variation along this axis while N treatment explained little (Table 2). Species that scored relatively high on

Table 2. Results of regression model that predicts functional parameters based on the species identity, nitrogen treatment, and the interaction between species and nitrogen. Abbreviations and conventions: FRBD (fine root biomass density (biomass per unit soil volume)); CRBD (coarse root biomass density (biomass per unit soil volume); % shoot (amount of shoot biomass relative to total biomass—same for crown, fine, and coarse); % fine xx–yy cm (amount of fine root biomass in the state strata relative to total fine root biomass 0–96 cm—same for coarse root biomass).

		Species		Nitrogen			Species *Nitrogen	
Parameter	r^2	F ratio	Prob > F	F ratio	Prob > F	Elev. – Amb.	F ratio	Prob > F
Root tissue density	0.73	8.77	<0.001	7.2	<0.01	+0.02 g cm^{-3}	1.2	ns
SRL	0.88	25.5	<0.001	13.2	<0.001	−20.6 cm g^{-1}	2.0	ns
Diameter	0.81	16.1	<0.001	2.1	ns		1.0	ns
C:N shoot	0.95	65.8	<0.001	7.2	<0.05	−2.5	2.3	<0.05
C:N fine	0.92	47.6	<0.001	0.3	ns		0.3	ns
C:N coarse	0.89	31.5	<0.001	2.9	ns		n/a	n/a
Shoot biomass	0.73	10.0	<0.001	6.7	0.01	+61.4 g m^{-2}	0.3	ns
Crown biomass	0.93	47.6	<0.001	8.9	<0.01	+47.7 g m^{-2}	0.6	ns
Fine biomass	0.94	67.4	<0.001	2.5	ns		0.5	ns
Coarse biomass	0.92	44.0	<0.001	2.8	ns		1.8	ns
% shoot	0.84	17.7	<0.001	0.1	ns		0.4	ns
% crown	0.86	23.0	<0.001	0.8	ns		0.3	ns
% fine	0.92	44.8	<0.001	0.2	ns		1.4	ns
% coarse	0.92	62.4	<0.001	0.76	ns		0.9	ns
FRBD 0–24 cm	0.94	56.9	<0.001	3.1	ns		1.0	ns
FRBD 24–56 cm	0.89	31.6	<0.001	1.0	ns		0.6	ns
FRBD 56–96 cm	0.89	31.7	<0.001	0.3	ns		0.0	ns
CRBD 0–24 cm	0.93	48.7	<0.001	3.5	ns		1.8	ns
CRBD 24–56 cm	0.76	11.7	<0.001	0.0	ns		0.4	ns
CRBD 56–96 cm	0.60	5.2	<0.001	0.3	ns		0.3	ns
% fine 0–24	0.91	38.0	<0.001	0.0	ns		0.8	ns
% fine 24–56	0.78	13.2	<0.001	1.0	ns		0.5	ns
% fine 56–96	0.94	58.3	<0.001	2.3	ns		2.3	0.03
% coarse 0–24	0.76	11.8	<0.001	2.1	ns		0.8	ns
% coarse 24–56	0.62	4.4	<0.001	0.12	ns		2.2	0.04
% coarse 56–96	0.49	3.2	<0.001	0.0	ns		0.3	ns
% moisture 0–24 cm	0.74	6.8	<0.001	8.3	<0.01	−10%	4.1	<0.001
% moisture 24–56 cm	0.68	7.2	<0.001	9.8	<0.01	−16%	ns	
% moisture 56–96 cm	0.69	7.4	<0.001	11.8	<0.01	−22%	0.8	ns
$[NO_3^-]$ + $[NH_4^+]$ 0–24 cm	0.71	9.1	<0.001	0.4	ns		1.5	ns
$[NO_3^-]$ + $[NH_4^+]$ 24–56 cm	0.64	5.3	<0.001	4.1	ns		1.1	ns
$[NO_3^-]$ + $[NH_4^+]$ 56–96 cm	0.81	14.2	<0.001	2.0	ns		1.5	ns
BG N	0.76	10.9	<0.001	5.1	<0.05	+1.4 g N m^{-2}	0.5	ns
AG N	0.47	2.8	<0.05	6.6	<0.05	+1.0 g N^{m-2}	0.3	ns
Total N	0.70	8.1	<0.001	7.2	<0.05	+2.3 g N^{m-2}	0.3	ns
Axis 1	0.96	103.7	<0.001	3.0	<0.1		0.9	ns
Axis 2	0.92	41.0	<0.001	5.4	<0.05	+0.50	0.4	ns
Axis 3	0.91	34.5	<0.001	0.1	ns		1.5	ns

Axis 1 were three C_4 grasses (*A. gerardii, S. scoparium*, and *B. gracilis*) and a C_3 forb (*S. rigida*), while the C_3 legumes (*P. villosum, L. capitata*), a C_3 forb (*A. cylindrica*), and a C_3 grass (*P. pratensis*) scored relatively low (Figures 3a, 3b). Species that scored high on Axis 1 had high tissue C:N ratios, high root tissue density, large amounts of shoot, crown and fine

Table 3. Number of replicates, means and standard errors of major functional traits of species, pooled across N treatments. Superscript letters refer to significant differences among species for a given trait.

