Functional traits, productivity and effects on nitrogen cycling of 33 grassland species

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Summary

1. Our goal was to determine the relationships among ecophysiological, whole-plant and ecosystem traits of a wide variety of grassland species grown under field conditions in the long term. We measured 87 traits for 33 species (32 perennial, one annual) grown in monoculture for 5 years on sandy soils, and determined the relationship among traits and their correspondence with current functional classifications.

2. Among non-legumes, species that produced and maintained large amounts of biomass had tough, low-activity leaves and roots, high root : shoot ratios, and low extractable inorganic nitrogen and N mineralization in their soils. The set of correlations among the functional traits of fine roots for non-legumes parallels the set of correlations for leaf functional traits. Low-N species maintained greater biomass than high-N species, more by producing tissues with low N concentrations and greater longevity than by acquiring more N. Greater relative production below ground, and the production of long-lived below-ground structures, were both important in determining the high root : shoot ratio of species.

3. For legumes, N_2 fixation not only led to greater above-ground biomass production, but also was associated with low fine root production; greater relative production of stem biomass; and accelerated ecosystem N cycling compared to non-legumes.

4. The measured traits, as condensed via principal components analysis, differentiated the 32 species into groups that corresponded with a common grassland functional classification scheme (C_3 grasses, C_4 grasses, forbs, legumes, woody species) as well as an alternative, continuous approach. For all traits, species can be arrayed well along two continuous axes. The first axis separates cool-season and warm-season legumes; the second low-N and high-N non-legumes.

5. These continuous classifications show the generality of the two strategies for dealing with low nitrogen availability (N_2 fixation and the low-N suite of traits) and extends the strategies to span organ-level traits to ecosystem processes including roots, whole-plant patterns of productivity, and nutrient cycling. The correlations of traits among species will also be useful in predicting a large number of important parameters associated with plant growth from the measurement of a few, key traits.

Key-words: Functional classifications, grasslands, nitrogen limitation, plant strategies, production

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Introduction

Understanding the processes structuring ecosystems and ecosystem responses to global change may require a comprehensive approach to plant trait relationships that spans organ-level traits to ecosystem processes, and includes roots, leaves, whole-plant patterns of productivity, and nutrient cycling. Much progress has been made in quantifying various components of these relationships (Chapin 1980; Garnier 1991; Grime *et al.* 1997; Poorter *et al.* 1995; Thompson *et al.* 1996; Tilman 1988; Wedin & Tilman 1990; Westoby & Leishman 1997), but these generally have not been under experimental field conditions, or have been on seedlings grown in controlled conditions.

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Moreover, the relationships have not included some important aspects of plant growth in natural ecosystems, including root dynamics and traits; patterns of biomass allocation and longevity; and the determinants of biomass productivity across fertility gradients. For example, across a broad range of species, photosynthesis, leaf respiration, leaf nitrogen concentrations and specific leaf area are all positively correlated among one another and negatively correlated with leaf longevity (Reich, Walters & Ellsworth 1997). It is not known whether analogous relationships exist for leaf traits and root traits (nutrient uptake, respiration, N concentrations, specific root length/density and longevity) across a wide variety of species. The described positive relationships between tissue density and fine root longevity (Ryser 1996) suggest that they might. Similarly, the degree to which root traits are integrated into whole-plant growth characteristics and the effects of plants on ecosystem nitrogen cycling have not been examined in a single comparative study, although various aspects have been examined (Craine *et al.* 2001; Eissenstat & Yanai 1997; Hendricks *et al.* 2000).

Species that grow well in infertile habitats have high root : shoot ratios (Chapin 1980). Yet, although much recent work has examined plasticity in root : shoot ratios (Müller, Schmid & Weiner 2000; Reynolds & D'Antonio 1996), it is unknown whether high ratios are due to differences in the relative longevity of above- and below-ground biomass or relative allocation of biomass below-ground, as is often assumed. Similarly, species differ in their ability to produce biomass on low-fertility soils, but the relative importance of nutrient uptake and nutrient-use efficiency among species grown under low nutrient conditions in producing greater biomass is still unresolved (Aerts & Chapin 2000).

A deeper understanding of the relationships among traits may also increase our ability to classify plant species functionally. Most functional classifications of plant species are based on empirical distributions within and among habitats, taxonomic relationships, physiological traits or above-ground organ traits (leaf, stem and/or seed traits; Curtis 1959; Leishman & Westoby 1992). However, few functional classifications explicitly incorporate below-ground traits or represent multiple process levels (Woodward, Smith & Shugart 1997). Consequently, our ability to predict and explain ecological scenarios may be limited by the lack of more encompassing functional classifications.

We report results of an experiment that ran 5 years, long enough to incorporate species effects on N cycling; studied enough species (32 perennial, one annual) to characterize a broad cross-section of the flora of a nitrogen-limited grassland; and measured a large suite of traits (87) that span many process levels. We used principal components analysis (PCA) to determine how ecophysiological, whole-plant and species-associated ecosystem traits were related across species. From the relationship of traits determined by PCA, we then sought to determine (i) whether there

are similar correlations among root functional traits as leaf functional traits (Reich *et al.* 1997); (ii) which traits were associated with the production and maintenance of large amounts of biomass under N-limited conditions; (iii) the relative roles of allocation and biomass longevity in determining root : shoot ratios; and (iv) the relative importance of nutrient uptake and nutrient-use efficiency in producing greater biomass. Lastly, we examine functional classifications of species that are derived from the PCA of functional traits, and compare those classifications to a common grassland classification (C_3 grass, C_4 grass, forb, legume, woody species).

Methods

EXPERIMENTAL DESIGN

This experiment was conducted at Cedar Creek Natural History Area, located on a glacial outwash sandplain in east-central Minnesota (Tilman 1988; www.lter.umn.edu). The 33 C_3 grasses, C_4 grasses, forbs, legumes and woody species surveyed in this study were established in fall 1992 on an abandoned agricultural field that had the top 60–80 cm of soil removed with a bulldozer (LTER experiment E111). On average, soils (0–20 cm) contained 0·46% C, 93% sand, 3% clay and 4% silt. To exclude mammalian herbivores, a 1·8-m-tall above-ground fence and a 1·2-m-deep below-ground fence was installed at the time of establishment.

Four replicate monocultures of each species were established at a seeding rate of 12 g m^{-2} with seed obtained from a local supplier (Prairie Restorations, Inc., Princeton, MN). Species names and functional classifications of the species used at Cedar Creek are given in Table 1. Authorities for species follow Moore (1973). Plots were $2·4 \times 1·5$ m for most species, and $1·2 \times 1·5$ m for others. Plot size was not an important determinant of any of the patterns discussed. Adjacent plots were separated by 25 cm deep in-ground sheet metal dividers. Each year, plots were weeded to maintain monoculture status and watered weekly as necessary during the 1997 growing season to ensure at least 2·5 cm weekly precipitation. Any plots that had experienced disturbance by gophers or had poor initial establishment were not sampled. In total, 114 plots were sampled with at least two replicates per species.

MEASUREMENTS

All parameters are referred to by a unique table-specific reference number. For example, parameters *101–162* are contained in Table 2; whereas parameters *201–225* are contained in Table 3. A superscript of ns following the reference number signifies that the relationship was not significant, though the trend may have been in the direction predicted.

A suite of metrics of ecophysiology and organ morphology [specific leaf area (SLA); specific root length (SRL); fine root specific respiration rate

Table 1. Species list, number of plots sampled, functional groups and species scores for each axis of the principal component analysis (PCA)

Species	n	Functional	Grass/Forb	Axis 1	Axis 2	Axis 3	Axis 4
Agropyron repens	4	C_3 grass	Grass	0·15	−0·52	−0·48	1·82
Agrostis scabra	2	C_3 grass	Grass	−0·22	−1·54	−0·80	2·13
Koeleria cristata	4	C_3 grass	Grass	0·47	−0·11	−0·76	−0·47
Poa pratensis	3	C_3 grass	Grass	0·43	−0·06	−1·26	1·17
Stipa spartea	4	C_3 grass	Grass	0·06	0·07	−0·29	−0·49
Andropogon gerardi	4	C_4 grass	Grass	0·21	1·62	−0·50	0·67
Bouteloua curtipendula	4	C_4 grass	Grass	0·30	0·77	−0·83	1·32
Calamovilfa longifolia	4	C_4 grass	Grass	−0·65	1·38	−0·15	−0·28
Panicum virgatum	4	C_4 grass	Grass	−0·08	0·74	−0·22	0·81
Schizachyrium scoparium	4	C_4 grass	Grass	0·26	1·87	−0·41	1·41
Sorghastrum nutans	4	C_4 grass	Grass	0·58	0·76	−0·82	0·63
Achillea millefolium	2	Forb	Forb	0·54	−1·21	−0·81	−1·65
Agastache foeniculum	2	Forb	Forb	0·52	−0·10	−0·84	0·27
*Ambrosia artemisiifolia**	4	Forb	Forb	0·58	−1·81	0·43	−0·20
Anenome cylindrica	4	Forb	Forb	0·48	−0·62	−0·22	−1·07
Aster azureus	3	Forb	Forb	0·32	−0·85	0·52	−0·56
Aster ericoides	2	Forb	Forb	−0·06	−0·30	−0·36	−0·42
Asclepias syriaca	4	Forb	Forb	0·09	−1·45	−0·39	0·34
Asclepias tuberosa	4	Forb	Forb	0·14	−0·59	−0·34	−0·09
Coreopsis palmata	4	Forb	Forb	0·16	0·71	−0·04	−1·03
Liatris aspera	2	Forb	Forb	0·71	0·84	−0·06	−0·15
Penstemon grandiflorus	4	Forb	Forb	0·11	−0·39	−0·37	−0·51
Potentilla arguta	2	Forb	Forb	0·03	0·48	0·34	−0·24
Rudbeckia serotina	3	Forb	Forb	0·19	−1·72	−0·33	−0·60
Solidago nemoralis	4	Forb	Forb	0·42	−0·24	−0·19	−1·36
Solidago rigida	4	Forb	Forb	0·50	0·88	−0·27	−0·89
Desmodium canadense	4	Legume	Forb	−0·28	−0·28	3·78	1·64
Lespedeza capitata	4	Legume	Forb	0·29	−0·34	2·00	−0·14
Lupinus perennis	3	Legume	Forb	−5·21	−0·32	−0·81	−0·24
Petalostemum purpureum	4	Legume	Forb	−0·67	−0·33	1·18	0·84
Petalostemum villosum	2	Legume	Forb	0·06	−0·53	1·57	−0·48
Corylus americana	4	Woody	Woody	0·29	1·47	0·81	−0·11
Quercus macrocarpa	4	Woody	Woody	−0·68	1·73	0·94	−2·07

*Annual.

(FSRR); photosynthesis per unit mass (P_s); stomatal conductance (g_s); leaf respiration per unit mass (R_s); and average root diameter] (*101–107*) were measured on the species in this experiment (M. Tjoelker, unpublished). We determined light-saturated rates of leaf net photosynthesis in the field using a portable photosynthesis system (CIRAS-1, PP Systems, Hitchin, UK). We conducted measurements on clear sunny days (25 June and 7, 21, 28 August 1997) at light-saturating conditions between 10·30 and 14·00 h CDT. We measured two to four mature leaves from the top of the canopy in each of two to four replicate plots for a species. Leaf area for SLA was determined with a video image analysis system (AgVision, Decagon Devices, Inc., Pullman, WA). Net photosynthesis rates were calculated on the basis of leaf mass.

To determine specific respiration rates of leaves and roots, we harvested intact shoots, including stems and leaves, from plots on the mornings of 17 and 18 June. Samples were transferred to a controlled-environment chamber (Conviron E15, Controlled Environments, Inc., Winnipeg, Canada) to measure dark respiration at a standard temperature (26·1 ± 0·6 °C) and CO_2 concentration (381 ± 15 µmol mol⁻¹ CO_2). Rates of net

CO_2 efflux were determined using infrared gas analysers (IRGA) and cuvettes (LCA-3 and PLC-C, Analytical Development Co. Ltd, Hoddesdon, UK), operated in an open configuration.

Aggregate root samples from soil cores (5 cm diameter, 20 cm depth) were collected for each of up to four plots per species (minimum of two plots for three species) across three dates (18, 20 and 21 August). Roots were washed from soil cores and kept moist at 26 °C prior to measurement, typically within 2·5 h of harvest. Net CO_2 efflux was determined on the fine root fraction at a standard temperature (25·7 ± 0·4 °C) and atmospheric CO_2 concentrations (366 ± 13 µmol mol⁻¹ CO_2) using IRGA and cuvettes as described above. We determined root lengths using a digital image analysis system (WinRhizo, Régent Instruments, Inc., Québec City, Canada). Root length and oven-dry mass measures were used to calculate specific root lengths.

Leaf longevity (*201*) was determined previously by Craine *et al.* (1999). Plant biomass and other associated measures were determined in early July and mid-August of 1997 by clipping to soil level all aboveground biomass in a previously unclipped 2·3 × 0·10 m strip (or two 1·15 × 0·10 m strips for 1·2 m wide plots).

Table 2. Component loadings for the PCA. A coefficient whose absolute value is >0·33 is equivalent to a correlation with the axis at $P < 0·05$