Species	n	Root tissue density (g cm^{-3})	SRL (m g^{-1})	Diameter (mm)	C:N shoot biomass	C:N fine root biomass	C:N coarse BG biomass	Shoot biomass (g m^{-2})	Crown biomass (g m^{-2})	Fine root biomass (g m^{-2})	Coarse BG biomass (g m^{-2})
A. millefolium	6	0.19 ± 0.03^{abcd}	76.95 ± 9.1^{cd}	0.31 ± 0.00^{b}	22.24 ± 2.25^{g}	39.89 ± 2.09^{cd}	53.12 ± 4.54^{b}	130.85 ± 13.78^{de}	81.05 ± 15.8^{d}	404.46 ± 68.02^{cde}	95.79 ± 32.94^{bc}
A. repens	6	0.18 ± 0.01^{abcd}	98.18 ± 7.22^{cd}	0.27 ± 0.01^{bc}	40.37 ± 1.77^{cd}	41.24 ± 1.53^{cd}	59.91 ± 2.03^{b}	320.79 ± 27.88^{abc}	130.42 ± 29.2^{cd}	450.39 ± 33.3^{cd}	720.97 ± 94.47^{a}
A. cylindrica	4	0.11 ± 0.02^{d}	76.59 ± 6.5^{cd}	0.39 ± 0.02^{a}	21.58 ± 1.01^{fg}	23.27 ± 1.62^{de}	46.48^{bc}	102.77 ± 47.54^{de}	12.41 ± 6.03^{d}	51.54 ± 21.19^{e}	2.85 ± 2.34^{c}
A. gerardi	6	0.24 ± 0.01^{abc}	61.00 ± 4.44^{cd}	0.30 ± 0.01^{b}	50.39 ± 1.25^{ab}	108.95 ± 5.56^{a}	98.32 ± 6.51^{a}	341.06 ± 20.01^{abc}	436.91 ± 42.24^{b}	1892.66 ± 150.95^{a}	47.38 ± 10.62^{c}
B. gracilis	6	0.25 ± 0.02^{ab}	102.71 ± 10.3^{c}	0.23 ± 0.01^{c}	35.15 ± 1.04^{de}	59.05 ± 2.21^{bc}	n/a	240.47 ± 19.35^{bcd}	460.22 ± 33.01^{ab}	822.36 ± 33.6^{b}	0 ± 0^{c}
K. cristata	6	0.19 ± 0.01^{abcd}	157.74 ± 16.88^{ab}	0.21 ± 0.01^{c}	30.49 ± 0.71^{ef}	49.84 ± 2.78^{bc}	n/a	194.34 ± 17.55^{bcde}	222.8 ± 16.29^{c}	664.56 ± 31.19^{bc}	0 ± 0^{c}
L. capitata	5	0.16 ± 0.02^{cd}	105.42 ± 19.31^{bc}	0.30 ± 0.04^{b}	20.45 ± 1.62^{fg}	14.66 ± 0.58^{de}	15.72 ± 1.52^{bc}	214.53 ± 84.39^{bcde}	36.14 ± 13.93^{d}	128.76 ± 24.65^{de}	247.88 ± 48.54^{b}
P. villosum	5	0.17 ± 0.01^{bcd}	84.21 ± 8.8^{cd}	0.30 ± 0.02^{b}	20.2 ± 2.00^{fg}	30.54 ± 3.28^{de}	41.19 ± 3.99^{bc}	161.42 ± 61.26^{cde}	28.83 ± 12.61^{d}	96.78 ± 32.24^{e}	174.62 ± 35.65^{bc}
P. pratensis	6	0.13 ± 0^{d}	207.06 ± 9.26^{a}	0.22 ± 0.01^{c}	26.96 ± 2.18^{fg}	31.45 ± 1.33^{de}	50.97 ± 4.10^{b}	54.94 ± 10.87^{e}	59.65 ± 11.71^{d}	207.74 ± 52.37^{de}	35.54 ± 6.85^{c}
S. scoparium	6	0.26 ± 0.02^{a}	102.69 ± 12.11^{c}	0.23 ± 0.01^{c}	51.95 ± 1.19^{a}	122.93 ± 11.29^{a}	n/a	351.01 ± 34.92^{ab}	461.47 ± 43.23^{a}	1637.08 ± 118.1^{a}	0 ± 0^{c}
S. rigida	6	0.25 ± 0.02^{ab}	47.25 ± 6.99^{d}	0.34 ± 0.01^{ab}	43.78 ± 2.55^{bc}	67.15 ± 3.45^{b}	69.04^{ab}	442.17 ± 44.79^{a}	249.92 ± 20.97^{c}	979.59 ± 47.54^{b}	3.01 ± 3.01^{c}
Species	n	% shoot (% of biomass)	% crown (% of biomass)	% fine (% of biomass)	% coarse (% of biomass)	FRBD 0–24 (mg cm^{-3})	FRBD 24–56 (mg cm^{-3})	FRBD 56–96 (mg cm^{-3})	CRBD 0–24 (mg cm^{-3})	CRBD 24–56 (mg cm^{-3})	CRBD 56–96 (mg cm^{-3})
A. millefolium	6	20 ± 2^{cde}	11 ± 0^{cd}	57 ± 1^{abc}	12 ± 2^{b}	1.26 ± 0.22^{cd}	0.2 ± 0.04^{c}	0.1 ± 0.02^{cd}	0.36 ± 0.15^{b}	0.03 ± 0.02^{b}	0 ± 0^{c}
A. repens	6	20 ± 1^{bcde}	8 ± 2^{d}	29 ± 3^{d}	44 ± 3^{a}	1.13 ± 0.12^{d}	0.22 ± 0.01^{c}	0.27 ± 0.01^{c}	2.73 ± 0.37^{a}	0.2 ± 0.06^{a}	0 ± 0^{bc}