		Axis 1	Axis 2	Axis 3	Axis 4
Organ level					
101	SLA	−0·14	−0·29	0·17	**0·54**
102	Aug SRL	0·32	**−0·40**	−0·26	−0·06
103	Aug FSRR	−0·22	**−0·78**	−0·01	−0·07
104	P_s/mass	−0·19	**−0·33**	−0·23	**0·57**
105	g_s	−0·23	**−0·45**	0·04	−0·04
106	R_s/mass	−0·14	**−0·66**	−0·13	0·30
107	Root diameter	**−0·39**	−0·14	0·23	**−0·49**
Tissue nitrogen					
108	Jul AG C : N	0·17	**0·73**	−0·29	−0·06
109	Jul BG C : N	0·17	**0·87**	−0·11	−0·08
110	Aug AG C : N	**0·39**	**0·79**	−0·16	0·05
111	Aug BG C : N	0·23	**0·84**	−0·08	0·08
Biomass					
112	Jul reproductive	**−0·93**	−0·09	−0·15	−0·06
113	Jul leaves	**−0·47**	**0·33**	**0·69**	−0·17
114	Jul stem	**−0·76**	0·07	**0·52**	−0·10
115	Jul root 0–10	0·11	**0·71**	0·09	0·29
116	Jul root 10–20	−0·26	**0·78**	0·31	0·01
117	Jul root 20–40	**−0·74**	**0·38**	0·16	−0·22
118	Jul root 40–60	**−0·78**	0·20	**0·38**	0·14
119	Jul root 60–80	**−0·69**	0·15	**0·49**	0·08
120	Jul root 80–100	**−0·93**	0·02	0·12	0·02
121	Aug reproductive	0·00	−0·14	**0·86**	0·21
122	Aug leaves	−0·04	0·31	**0·72**	−0·27
123	Aug stem	−0·05	0·12	**0·93**	−0·02
124	Aug 0–20 Coarse	−0·14	**0·34**	**0·65**	0·25
125	Aug 0–20 Fine	0·22	**0·66**	−0·32	**0·37**
Relative biomass					
126	Jul %Stem	**−0·37**	**−0·34**	**0·55**	−0·17
127	Jul %Fruit	**−0·78**	−0·28	−0·20	−0·08
128	Jul %Leaves	0·19	**−0·60**	0·23	**−0·48**
129	Jul %0–10	**0·46**	0·27	**−0·46**	**0·35**
130	Jul %10–20	0·24	**0·52**	−0·06	0·08
131	Jul %20–40	**−0·55**	**0·38**	0·15	−0·23
132	Jul %40+	**−0·47**	−0·04	**0·35**	0·10
133	Jul beta	**−0·47**	0·03	**0·47**	−0·07
134	Aug %Fruit	0·09	**−0·52**	**0·52**	0·00
135	Aug %Leaves	0·05	**−0·38**	0·12	**−0·67**
136	Aug %Stem	0·00	−0·24	**0·80**	−0·24
137	Aug %Coarse 0–20	**−0·53**	0·16	0·16	**0·33**
138	Aug %Fine 0–20	**0·33**	0·30	**−0·66**	0·25
Production					
139	MJ Ingrowth	0·02	**0·52**	−0·20	0·31
140	JA Ingrowth	−0·13	**0·61**	0·08	−0·01
141	SO Ingrowth	−0·12	0·09	−0·16	**−0·35**
142	Stem/year	**−0·50**	−0·12	**0·77**	0·10
143	%Stem/year	−0·30	**−0·33**	**0·78**	−0·07
144	%Leaf/year	0·14	0·06	0·26	**−0·64**
145	%Fruit/year	**−0·74**	−0·29	0·27	−0·04
146	%Coarse/year	−0·27	**0·40**	0·29	0·30
147	%Fine/year	**0·39**	0·10	**−0·74**	**0·33**
Soil nitrogen					
148	Jul NO_3^- 0–10	**−0·59**	**−0·60**	0·20	0·17
149	Jul NO_3^- 10–20	−0·32	**−0·61**	0·18	**0·39**
150	Jul NO_3^- 20–40	**−0·56**	**−0·43**	0·04	0·26
151	Jul NO_3^- 40–60	**−0·91**	−0·16	−0·14	0·04
152	Jul NH_4^+ 0–10	**−0·49**	−0·02	0·29	0·00
153	Jul NH_4^+ 10–20	−0·26	−0·08	**0·74**	0·27
154	Jul NH_4^+ 20–40	−0·10	0·23	0·08	0·01
155	Jul NH_4^+ 40–60	0·18	0·07	**0·40**	0·17
156	Aug NO_3^-	**−0·93**	−0·22	0·09	0·09
157	Aug NH_4^+	**−0·63**	−0·31	**0·35**	0·08
158	N min AM	**−0·40**	−0·25	**−0·33**	0·11
159	N min MJ	**−0·78**	**−0·39**	−0·02	0·12
160	N min JJ	**−0·69**	−0·21	0·04	0·23
161	N min JA	**−0·69**	**−0·33**	**0·43**	0·16
162	N min AO	**−0·52**	−0·35	0·48	0·41

The previous years' litter was removed from above-ground biomass samples, and above-ground biomass was separated into leaves, stems and reproductive parts (flowers, seeds and associated stems) (*112–114, 121–123*).

Below-ground biomass was sampled at both harvests. In July, three soil cores 5 cm in diameter and 100 cm deep were taken per plot and divided 0–10, 10–20, 20–40, 40–60, 60–80 and 80–100 cm (*115–120*). In August, three cores per plot were taken for a depth of 0–20 cm. For both harvests, all cores in a plot for a given depth interval were composited and then washed over a 1·3 mm screen. In August, roots were also separated into fine roots (<2 mm) and coarse below-ground biomass (roots and rhizomes >2 mm, crowns, corms) (*124 125*). All biomass was dried at 60 °C for a minimum of 96 h, weighed, and ground in a Wiley mill. Roots from the July harvest were composited 0–20, 20–40 and 40–100 cm and then ground. Tissue C and N concentrations for each fraction were determined with a Carlo-Erba NA1500 analyser. As tissue N concentrations can be biased by contamination with mineral soil, particularly for roots, we report tissue C : N ratios of biomass fractions (*209–219*). The N content of recently senesced leaves of 15 species was determined in August (*208*) in a manner similar to the live biomass.

Fine root production (*139–141*) was estimated with an ingrowth technique. At three times during the year (May, July and August) a soil core 5 cm in diameter was taken from a random location within the plot to a depth of 25 cm. Each hole was then refilled with a common root-free soil taken from an area of the garden that underwent the same initial treatment as all the plots and kept unvegetated for the entire experiment. The ingrowth cores were resampled after 41, 40 and 56 days by removing a 20 cm deep, 3·75 cm diameter core from the centre of the ingrowth core. The roots in the core were washed free of soil, coarse roots (>2 mm) removed, and the root biomass dried and weighed in the same manner as other root samples.

Extractable soil NO_3^- and NH_4^+ were determined for 0–10, 10–20, 20–40 and 40–60 cm in July (*148–155*) and 0–20 cm in August (*156–157*) with a 0·01 M KCl extraction (Tilman & Wedin 1991a). Rates of N mineralization (*158–162*) were determined by *in situ* soil incubation using 1·9 cm diameter PVC plastic tubes that were inserted 15 cm into the soil. Monthly incubations began in mid-April and continued to mid-August. The last incubation ran from mid-August until early October. NO_3^- and NH_4^+ concentrations for the incubations were determined with a 1 M KCl extract (Wedin & Tilman 1990).

CALCULATED PARAMETERS

An exponential decay constant for the dependence of root biomass on depth (*133*) was computed for each species by fitting an exponential function to cumulative biomass and soil depth data (Jackson *et al.* 1996). We calculated maximal total biomass (*204*) as the sum of

Table 3. Other correlations (*r*) with PCA axes 1–4 (legumes were excluded from the analysis of correlations with axis 2)

			Axis 1		Axis 2		Axis 3		Axis 4	
		n	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Organ level										
201	Leaf longevity	14	0·50	<0·1	0·60	<0·05	−0·07	>0·1	0·10	>0·1
202	Fine root longevity	33	0·25	>0·1	0·43	<0·05	−0·31	<0·1	0·33	<0·1
203	Aug CSRR	33	0·02	>0·1	−0·33	<0·1	0·05	>0·1	−0·13	>0·1
Whole plant										
204	Max total biomass	33	−0·59	<0·001	0·83	<0·001	0·55	<0·01	0·02	>0·1
205	Max total plant N	33	−0·78	<0·001	0·65	<0·01	0·50	<0·01	0·12	>0·1
Relative biomass										
206	July R : S	33	0·48	<0·01	0·57	<0·01	−0·38	<0·05	0·34	<0·1
207	August R : S	33	−0·23	>0·1	0·48	<0·05	−0·49	<0·01	0·49	<0·01
Tissue nitrogen										
208	C : N senesced leaves	15	0·88	<0·001	0·53	<0·1	−0·08	>0·1	0·26	>0·1
209	Aug C : N reproductive	24	−0·15	>0·1	0·31	>0·1	−0·18	>0·1	0·15	>0·1
210	Aug C : N leaves	28	0·07	>0·1	0·78	<0·001	−0·09	>0·1	0·25	>0·1
211	Aug C : N stems	28	0·36	<0·1	0·48	<0·01	−0·07	>0·1	−0·04	>0·1
212	Aug C : N fine	28	−0·21	>0·1	0·70	<0·001	−0·08	>0·1	0·20	>0·1
213	Aug C : N coarse	24	−0·04	>0·1	0·76	<0·001	0·30	>0·1	0·04	>0·1
214	Jul C : N reproductive	27	0·19	>0·1	0·42	>0·1	0·08	>0·1	−0·07	>0·1
215	Jul C : N leaves	13	0·39	>0·1	0·75	<0·001	0·18	>0·1	−0·13	>0·1
216	Jul C : N stems	28	0·18	>0·1	0·31	>0·1	−0·06	>0·1	0·13	>0·1
217	Jul C : N 0–20	28	−0·23	>0·1	0·89	<0·001	0·33	<0·1	0·00	>0·1
228	Jul C : N 20–40	25	−0·41	<0·05	0·79	<0·001	0·43	<0·05	−0·33	>0·1
219	Jul C : N 40–100	27	−0·45	<0·05	0·62	<0·001	0·12	>0·1	0·13	>0·1
Production										
220	Annual total productivity	33	−0·81	<0·001	0·69	<0·001	0·47	<0·01	−0·03	>0·1
221	Total plant N uptake	33	−0·90	<0·001	0·11	>0·1	0·27	>0·1	0·02	>0·1
222	Plant N loss rate	33	−0·90	<0·001	0·06	>0·1	0·26	>0·1	−0·01	>0·1
223	Total above-ground production	33	−0·61	<0·001	0·42	<0·05	0·71	>0·001	−0·05	>0·1
224	Total below-ground production	33	−0·90	<0·001	0·77	<0·001	0·03	>0·1	0·01	>0·94
225	%Above-ground production	33	−0·32	<0·1	−0·18	>0·1	0·68	<0·001	−0·44	<0·01

below-ground biomass to 100 cm measured in July and above-ground biomass measured in August, except for *Lupinus perennis*, for which July above-ground biomass was used due to the earlier phenology of *L. perennis*. Maximal total plant N (*205*) was calculated as the sum of the calculated N content of each biomass fraction in August, except for *L. perennis* for which the July data were used.

Total productivity (*220*) and relative production of different biomass fractions (*143–147*) required calculating the productivity for each of the fractions. Annual fine root production was calculated as the sum of the fine root production in the 0–20 and 20–100 cm strata. Annual fine root production at 0–20 cm was equal to the sum of fine root production in the ingrowth cores. The production of fine roots deeper than 20 cm was calculated by assuming that the root biomass below 20 cm had the same relative proportion of coarse and fine material as calculated in August at 0–20 cm, and that these roots had the same turnover rates as fine roots at 0–20 cm. The longevity of fine roots 0–20 cm (*202*) was calculated as the ratio of August fine root biomass at 0–20 cm to fine root production from ingrowth cores (*139–141*).

With no data on turnover or production of coarse below-ground biomass, we conservatively calculated annual production of coarse below-ground biomass by assuming equal coarse below-ground biomass production among years; no turnover of coarse below-ground biomass; and scaled coarse below-ground biomass to 100 cm assuming equivalent coarse : fine ratios in the 20–100 cm stratum and the 0–20 cm stratum. Leaf and reproductive biomass production were assumed to be equivalent to the greater of the July or August biomasses for leaves and reproductive parts (*112, 113, 121, 122*), respectively. Stem production (*142*) was calculated in a similar manner, except for the two woody species where, as with coarse below-ground biomass, stem biomass was divided by five to equalize production among years. Relative production of a biomass fraction or set of fractions was calculated as the ratio of production of a biomass fraction to total biomass production (*143–147, 225*).

Annual net N uptake (*221*) was calculated as the sum of the amounts of N incorporated into new production (N demand) for each biomass fraction. The N demand for a fraction was calculated as the product of the N concentration as measured in August and the

annual production of each fraction, except for leaves for which senesced leaf tissue N was estimated from August green leaf N concentrations (%N senesced leaves = −0·510 + 1·032 × August %N leaves; r^2 = 0·90, based on 15 species for which %N of senesced leaves was measured; July %N leaves was used for *L. perennis*). Plant N-loss rate (*222*) was calculated in a similar manner to annual N uptake, except that the N incorporated into stem or coarse below-ground biomass production was not considered to be lost from the plant.

DATA ANALYSIS

All statistical analyses were performed in JMP 3·2 (SAS Institute, Carey, NC). We analysed 62 traits by principal component analysis (PCA): seven ecophysiological/ morphological parameters (*101–107*); four measurements of plant tissue C or N of above-ground or below-ground biomass (*108–111*); 18 measurements of biomass pools or production rates of different plant fractions (*112–125*, *139–142*); 18 measurements of the relative size of these plant biomass pools or their production relative to total production (*126–138*, *143–147*); and 15 measurements of N associated with soils (*148–162*). The PCA weights all traits equally, and does not presuppose directional or causal relationships among traits. The first four resultant axes were determined to be the most biologically significant and then rotated using the Varimax rotation protocol to strengthen contrasts and aid in interpretation. For a parameter of a given rotated axis, a coefficient of absolute value >0·33 corresponds to a probability of P < 0·05 that a parameter is significantly correlated with the resultant axis.

As discussed below, the second axis primarily differentiates non-legumes in their trait relationships. Axes 1 and 3 account for most of the variation associated with legumes; scores on axis 2 of legumes are all close to zero. In order to provide the strongest analysis of how non-legumes differ in their trait relationships, we removed legumes from the data set and re-examined the correlations between the variables used in the PCA with the original scores of species on axis 2. This provides an average absolute value of the eigenvector coefficients that is approximately equal to the original coefficient (about 0·08 higher on average, or the equivalent of significance changing from P < 0·05 to P < 0·01). Yet individual variables that are also strongly associated with legumes and axis 2 had much higher correlations, such as August coarse below-ground biomass (*124*) (r = 0·32, P < 0·07 *vs* r = 0·66, P < 0·001). Overall, the loadings of variables on the PCA and their subsequent correlation coefficients were well correlated (r = 0·96, P < 0·001), but 10 more variables came to be considered as significant and one lost significance.