A. cylindrica	4	61 ± 1^{a}	7 ± 0^{d}	31 ± 1^{d}	1 ± 1^{c}	0.16 ± 0.05^{e}	0.04 ± 0.04^{c}	0 ± 0^{cd}	0.01 ± 0.01^{b}	0 ± 0^{b}	0 ± 0^{bc}
A. gerardi	6	13 ± 1^{e}	16 ± 1^{bc}	70 ± 2^{a}	2 ± 0^{bc}	3.73 ± 0.16^{a}	1.78 ± 0.2^{a}	1.08 ± 0.15^{a}	0.09 ± 0.05^{b}	0.07 ± 0.03^{b}	0.01 ± 0.01^{bc}
B. gracilis	6	16 ± 1^{de}	30 ± 1^{a}	54 ± 1^{c}	0 ± 0^{c}	2.43 ± 0.06^{b}	0.51 ± 0.09^{bc}	0.2 ± 0.03^{cd}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{c}
K. cristata	6	18 ± 1^{cde}	21 ± 1^{b}	62 ± 2^{abc}	0 ± 0^{c}	2.22 ± 0.1^{b}	0.37 ± 0.03^{bc}	0.04 ± 0.01^{cd}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{c}
L. capitata	5	28 ± 7^{bc}	5 ± 1^{d}	24 ± 4^{d}	43 ± 5^{a}	0.14 ± 0.05^{e}	0.13 ± 0.02^{c}	0.13 ± 0.01^{cd}	0.53 ± 0.11^{b}	0.31 ± 0.06^{a}	0.05 ± 0.02^{a}
P. villosum	5	32 ± 3^{b}	6 ± 2^{d}	20 ± 1^{d}	43 ± 5^{a}	0.08 ± 0.02^{e}	0.1 ± 0.04^{c}	0.11 ± 0.04^{cd}	0.52 ± 0.12^{b}	0.12 ± 0.01^{b}	0.03 ± 0.01^{ab}
P. pratensis	6	16 ± 2^{de}	17 ± 3^{bc}	56 ± 5^{bc}	11 ± 2^{b}	0.85 ± 0.22^{de}	0.01 ± 0^{c}	0 ± 0^{d}	0.15 ± 0.03^{b}	0 ± 0^{b}	0 ± 0^{c}
S. scoparium	6	15 ± 2^{e}	19 ± 1^{b}	67 ± 2^{ab}	0 ± 0^{c}	3.78 ± 0.35^{a}	1.49 ± 0.22^{a}	0.65 ± 0.04^{b}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{c}
S. rigida	6	26 ± 2^{bcd}	15 ± 1^{bc}	59 ± 2^{abc}	0 ± 0^{c}	2.03 ± 0.14^{bc}	0.79 ± 0.04^{b}	0.6 ± 0.04^{b}	0.01 ± 0.01^{b}	0 ± 0^{b}	0 ± 0^{c}
Species	n	% fine 0–24 (% of fine)	% fine 24–56 (% of fine)	% fine 56–96 (% of fine)	% coarse 0–24 (% of coarse)	% coarse 24–56 (% of coarse)	% coarse 56–96 (% of coarse)	% moisture 0–24	% moisture 24–56	% moisture 56–96	
A. millefolium	6	80 ± 3^{bc}	13 ± 1^{bc}	7 ± 2^{cd}	82 ± 16^{ab}	18 ± 17^{ab}	0 ± 0^{a}	3 ± 1^{b}	5 ± 1^{bcd}	6 ± 1^{bcd}	
A. repens	6	69 ± 2^{c}	14 ± 1^{c}	17 ± 1^{b}	92 ± 3^{a}	7 ± 3^{ab}	0 ± 0^{a}	4 ± 0^{b}	6 ± 0^{abc}	7 ± 1^{abcd}	
A. cylindrica	4	83 ± 8^{bc}	13 ± 8^{ab}	5 ± 4^{cd}	50 ± 29^{abcd}	0 ± 0^{b}	0 ± 0^{a}	10 ± 4^{a}	8 ± 1^{ab}	10 ± 3^{ab}	
A. gerardi	6	57 ± 2^{e}	27 ± 1^{e}	16 ± 1^{b}	37 ± 18^{bcd}	42 ± 18^{a}	4 ± 3^{a}	4 ± 1^{b}	3 ± 1^{cd}	4 ± 1^{cd}	
B. gracilis	6	78 ± 3^{bc}	16 ± 2^{bc}	6 ± 1^{cd}	0 ± 0^{d}	0 ± 0^{b}	0 ± 0^{a}	3 ± 0^{b}	5 ± 1^{bcd}	8 ± 1^{abc}	
K. cristata	6	85 ± 1^{ab}	14 ± 1^{ab}	1 ± 0^{d}	0 ± 0^{d}	0 ± 0^{b}	0 ± 0^{a}	2 ± 0^{b}	4 ± 1^{bcd}	7 ± 1^{abc}	
L. capitata	5	32 ± 4f	33 ± 3f	35 ± 3^{a}	59 ± 4^{abc}	36 ± 4^{ab}	5 ± 2^{a}	6 ± 2^{ab}	5 ± 1^{bcd}	5 ± 1^{bcd}	
P. villosum	5	31 ± 6f	33 ± 3f	36 ± 3^{a}	76 ± 3^{ab}	19 ± 2^{ab}	5 ± 2^{a}	3 ± 1^{b}	5 ± 1^{bcd}	6 ± 2^{bcd}	
P. pratensis	6	99 ± 0^{a}	1 ± 0^{a}	0 ± 0^{d}	100 ± 0^{a}	0 ± 0^{b}	0 ± 0^{a}	6 ± 1^{ab}	10 ± 1^{a}	12 ± 1^{a}	
S. scoparium	6	64 ± 3^{de}	25 ± 3^{de}	11 ± 1^{bc}	0 ± 0^{d}	0 ± 0^{b}	0 ± 0^{a}	4 ± 0^{b}	5 ± 1^{bcd}	7 ± 1^{abcd}	