In addition to the 62 variables of the PCA, two other types of variable were examined as part of the relationship of traits among species. The first data type is metrics that were not measured on all of the species [leaf longevity (*201*); N content of senesced leaves

(*208*); coarse below-ground biomass respiration rates (*203*); tissue C : N of certain biomass fractions (*209*, *211*, *213*, *214*, *216*)] and therefore could not be included in the PCA. The average C : N of above- and below-ground biomass for each harvest (*108–111*) were included in the PCA, while the C : N ratios of all individual fractions (*209–219*) were later correlated with the PCA axes. Other organ-level measures (*202*), whole-plant biomass and relative biomass measures (*204–207*), and measures of productivity and N utilization (*220–225*) were not included in the PCA, as these were calculated from metrics included in the PCA and their inclusion would have unnecessarily emphasized constituent factors.

For both measured and calculated parameters not included in the PCA, a pairwise correlation coefficient and the significance of the correlation were determined for each trait and PCA axes 1, 3 and 4 to determine if these traits and axes were associated with one another. Legumes were removed from the data set when examining the relationship between additional metrics and axis 2. For both PCA and additional correlations, we generally consider a trait to be a part of a given suite of traits if it is statistically significant (P < 0·05), although in particular circumstances we relax this condition.

Results

The first four axes of the PCA accounted for 59% of the explainable variation of the data set (6·3% expected). Axes 1 and 3 distinguish cool- and warm-season legumes from each other and from non-legumes. Axis 2 differentiates non-legumes based on a suite of traits at multiple process levels associated with the production the maintenance of large amounts of biomass on low-N soils. Axis 4 differentiates grasses and forbs, primarily on morphological traits.

AXIS 1: COOL-SEASON LEGUME

Axis 1 differentiates *L. perennis* from all other species (Table 1; Fig. 1a). A simple binary scoring of species as *L. perennis* or other species explained 80% of the variation on this axis (data not shown). Axis 1 was the most important of the four axes, explaining 21·6% of the total variation in the data set (36·6% of the explained variation).

Lupinus perennis is unique among the species we examined in that it is a strong N_2-fixer with a strict cool-season phenology. N_2-fixation rates in *L. perennis* plots were apparently sufficient, annually or cumulatively, to provide 35 g N m^{-2} more for production purposes in the fifth growing season than the typical non-legume (*221*, *222*). *Lupinus perennis* had at least twice the total biomass production of the most productive non-legume, and six times greater biomass production than typical non-legumes (*220*).

Lupinus perennis builds primarily coarse roots (*137*) that penetrate deeply (*119*, *120*, *133*), presumably for
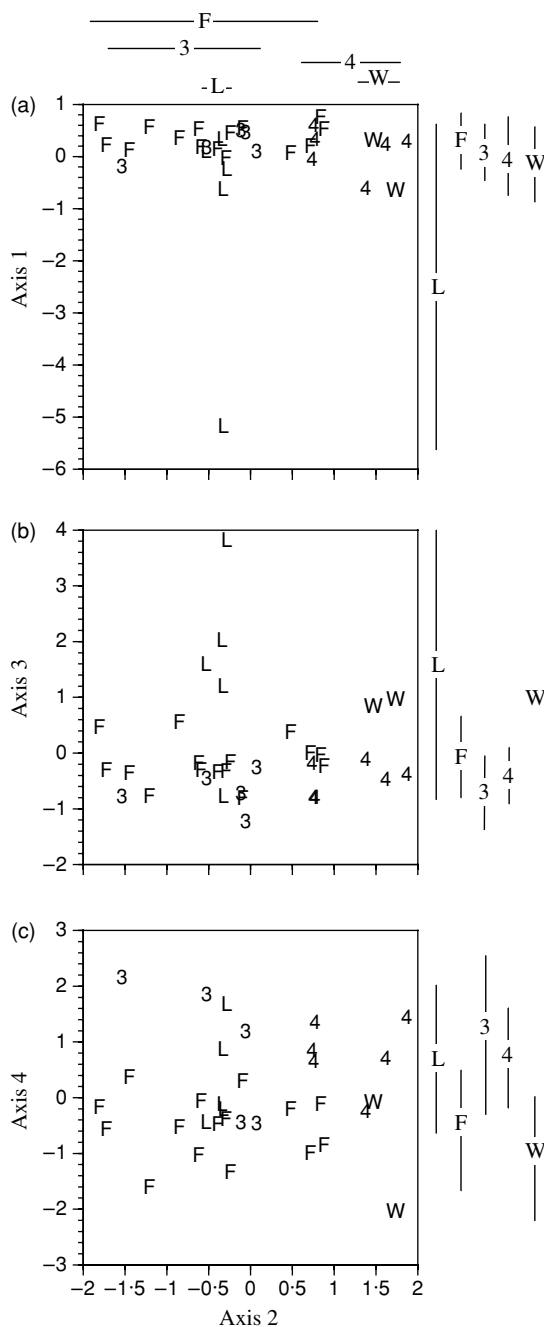
Fig. 1. (a–c) Principal component axes 1, 3 and 4 plotted *vs* axis 2. Symbols are based on current Cedar Creek functional classification: forb (F); C$_3$ grass (3); C$_4$ grass (4); woody species (W); legume (L).

water acquisition. *Lupinus perennis* had greater relative production of reproductive biomass (*145*) and less relative production of fine roots (*147*) than other species. *Lupinus perennis* leaves had the shortest longevity of any leaves measured by Craine *et al*. (1999), and one of the shortest fine root longevities (*202*[ns]), both approximately 4 weeks. Its low C : N ratio for senesced leaves (*208*) and fine roots (*212*[ns]), along with their short longevity, are associated with high rates of N mineralization (*158–162*) and extractable NO$_3^-$ (*148, 149*[ns]*, 150, 151, 156*) throughout the growing season.

AXIS 2: HIGH- AND LOW-N NON-LEGUMES

Axis 2 was the second most important axis, explaining 16·9% of the total variation. It differentiates the non-legumes in their trait relationships, especially in their ability to maintain large amounts of biomass (Fig. 1). Total peak biomass (*204*) was positively correlated with axis 2 ($r = 0.83$, $P < 0.001$), ranging from 72 to 704 g for the central 80% of the non-legumes. Legumes were in the centre of the distribution of axis 2, as their differences are contained in axes 1 and 3 (Fig. 1). Consequently, this section discusses the differences among non-legumes, and does not address how legumes differ from non-legumes. For simplification, the word 'species' in this section refers to non-legume species.

Relationships among ecophysiological traits

Those species that maintain high biomass also have low concentrations of N in above- and below-ground tissues (*108–111*), and generally in each individual fraction (*209*[ns]*, 210–213, 214*[ns]*, 215, 216*[ns]*, 217–219*) (Table 3).

The pattern seen in leaf functional traits across the gradient of species in axis 2 follows the five-part correlation seen by Reich *et al*. (1997): photosynthesis (*104*), leaf respiration (*106*) and SLA (*101*) were all negatively correlated with axis 2; and leaf longevity (*201*) and leaf C : N ratio (*210, 215*) were positively correlated with axis 2.

The existence of a set of correlations for analogous functional parameters for roots has not been demonstrated previously. Species that scored high on axis 2 had high root C : N (*109, 111, 212, 213, 217–219*); low FSRR (*103, 203*[ns]); and high fine root longevity (*202*). Roots changed little in diameter along axis 2 (*107*[ns]), but the roots of low-N species have lower specific root length (more mass per unit root length) (*102*), together indicative of greater tissue density. This four-way set of correlations among the root functional parameters (low respiration, low SRL/high tissue density, high longevity, high tissue C : N) is analogous to the correlations observed here and by Reich *et al*. (1997) for leaves (low photosynthesis, low respiration, low SLA, high longevity, high tissue C : N).

Among all species, fine root longevity was positively associated with the C : N ratio of fine roots (Fig. 2). Fine root longevity and fine root specific respiration rate [log (fine root longevity) = 1·59 − 1·72 × (FSRR), $r = -0.48$, $P = 0.005$; type II regression] and diameter [log (diameter) = −5·59 − 3·51 × log (diameter), $r = -0.38$, $P = 0.03$; type II regression] were significantly related, but not specific root length or tissue density ($P > 0.1$).

Biomass production

Low-N species had greater leaf, fine root and coarse below-ground biomass (*113, 115, 116, 117, 122*[ns]*, 124, 125*) than high-N species. From high- to low-N species,
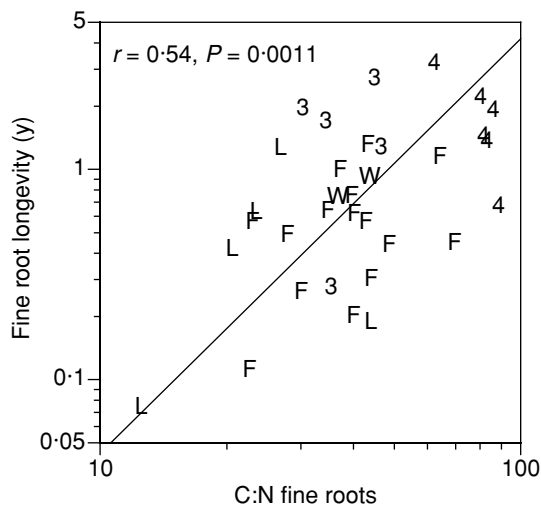
Fig. 2. Relationship between C : N ratio of fine roots in August and the longevity of fine roots. The equation for the reduced major axis (type II regression): log fine root longevity $= -3.32 + 1.95 \times \log(C : N$ fine roots).

above-ground biomass increases less than below-ground biomass, and the ratio of root biomass to shoot biomass is greater for low-N species (*206, 207*). Fine root production is greater for low-N species (*139, 140*) and, with the greater longevity of roots of low-N species, this leads to greater fine root biomass, especially at shallow depths (*125*).

Low-N species produced more biomass below-ground (*124, 139, 140*), but low-N species also had greater leaf production (*113, 122*[ns]). With greater rates of production in both above- and below-ground fractions for low-N species, it is necessary to examine ratios of production to determine differences in the relative production of biomass of the different fractions. Relative production of stem biomass in low-N species was smaller than in high-N species (*143*), and relative production of coarse below-ground biomass was greater (*146*). Although low-N species had greater relative below-ground coarse biomass production, this comprises a small fraction of productivity. Patterns of the relative production of biomass above- and below-ground do not support the assertion that low-N species produce a greater proportion of their biomass below ground. The relative production of leaves and fine roots were not correlated with axis 2 (*144*[ns], *147*[ns]). Similarly, the relative production of biomass above- as well as below-ground were not significantly correlated with axis 2 (*230*[ns]). Together, high- and low-N plants differed little in their major relative above- *vs* below-ground production patterns, or in the relative production of leaves *vs* fine roots.

Although differences in patterns in biomass allocation above-ground/below-ground were not associated with axis 2, allocation patterns were important among herbaceous non-legumes in differences in root : shoot ratios. Differences in root : shoot ratio among species were due to greater biomass allocation below-ground,

as well as greater allocation to coarse root biomass and greater relative longevity of fine roots to leaves (Fig. 3a–c). The biomass that is produced below ground in low-N plants has a greater proportional longevity than the below-ground structures of high-N species, both because more of it tends to be coarse structures, and because the fine roots of low-N species turn over more slowly. The ratio of fine root to leaf longevity is greater in high root : shoot ratio plants (Fig. 3a), which would also cause biomass to accumulate disproportionately below ground.

Plant N dynamics

Low-N species have lower per unit mass nutrient demands (higher C : N, *108–111*), but higher production rates under nutrient-poor conditions than high-N species, leading to similar annual net N uptake after the 5 years of growth in this study (*221*[ns]). Stand-level N loss via tissue turnover is not correlated with axis 2 (*222*[ns]). After 5 years, stands of both high- and low-N species acquired N at similar absolute annual rates, and there was no relationship between peak biomass and stand-level N uptake (Fig. 4a). Instead, the larger biomass of low-N species is accomplished by higher C : N ratios and greater longevity of tissues (Fig. 4b,c). This contrasts with legumes, that produce more biomass largely by having higher N uptake (Fig. 4a)

Ecosystem N dynamics

Differences in functional traits among species along axis 2 had consequences for soil N availability and cycling. In the soils beneath low-N species, NO_3^- concentrations were lower in July, especially at shallow depths, and tended to be lower in August (*148–150, 156*[ns]) compared to high-N species. Decreased NO_3^- concentrations were associated with accumulation of N in plant material (*205*) and decreased rates of net N mineralization (*158*[ns], *159, 160*[ns], *161, 162*).

AXIS 3: WARM-SEASON LEGUMES

Axis 3 was similar in importance to axis 2, explaining 15·6% of the total variation in the data set. Of the variation along axis 3, 68% is explained by a binary classification of species: the four warm-season legume species (*Desmodium canadense*, *Lespedeza capitata*, *Petalostemum purpureum*, *Petalostemum villosum*) *vs* the other 29 species. The two woody species were intermediate between warm-season legumes and other species in their scores, but removing the woody species from the data set did not change the significance of any parameters, except the C : N ratio of stems in July (data not shown).