Table 3. Continued.

Species	n	Root tissue density (g cm^{-3})	SRL (m g^{-1})	Diameter (mm)	C:N shoot biomass	C:N fine root biomass	C:N coarse BG biomass	Shoot biomass (g m^{-2})	Crown biomass (g m^{-2})	Fine root biomass (g m^{-2})	Coarse BG biomass (g m^{-2})
S. rigida	6	59 ± 1^{e}	23 ± 1^{e}	18 ± 1^{b}	17 ± 17^{cd}	0 ± 0^{b}	0 ± 0^{a}	2 ± 0^{b}	2 ± 0^{d}	2 ± 0^{d}	
Species	n	$[NO_3^-]$ + $[NH_4^+]$ 0–24 (mg/kg)	$[NO_3^-]$ + $[NH_4^+]$ 24–56 (mg/kg)	$[NO_3^-]$ + $[NH_4^+]$ 56–96 (mg/kg)	BG Biomass N (g N m^{-2})	AG Biomass N (g N m^{-2})	Total Plant N (g N m^{-2})	Axis 1	Axis 2	Axis 3	
A. millefolium	6	0.07 ± 0.07^{b}	0.28 ± 0.09^{c}	0.43 ± 0.09^{c}	5.98 ± 0.97^{bcde}	2.66 ± 0.2^{ab}	8.65 ± 1.01^{bcde}	–0.78 ± 0.33^{d}	–0.71 ± 0.33^{de}	0.14 ± 0.26^{bc}	
A. repens	6	0.06 ± 0.07^{b}	0.53 ± 0.05^{bc}	0.44 ± 0.07^{c}	11.32 ± 0.85^{a}	3.63 ± 0.41^{ab}	14.95 ± 1.21^{a}	–1.63 ± 0.21^{de}	1.74 ± 0.41^{b}	4.09 ± 0.5^{a}	
A. cylindrica	4	2.09 ± 0.7^{a}	1.89 ± 1.04^{abc}	3.12 ± 0.88^{ab}	1.14 ± 0.46^{e}	2.15 ± 1.01^{ab}	3.29 ± 1.45^{e}	–4.26 ± 0.48^{f}	–1.82 ± 0.56^{de}	–2.76 ± 0.38^{d}	
A. gerardi	6	0.01 ± 0.02^{b}	0.21 ± 0.07^{c}	0.18 ± 0.05^{c}	10.44 ± 0.95^{ab}	3.05 ± 0.17^{ab}	13.48 ± 0.94^{ab}	4.61 ± 0.34^{a}	1.92 ± 0.47^{b}	–0.96 ± 0.23^{c}	
B. gracilis	6	0.10 ± 0.05^{b}	0.32 ± 0.1^{bc}	0.45 ± 0.08^{c}	10.25 ± 0.66^{ab}	3.10 ± 0.27^{ab}	13.34 ± 0.75^{ab}	2.17 ± 0.24^{bc}	–1.77 ± 0.26^{de}	0.13 ± 0.07^{bc}	
K. cristata	6	0 ± 0.02^{b}	0.37 ± 0.06^{bc}	0.60 ± 0.19^{c}	7.98 ± 0.52^{abcd}	2.87 ± 0.25^{ab}	10.85 ± 0.77^{abcd}	0.90 ± 0.14^{c}	–2.36 ± 0.26^{e}	0.26 ± 0.15^{bc}	
L. capitata	5	2.47 ± 0.86^{a}	1.54 ± 0.44^{abc}	1.66 ± 0.45^{bc}	12.19 ± 2.18^{a}	4.28 ± 1.51^{a}	16.46 ± 3.42^{a}	–4.14 ± 0.36^{f}	4.01 ± 0.52^{a}	–0.78 ± 0.54^{c}	
P. villosum	5	1.41 ± 0.49^{ab}	2.33 ± 1.11^{ab}	2.82 ± 0.97^{ab}	3.94 ± 1.17^{cde}	3.22 ± 0.96^{ab}	7.16 ± 2.11^{cde}	–4.07 ± 0.64^{f}	2.94 ± 0.76^{ab}	–1.06 ± 0.31^{c}	
P. pratensis	6	1.01 ± 0.29^{ab}	2.86 ± 0.55^{a}	4.18 ± 0.4^{a}	3.86 ± 0.91^{de}	0.99 ± 0.24^{b}	4.85 ± 1.08^{de}	–2.98 ± 0.21^{ef}	–4.33 ± 0.14^{f}	0.69 ± 0.18^{b}	
S. scoparium	6	0.06 ± 0.09^{b}	0.16 ± 0.04^{c}	0.23 ± 0.08^{c}	10.21 ± 0.89^{ab}	3.07 ± 0.35^{ab}	13.28 ± 0.94^{ab}	4.72 ± 0.31^{a}	–0.29 ± 0.21^{cd}	0.03 ± 0.15^{bc}	
S. rigida	6	–0.03 ± 0.01^{b}	–0.01 ± 0.03^{c}	0.02 ± 0.05^{c}	8.71 ± 0.57^{abc}	4.65 ± 0.59^{a}	13.36 ± 0.98^{abc}	2.67 ± 0.16^{b}	1.21 ± 0.12^{bc}	–1.01 ± 0.08^{c}	

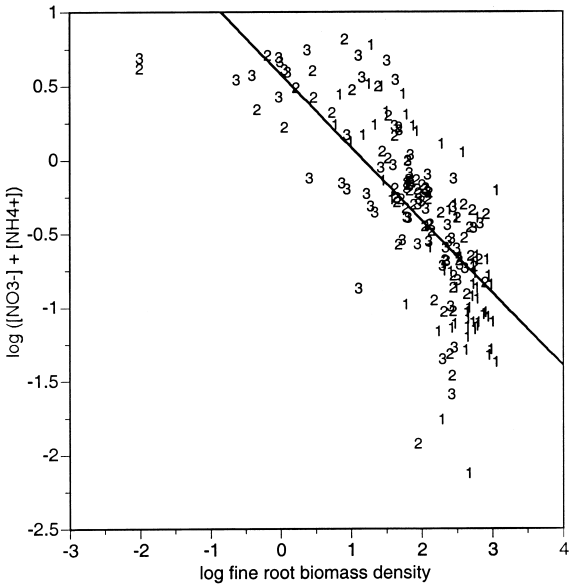

Figure 1. Relationship between log fine root biomass density and log extractable NO_3^- and NH_4^+ for all species and depths. Symbols refer to strata (1 = 0–24 cm, 2 = 24–56 cm, 3 = 56–96 cm). See Table 4 for model results.

Table 4. Results of the models that predict gravimetric soil moisture and log of inorganic nitrogen concentrations in the soil. Predictor variables include log fine root biomass density, an interaction between nitrogen treatment and fine root biomass density, and a categorical classification of depth (0–24 cm, 24–56 cm, 56–96 cm), as well as analogous variables for coarse root biomass density. For % moisture of the soil, $r^2 = 0.46$ and for log of inorganic nitrogen concentrations in the soil, $r^2 = 0.61$.

	% Moisture		log ($[NO_3^-]$ + $[NH_4^+]$)	
Parameter	F ratio	Prob > F	F ratio	Prob > F
log fine	67.3	<0.001	233.9	<0.001
nitrogen * log fine	0.19	ns	1.7	ns
depth * log fine	3.4	0.04	0.3	ns
log coarse	2.8	ns	2.7	ns
nitrogen * log coarse	13.7	<0.01	1.2	ns
depth * log coarse	0.3	ns	2.3	ns

root biomass, and lower concentrations of soil inorganic nitrogen at all three depths (Tables 3 and 5).

Axis 2 explained 20% of the explainable variation (3.1% expected) (Table 5) and represents a continuous axis of separation for species that involves many traits (Figure 3a), but generally separates species based on the depth distribution of root biomass. "Deep species" scored high on Axis 2 and included C_3 legumes (*L. capitata, P. villosum*) and a C_4 grass (*A. gerardii*). "Shallow species" included C_3 grasses (*P. pratensis, K. cristata*), and a C_4 grass (*B. gracilis*)

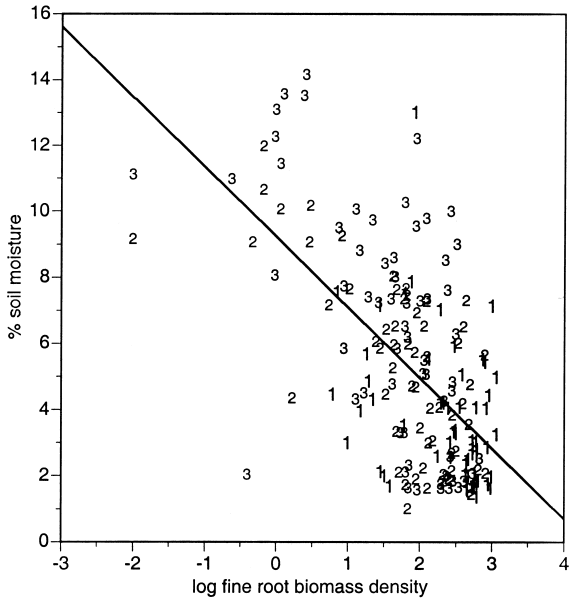

Figure 2. Relationship between log fine root biomass density and % soil moisture. Symbols refer to strata (1 = 0–24 cm, 2 = 24–56 cm, 3 = 56–96 cm). See Table 4 for model results.

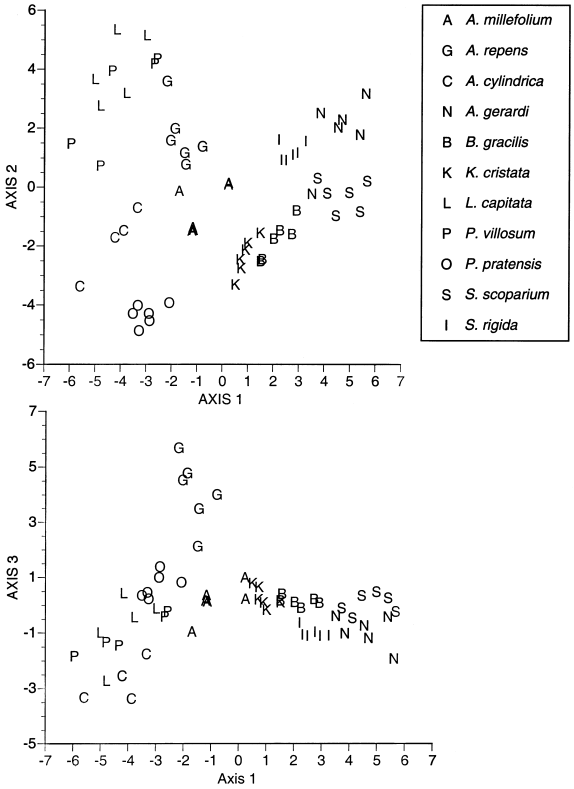

Figure 3. Scores of individual species on Axes 1, 2, and 3 from the principal components analysis.

(Figure 3, Table 3). The "deep species" had lower SRL, larger root diameter, similar biomass C:N ratios as shallow species, higher shoot and coarse biomass, more fine root (relative and absolute) and coarse belowground biomass (absolute) below 24 cm, as well as greater aboveground and belowground N (Table 5). The deep species had similar amounts of shallow soil moisture and inorganic nitrogen as shallow species, but less water and inorganic nitrogen was present at depth.

Axis 3 is relatively minor compared to the first two axes, explaining only 9.1% of the explainable variation (3.1% expected) (Table 5) and appears primarily to separate the high-nitrogen species based on whether they are rhizomatous or not (Figure 3b). The trait that correlated most strongly with Axis 3 is coarse belowground biomass in the upper soil horizon (Table 5). The rhizomatous high-nitrogen species also had low-diameter roots, higher relative and absolute amounts of coarse belowground biomass, especially at shallow depth, greater belowground N, lower shallow soil moisture, and lower inorganic nitrogen concentrations at all depths (Table 5).

N fertilization

For the vast majority of parameters, all species responded similarly to N fertilization (i.e., there were few species × N interactions, Table 2). Thus, overall, fertilization with nitrogen increased shoot biomass and nitrogen as well as crown biomass (Table 2), but did not affect fine or coarse root biomass, suggesting a higher relative allocation to light acquisition and/or reproduction. The N treatment increased average root tissue density of species, but otherwise, did not lead to significant changes in root system construction. There were no changes in diameter, tissue nitrogen concentrations, biomass, or placement of root biomass in the soil profile. Belowground biomass N was higher, but mostly due to greater crown biomass. Addition of fertilizer increased the supply rate of N on an annual basis, yet there were no significant changes in soil inorganic N concentrations (Table 2). Additional N in the biomass of fertilized plants only accounted for 2.3 g m^{-2} of the 6.7 g m^{-2} added by fertilization (Table 2). The rest of the N must have been incorporated into previous production, lost at different times, or immobilized by microbes.