In total, 16 of the 21 components that load high for this axis relate to absolute or relative biomass and biomass production. The highest loading component for axis 3 was August stem biomass (*123*). This factor
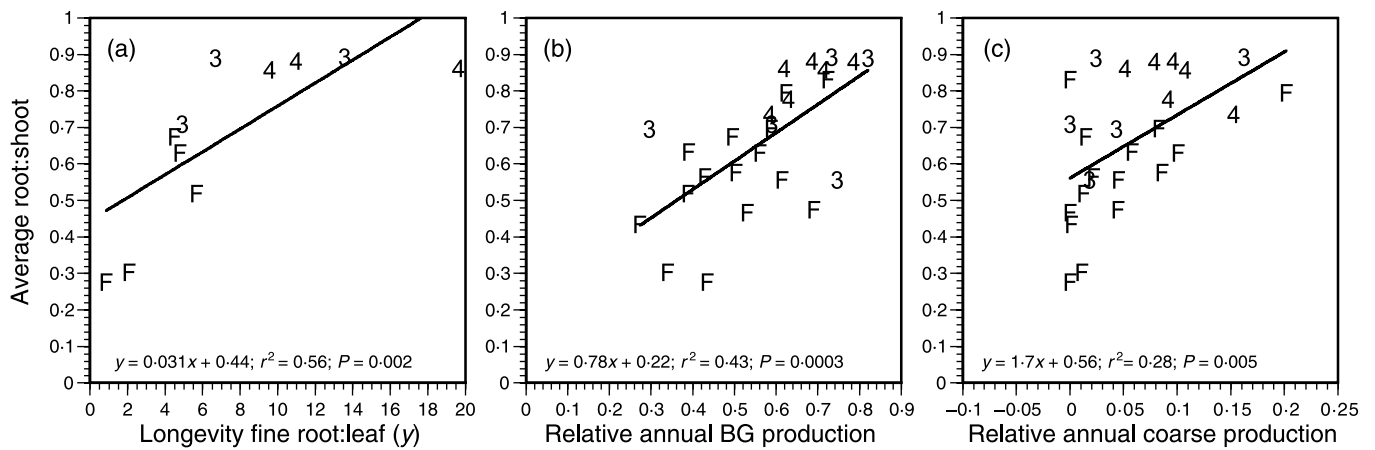
Fig. 3. Relationships between root : shoot ratios of non-legumes and the ratio of fine root to leaf longevity (a); relative production of below-ground biomass (b); and relative production of coarse root biomass (c).
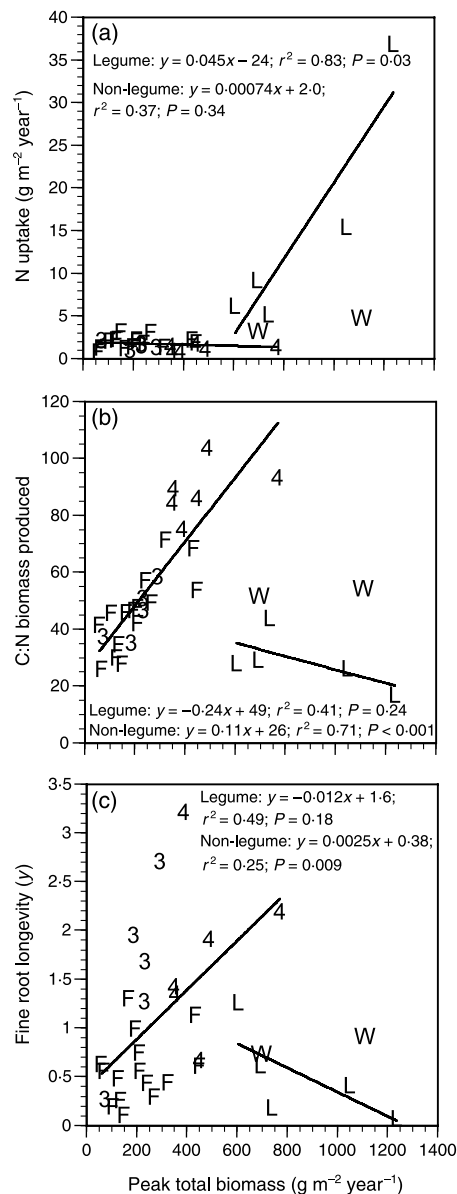

Fig. 4. Relationships between peak total biomass (larger biomass from July and August harvest) and annual N uptake (a); average C : N ratio of biomass produced (b); and fine root longevity (c).

alone explains 86% of the variation along this axis ($P < 0.001$). In general, the warm-season legumes had more stem biomass in July and August (*114*, *123*) than non-legumes, larger relative rates of stem production (*142*, *143*), more leaf biomass in July and August (*113*, *122*), and more August reproductive biomass (*121*). In addition, coarse below-ground biomass production and biomass (*124*) were greater, as well as relative stem production rates (*143*). Fine root biomass and relative fine root production were smaller (*125*[ns], *147*).

AXIS 4: GRASSES AND FORBS

Axis 4 largely separates the grasses and forbs (Fig. 1). Axis 4 is a relatively minor axis, associated with fewer traits than axes 1–3 and explaining only 7·0% of the total variation in the data set. In general, grasses and forbs differed in certain ecophysiological and morphological traits as well as the relative production rates and biomass of leaves. The fine roots of grasses are thin (*107*) and more dense [similar SRL with smaller diameter (*102*[ns], *107*)]. Photosynthetic rates (*104*) and SLA (*101*) of grasses exceeded those of forbs. Differences between grasses and forbs were not associated with differences in production and maintenance of large amounts of biomass. The fraction of production above ground relative to total production was less for grasses than non-grasses (*230*).

Discussion

STRATEGIES FOR PRODUCTION AND MAINTENANCE OF BIOMASS ON LOW-NUTRIENT SOILS

Previous work on plant strategies and the relationships among functional traits has generally been limited to short-term studies on young plants, or to a few species, or has not included below-ground and/or ecosystem traits. Yet when the results of this study are viewed in conjunction with previous work, it is clear that there are two general strategies that confer the ability to produce

and maintain large amounts of biomass in low-nutrient soils, and these extend to include a wide variety of organ-level, whole-plant and ecosystem traits.

The first strategy is to avoid nutrient limitation entirely by accessing a unique source of the limiting nutrient. The differences among *L. perennis* (axis 1), the warm-season legumes (axis 3) and non-legumes (axis 2) reveal that symbioses with N_2-fixing bacteria allow species to have an integrated suite of traits that includes high nutrient concentrations, large amounts of above-ground biomass production and accelerated nitrogen cycling. This is not a new idea, but it is clear that there are large interspecific differences among legumes that are associated with N_2-fixation capacity, and N_2-fixation is associated with a broad suite of traits. It is important to note that differences among legumes can be as great as differences between legumes and non-legumes. For example, the C : N of leaves (24) and C : N of above-ground biomass (30) in August of *L. capitata* is higher than many non-fixing forbs (data not shown), while some of the greatest differences in traits among species were between cool- and warm-season legumes.

The second general strategy that confers the ability to produce and maintain large amounts of biomass in low-nutrient environments is associated with production of low-nutrient biomass and the reduction of N availability (Fig. 5). The suite of traits for the low nutrient strategy involves low rates of physiological activity and tough, dense, long-lived tissues that have low nutrient concentrations both above- and below-ground. After 5 years, low-N species had higher total biomass, not because more N was acquired or less was lost on an annual basis, but instead because more biomass was produced per unit N and roots and leaves lived longer (Fig. 4). The low N concentrations of the biomass are tightly associated with lower net N mineralization and lower nitrate availability. The relationships among species in tissue N concentration, biomass, and available N appear tightly constrained (Fig. 5).

Differential biomass longevity between leaves and roots provides an alternative mechanism to resolve conflicting patterns of relative root : shoot allocation in seedlings and more mature plants. For example, Gleeson & Tilman (1994) found no consistent differences in root : shoot ratio of seedlings, but large differences in mature plants (Gleeson & Tilman 1990; Gleeson & Tilman 1994) and suggested that the differences in these root : shoot ratios were due to allocational plasticity and not the result of differences in turnover rates. As both differences in allocation and relative longevity could increase the root : shoot ratio, caution should be used in assuming that root : shoot ratio serves as a good surrogate for allocation.

Although there are differences between these results and previous studies, at this point it is more important to emphasize the similarities in the results of the many studies that have been conducted on the relationships
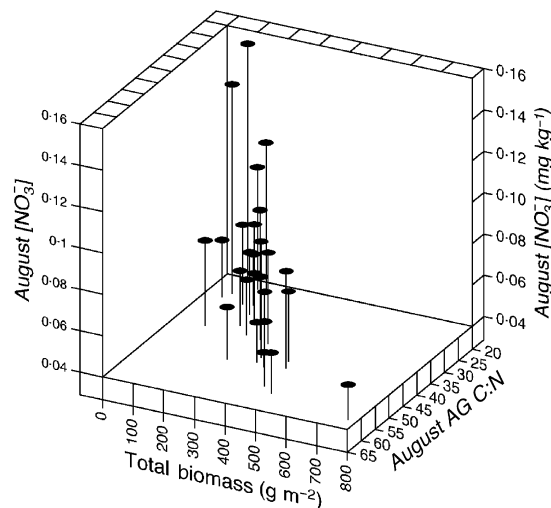

Fig. 5. Three-way relationships among peak biomass (larger biomass from July and August harvest); C : N ratio of August biomass; and extractable concentrations of NO_3^- in August for all non-legumes.

of plant traits. Besides the few key differences in the allocation and production of biomass focused on above, the low-N strategy discussed here corresponds to strategies and trait relationships discussed by others (Aerts & Chapin 2000; Chapin 1980; Grime 1977; Grime *et al.* 1997; Hobbie 1992; Poorter, Remkes & Lambers 1990; Reich *et al.* 1997; Tilman 1988; Tilman & Wedin 1991b). It incorporates the toughness, longevity and low rates of activity first emphasized by Grime (1977) and Chapin (1980), while also incorporating the feedbacks to N availability of Tilman (1988). For example, species that have low nutrient concentrations were found by Grime *et al.* (1997) to sustain yield better under limiting supplies of nutrients, and had low leaf longevity and high decomposition rates. Wedin & Pastor (1993) found that species with high C : N concentrations had low rates of N mineralization. Others have emphasized aspects of this strategy that differ from our results here, such as lower rates of nutrient loss from low-N species (Berendse, Oudhof & Bol 1987) but, taken as a whole, most show similar patterns across and within process levels.

CATEGORICAL AND CONTINUOUS FUNCTIONAL CLASSIFICATIONS

Analysis of individual functional traits revealed numerous traits that differed significantly among Cedar Creek's categorical functional classifications (Table 4). C_3 grasses, C_4 grasses, forbs, legumes and woody species were well differentiated in the space described by the four PCA axes. Legumes are well differentiated from non-legumes by axes 1 and 3; C_4 grasses and woody species are distinguished from other species by axis 2; and forbs and grasses are offset from one another on axis 4.

Table 4. Means and standard errors of PCA scores for each standard functional classification

		F ratio	P	C$_3$ grass (5)	C$_4$ grass (6)	Forb (15)	Legume (5)	Woody (2)
363	Axis 1	2·5	<0·1	0·18 ± 0·13[a]	0·10 ± 0·17[a]	0·31 ± 0·06[a]	−1·16 ± 1·03[a]	−0·20 ± 0·48[a]
364	Axis 2	8·9	<0·001	−0·43 ± 0·29[b]	1·19 ± 0·2[a]	−0·42 ± 0·23[b]	−0·36 ± 0·04[b]	1·60 ± 0·13[a]
365	Axis 3	9·0	<0·001	−0·72 ± 0·16[b]	−0·49 ± 0·12[b]	−0·20 ± 0·1[b]	1·54 ± 0·74[a]	0·87 ± 0·06[ab]
366	Axis 4	5·6	<0·01	0·83 ± 0·56[a]	0·76 ± 0·25[a]	−0·54 ± 0·15[b]	0·32 ± 0·4[ab]	−1·09 ± 0·98[ab]

In parentheses, number of species per functional group. Superscript letters refer to *post hoc* comparisons for a trait among functional groups.

Examination of component data and the PCA axes suggests that it may be helpful to divide legumes into cool- and warm-season legumes. Legumes encompass a surprisingly broad range of phenology and productivity. *Lupinus perennis* had the earliest spring growth of any species studied, while *L. capitata* was one of the last species to produce green leaves in the spring with a phenology similar to C$_4$ grasses (Craine *et al.* 1999). Peak biomass and patterns of availability and mineralization of N are also offset seasonally. *Lupinus perennis*, the only cool-season legume we sampled, also had higher reproductive biomass production, total productivity, annual N uptake, and N mineralization rates than the average warm-season legume (data not shown). Although *L. perennis* may be unique among the species sampled in this study, other cool-season legumes have traits similar to *L. perennis*. *Vicia villosa*, *Lathyrus venosus* and *Trifolium repens* produce most of their biomass in the spring and fall, and have high tissue N concentrations and potential productivity (Knops *et al.* 2002; Ritchie, Tilman & Knops 1998).

In contrast to categorical classifications of non-legumes based on photosynthetic pathway and monocot–dicot classification, non-legumes can be arrayed along a single continuous axis that incorporates C$_3$ grasses, C$_4$ grasses and forbs. Grasses and forbs are offset on axis 4 largely because of certain morphological and allocational traits, not by traits associated with growth and production of biomass on low-N soils (axis 2). Although C$_4$ grasses scored consistently high on axis 2, several forb species (*Liatris aspera* and *Solidago rigida*) scored as high on axis 2 as many of the C$_4$ grasses. The categorical differences among these functional classifications could also be expressed as a single continuous distribution of C$_4$ grasses, C$_3$ grasses and forbs (Fig. 1). There was more variation within C$_3$ grasses and forbs than between the two classifications (Table 4), such that with regard to N status and successful growth on low-N soils, forbs and C$_3$ grasses are not well differentiated classifications. Other traits, such as root diameter (axes 3 and 4), may be necessary to explain differences in performance between the two functional groups.

Further research on additional traits and species is still needed, and more complex ecological questions require continued development of our understanding of trait variation across a range of supplies for different resources. Still, the relationships among traits that we have described demonstrate that these species have suites of correlated traits. This means that knowledge of the value of one variable can be used to predict a large number of other traits. For example, the leaf N of non-legumes allows a species to be arrayed along a functional axis (e.g. axis 2) and allows *a priori* quantitative prediction of root traits such as root N concentration and longevity. Although the functional classifications derived from the PCA appear to be more parsimonious than the common grass–forb, C$_3$–C$_4$ species classifications, further testing is required to determine the relative utility of categorical and continuous functional classifications in explaining and predicting different ecological patterns. The grass–forb, C$_3$–C$_4$ classification is easy to assign to species, yet the additional effort in measuring key traits and arraying species into continuous classifications may provide important additional predictive power.

The species examined here represent a range of strategies that are all viable in grasslands and savannas. Although relative abundance will vary among species, all species are able to coexist in grasslands. When grown without interspecific competition, the two low-N strategies do best when productivity is strongly N-limited. High-N species would presumably grow better as N supply increases, congruent with the findings of Craine *et al.* (2001) which showed that species with low tissue density and related traits increased in relative abundance with N fertilization at Cedar Creek. The relative importance of measured traits in explaining relative abundance when interspecific competition is present requires further research that examines the relationships between relative abundance and key traits for the traits examined here, as well as other traits such as seed size and reproductive output.

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