Table 5. Results of the PCA for 32 functional traits that were measured on all 11 species. FRBD = fine root biomass density (g cm^{-3} soil) and CBGBD = coarse belowground biomass density (g cm^{-3} soil). The results of pairwise correlations (†) between C:N of coarse root biomass, and biomass nitrogen were included since data were not collected for all individual mesocosms. For the correlations, correlation coefficients are not comparable to component loadings and italicized. Probability significance is denoted as follows: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. The amounts of variance explained by the Axes 1, 2, and 3 relative to the total variance explained by the PCA were 34.8%, 20.3%, and 9.1%, respectively (3.1% expected by chance).

Eigenvectors	Axis 1	Axis 2	Axis 3
Root tissue density	0.23	0.07	0.03
SRL	–0.08	–0.23	0.18
Diameter	–0.07	0.15	–0.29
C:N shoot	0.25	0.04	0.11
C:N fine	0.26	0.01	–0.02
†C:N coarse	*0.83****	*0.11*	*0.11*
Shoot biomass	0.19	0.19	0.11
Crown biomass	0.27	–0.01	0.01
Fine root biomass	0.28	0.05	–0.04
Coarse BG biomass	–0.10	0.18	0.45
% shoot	–0.15	0.05	–0.22
% crown	0.18	–0.19	0.03
% fine	0.23	–0.18	–0.08
% coarse	–0.20	0.22	0.21
FRBD 0–24 cm	0.28	–0.03	0.01
FRBD 24–56 cm	0.25	0.09	–0.09
FRBD 56–96 cm	0.23	0.15	–0.06
CBGBD 0–24 cm	–0.08	0.15	0.47
CBGBD 24–56 cm	–0.12	0.27	0.16
CBGBD 56–96 cm	–0.10	0.24	–0.04
% fine 0–24 cm	0.02	–0.34	0.12
% fine 24–56 cm	0.05	0.30	–0.16
% fine 56–96 cm	–0.08	0.33	–0.07
% coarse 0–24 cm	–0.17	0.03	0.19
% coarse 24–56 cm	–0.01	0.19	–0.13
% coarse 56–96 cm	–0.04	0.22	–0.11
% moisture 0–24 cm	–0.09	–0.07	–0.15
% moisture 24–56 cm	–0.16	–0.20	0.08
% moisture 56–96 cm	–0.12	–0.26	0.07
$[NO_3^-]$ + $[NH_4^+]$ 0–24 cm	–0.19	0.03	–0.25
$[NO_3^-]$ + $[NH_4^+]$ 24–56 cm	–0.19	–0.09	–0.13
$[NO_3^-]$ + $[NH_4^+]$ 56–96 cm	–0.21	–0.13	–0.13
†BG N	*0.49****	*0.48****	*0.40***
†AG N	*0.22*	*0.60****	*0.16*
†Total N	*0.45****	*0.56****	*0.36***

Discussion

Trait relationships and resultant functional classifications

Tallgrass vs. shortgrass species

Although the individual PCA axes can be used as a functional classification schemes themselves, it is probably more useful to interpret the results of the PCA further by looking at the pattern of species distributions in all three axes at once in conjunction with the average traits for individual species. Examination of the three PCA axes and the interspecific differences for individual traits revealed similar root systems for the two tall, non-legume species, *S. rigida* (C_3 forb) and *A. gerardii* (C_4 grass). Both are characteristic of productive tallgrass prairie and maintained a large standing root biomass that extracts nitrogen and water from a large, deep volume of soil, in which inorganic nitrogen is present in low concentrations. The fine roots of these species have high tissue density and low tissue N (Tables 3 and 5), traits associated with long root lifespans in other studies (Weaver and Zink 1946b; Ryser (1996, 1996)). In analyses of minirhizotron images from these monocultures from September 1997 to June 1999, no root death was observed for these two species by the middle of the third growing season (data not shown). Multi-year longevity was also seen for these species by Weaver and Zink (1946b) as well as minirhizotron data that we've collected on C_4 grasses grown in the field at Cedar Creek (Craine, unpublished). At a given rate of production, longer lifespan of the fine root biomass increases standing fine root biomass and length, increasing the capacity of plants to acquire large amounts of nitrogen that may be present in low concentrations throughout the soil profile (Tinker and Nye 1977; Yanai et al. 1995; Silberbush and Barber 1983). This provides uptake to lower inorganic N concentrations and to minimize ecosystem N loss (Tables 3 and 5). These low inorganic soil N concentrations may also reflect slow decomposition and microbial immobilization of N in the high C:N senesced roots and shoots of these species (Wedin and Pastor 1993). Root systems of these species are not only large, but also deep relative to those of other grasses (Tables 3 and 5).

The two native short grasses, *K. cristata* and *B. gracilis*, differ in their placement on the landscape and in drought tolerance (Weaver 1968; Coupland 1950; Clarke et al. 1943; Albertson and Weaver

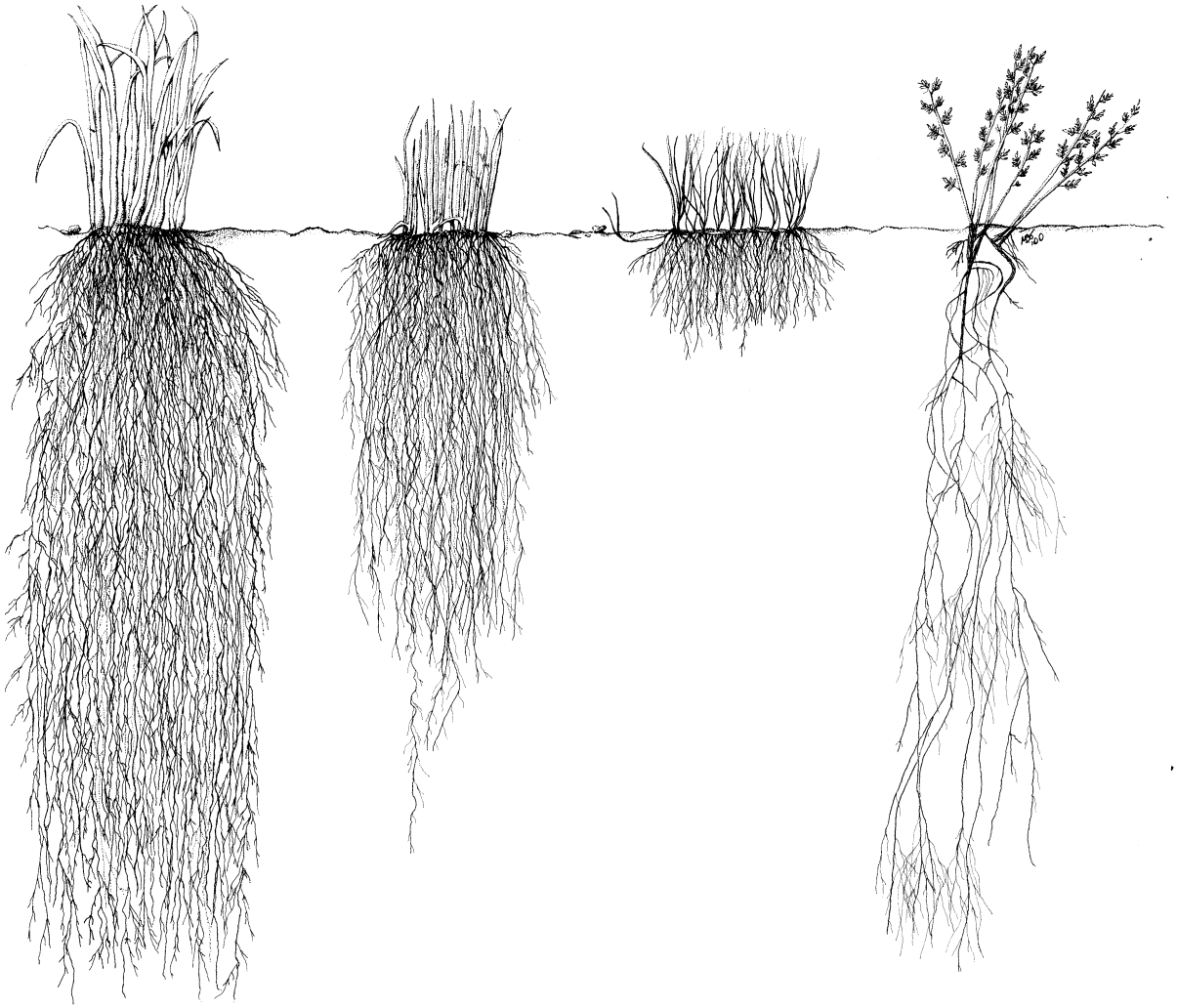

Figure 4. Artistic representation of the root systems of representative tallgrass, shortgrass, high-N rhizomatous and warm-season legume species. Plants were grown in the same manner as those studied in this experiment, but washed out entire. Root systems are depicted to 96 cm.

1944), yet shared many similar traits (Figure 4). They lacked coarse belowground biomass and reproduced vegetatively through root offshoots, resulting in a bunch morphology (Tables 3 and 5). The fine roots of these shortgrass species were finer than those of the tallgrass species and tended to have lower tissue density (Tables 3 and 5). Due to the shallow root distribution of these species, moisture and inorganic nitrogen levels were low in the shallow horizon, but high in deeper soils (Tables 3 and 5).

S. scoparium can be considered to be functionally intermediate between tallgrass and shortgrass dominants. *S. scoparium* is intermediate in its height and its degree of rhizomatousness (Weaver 1968) as well as many of its root functional traits, such as rooting depth, soil water uptake, and inorganic nitrogen levels (Tables 3 and 5).

Hi-N rhizomatous species

P. pratensis (C_3 grass), *A. millefolium* (C_3 forb), and *A. repens* were similar in their traits (Figure 4, Tables 3 and 5), except *A. repens* had large amounts of coarse biomass below 24 cm, leading to a different score on Axis 3 (Table 5). This difference appeared to be an artifact of the experiment as some of *A. repens*' rhizomes that contacted the sides of the con-

Table 6. General summary of main traits that differentiate functional classifications of 11 species in this study.

Parameter	Tallgrass	Shortgrass	Hi-N Rhizomatous	Legume
Root tissue density	High			Low
Diameter		Low		
Fine root biomass	High			Low
Coarse bg biomass	Low		Mid-High	High
Depth of fine roots	Deep	Shallow	Shallow	Deep
Tissue %N	Low		High	High
Shallow inorganic N	Low	Low	Low	High
Deep inorganic N	Low	High	High	High

tainer grew downward. Taking this into account the three rhizomatous species have shallow fine root distributions with a large relative investment in shallow rhizomes. Although all three species had shallow root systems relative to *S. rigida* and *A. gerardii*, they differed in depth distribution. *A. repens* had fine roots in the deep horizon (these did not appear to be an artifact of growing in containers) while *P. pratensis* had only 1% of its fine root biomass deeper than 24 cm (Table 3). The tissue N of all fractions for these rhizomatous species was higher than for the other nonlegume species (Table 3). Due to the shallow distribution of fine root biomass, moisture and NO_3^- levels were low in shallow soils, but high at depth (Tables 3 and 5).

Legumes

The two legume species shared many similar traits. In general, root systems of the two legumes deplete water evenly throughout the soil profile, with little capacity for acquisition of inorganic nitrogen throughout the soil profile. Due to their rhizobial associations, these species can accumulate as much nitrogen as other species with much less fine root biomass (Tables 3 and 5). Their fine roots were evenly distributed throughout the soil profile (Figure 4) and, as a result, soil moisture was constant throughout the soil profile (Tables 3 and 5). Inorganic nitrogen levels in soil solution were higher than for most species (Tables 3 and 5). This pattern probably reflects both reduced N uptake and increased soil N mineralization for the legumes.

Anenome cylindrica

A. cylindrica (C_3 forb) was unique among the species with its low biomass and small root system (Tables 3 and 5). This species may represent the functional strategy of interstitial species (Collins et al. 1993) or early-season forbs. On the other hand, it may have performed poorly under the conditions of our experiment, including more over-winter mortality than observed for the other species. As such it is difficult to generalize from the results of this individual species.

In summary, the major classifications of species derived from this data set include: 1) a continuous distribution of species from tallgrass to shortgrass species, 2) shallow-rooted, strongly-rhizomatous high-nitrogen grasses and forbs, and 3) legumes (Figure 3, Table 6). The two tallgrass species are characterized by having high density, low-N fine roots that extend deep into the soil and reduce soil moisture and extractable N to low levels. Biomass is high aboveground and belowground, but there is little or no coarse belowground biomass produced. The two shortgrass species have fine roots that do not extend deep into the soil and extractable N is low shallow, but high deep. *S. scoparium* was intermediate between tallgrass and shortgrass species. The three high-N, rhizomatous species produce large amounts of rhizomes and have a shallow fine root system that does not acquire deep resources. Tissue N concentrations are high aboveground and belowground. The two legumes produce little fine root biomass, large amounts of coarse roots, have high tissue N concentrations, and high extractable N both shallow and deep in the soil.

Relation to other functional classifications

Our results are generally consistent with Weaver's classification of root systems for grassland plants (Weaver 1958a). The results of our study support Weaver's differentiation of grasses into tall, mixed, and short, classifications made both on distributional data as well as on measurements of species' root systems. We also see clear separation of legumes and

rhizomatous forbs (Weaver 1958a). In other aspects, the grassland functional groups of Weaver and others should be revised. In Weaver's classification system, all forbs that reproduced vegetatively were contained in one classification such that *A. millefolium* and *S. rigida* would have been considered the same root type, even though deep and shallow-rooted forbs appear to be as different as tallgrass and shortgrass grass species. In general, we found little support for functional dichotomies between C_3 and C_4 species or between grasses and forbs. *S. rigida* (C_3 forb) and *A. gerardii* (C_4 grass) were more similar to one another than to other C_3 forbs or C_4 grasses. A similar situation existed for *B. gracilis* (C_4 grass) and *K. cristata* (C_3 grass).

In general, weak separation between grasses and forbs is common in functional classifications that are based on suites of functional traits (Craine et al. (in press); Grime et al. 1997; Diaz and Cabido 1997), as opposed to largely morphologically-based or *post hoc* classifications (Leishman and Westoby 1992; Kindscher and Tieszen 1998).

Belowground biomass and resource utilization

When grown under common conditions, species differed in their production and placement of root biomass in the soil profile, leading to consistent patterns of aboveground production, resource utilization, and ecosystem nitrogen retention. Two similar experiments showed similar species rankings for allocation (Weaver and Zink 1946a) and tissue N concentrations (Tilman and Wedin 1991) but reported less root biomass. Shoot biomass in our experiment was intermediate between values reported in the other two experiments. This comparison suggests that there is a robust basis for species rankings, but that the actual values of biomass and allocation are sensitive to factors such as genotype, resource supply, soil properties, and experimental design.

Our research supports the importance of differentiating coarse and fine root biomass (Körner 1984; Coutts 1987; Eissenstat 1997). While fine roots directly acquire water and nitrogen, coarse belowground biomass should be less directly responsible for resource acquisition than placing fine roots in deeper pools of soil water while economically transporting large amounts of water, storing carbohydrates and mineral nutrients, or supporting vegetative reproduction (Fitter 1996).

Production and maintenance of large amounts of fine root biomass not only reduced moisture and inorganic nitrogen concentrations, but also led to decreased ecosystem N loss. Other experiments at CCNHA have shown that the concentrations of inorganic nitrogen below the rooting zone is lower in experimental plots with high amounts of fine root biomass (Tilman et al. 1996).

Some recent studies have emphasized nitrogen's role in limiting primary productivity in tallgrass prairie (Tilman 1988; Wedin 1995), while others have emphasized the interaction of N and water availability (Schimel et al. 1991; Hooper and Johnson 1999). There was no indication that deep roots mainly acquired water and shallow roots mainly acquired nitrogen. We were unable to differentiate between the allocation of roots for the acquisition of water versus nitrogen. There were similar negative relationships between root biomass and both water and nitrogen levels in the soil, whether shallow or deep in the soil profile. Although a few early grassland studies discussed the depth profiles of nitrate acquisition by native grasses (Weaver et al. 1922), explanations for the patterns of productivity and species distributions in grasslands generally emphasized the role of water.

For non-legumes, the construction of root systems coincided with the patterns of resource availability where the species are most common. As opposed to shrublands that rely on deep soil water (Sala et al. 1989), grassland productivity does not depend on water deeper than 2 m in non-drought years (Albertson and Weaver 1944). Most of the soil organic matter and therefore nitrogen mineralization occurs at similar depths (< 2 m) and water and nitrogen resource profiles are similar within a grassland. The deep root systems of the *A. gerardii* and *S. rigida* coincide with the typically deep organic matter and large soil volume where water and nitrogen are available to plants in the tallgrass praries. The root systems of *S. rigida* can extend greater than 1.5 m while the roots of *A. gerardii* go deeper than 2 m (Sperry 1935). Both species' root systems decline in an exponential fashion with depth. During mild droughts, species such as *A. gerardii* are able to maintain productivity by accessing deeper soil resources, while most shallow rooted species go dormant or die (Albertson and Weaver 1944).

The two shortgrass species, *K. cristata* (C_3 grass) and *B. gracilis* (C_4 grass) occupy environments with shallow resource profiles (Albertson and Weaver 1944). *K. cristata* is widely distributed in North

America (Coupland 1950) and frequently occurs between taller bunch grasses in tallgrass, mixed grass and palouse prairies (Weaver 1968; Coupland 1950). *B. gracilis*, in contrast, is a dominant species of the arid shortgrass prairie. The rooting depth of these species generally tracks shallow water storage associated with growing season precipitation (Weaver 1968; Sala et al. 1989). The lack of deep roots and the presence of deep resources implies a limit to the ability of shortgrass species to utilize resources at depth.

In contrast to the grasses, many legumes and some forbs appear to rely primarily on deep resources (Nedrow 1937). Both of the legumes we studied fit this pattern. These deeply rooted legumes and forbs are also more productive during droughts when the shallow water table was depleted but deep water was still present (Weaver et al. 1935b).

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