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Variation in growth rate and ecophysiology among 34 grassland and savanna species under contrasting N supply: a test of functional group differences

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Summary

• We tested the hypothesis that biological trait-based plant functional groups provide sufficient differentiation of species to enable generalization about a variety of plant ecophysiological traits or responses to nitrogen (N).

• Seedlings of 34 North American grassland and savanna species, representing 5 functional groups, were grown in a glasshouse in an infertile soil with or without N fertilization.

• Forbs, C_3 and C_4 grasses, on average, had similar relative growth rates (RGR), followed in declining order by legumes and oaks, but RGR varied greatly among species within functional groups. All measured attributes differed significantly among functional groups, of these, only RGR and photosynthesis differed among functional groups in response to N. All groups, except the legumes, had significantly greater photosynthetic and respiration rates at elevated N supply. Principal components analyses and cluster analyses yielded groupings that corresponded only moderately well to the biologically based *a priori* functional groupings.

• Variation in RGR among species and treatments was positively related to net CO_2 exchange (photosynthesis and respiration) and net assimilation rate, but unrelated to leaf area ratio. Photosynthetic and respiration rates were related to tissue %N among treatments and species. Our data indicate that RGR and related traits differ among the functional groups in significant ways, but in a complex pattern that does not yield simple generalizations about relative performance, controls on RGR, or response to resource supply rate.

Key words: functional types, functional groups, nitrogen, relative growth rate, ecophysiology.

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Introduction

The potential utility of considering plant species within differing functional groups or types (hereafter used interchangeably) has been increasingly examined (Grime, 1979; Pearcy & Ehleringer, 1984; Garnier, 1992; Smith *et al.*, 1996; Lavorel *et al.*, 1997; Reich *et al.*, 1998b; Campbell *et al.*, 1999; Diaz *et al.*, 1999; Wand *et al.*, 1999; Craine *et al.*, 2002). If the use of functional types allows us to more easily characterize the attributes or responses of vegetation, it will enable higher order conceptual and more complex quantitative models at a range of scales. It is common sense to assume that functional groups will only be of use if the members of one group differ consistently on average from those of another group with respect to a single or set of target traits or responses. As yet, however, there is no definite set of rules that allows us to judge when functional types will be useful and when not.

In this paper, we will refer to both traditional *a priori* groupings based largely on single biological traits of species and to posthoc classification schemes (e.g. plant functional

types) that attempt to group plant species based on their responses to specific environmental factors (Lavorel *et al.*, 1997). The traditional *a priori* groupings are typically defined by discrete and measurable biological trait differences (e.g. whether a plant fixes nitrogen (N) or not; has perennial woody tissues or not; has a given photosynthetic pathway or not). Thus, these class distinctions are real, but an unresolved question is which of these single-trait groupings enable successful prediction of responses to environmental variation in climate, atmospheric chemistry or site disturbances (Lavorel *et al.*, 1997; Wand *et al.*, 1999; Reich *et al.*, 2001b). Although the limitations of traditional single-trait groupings has spurred strong interest in more complex and sophisticated (multitrait) classification schemes, in many cases geared specifically to global change, the advantages of the traditional groups have also been demonstrated (Wedin & Tilman, 1993; Reich *et al.*, 2001a), and the underlying trait variation that might lead to success or failure of predictions based on such groups often remains undocumented. In this study, we sought to examine whether membership in different *a priori* biological trait-based functional groups results in clear distinction in a number of seedling traits among sympatric plant species.

The species pool from temperate grasslands and savannas offers one opportunity to examine functional group differences, because a variety of classically defined groups are important there. In this paper, we explore such patterns for perennial species from the central North American grassland-savanna ecotone. Grasslands and savannas are inhabited by plant species with diverse sets of traits, including the contrasting woody vs herbaceous species types. Within the herbaceous group, we distinguish between grasses and forbs. Within the former group we contrast C_3 vs C_4 grasses and within the latter we contrast species that either do or do not fix N (with the aid of root symbionts). Differences among these functional groups have been shown to have significant impacts on the composition, succession, N dynamics and productivity of experimental and natural communities in this region (Tilman, 1988; Tilman & Wedin, 1991; Wedin & Tilman, 1993; Reich *et al.*, 2001a). It is still unclear, however, whether and how these functional types differ in a set of important tissue and plant traits, including relative growth rate (RGR), seed size, allocation, morphology, metabolism or chemistry (Poorter *et al.*, 1990; Reich *et al.*, 1998a,c; Van der Werf *et al.*, 1998).

Although the precise nature of the relationships is sometimes hazy and the strength of the relationships highly variable, traits such as seed size, plant size, growth rate, tissue chemistry, leaf and root morphology, and net CO_2 exchange characteristics have been shown many times to be related to the ecology and distribution of plant species (Grime, 1979; Tilman, 1988; Westoby, 1998; Diaz *et al.*, 1999; Hodgson *et al.*, 1999). Therefore, these traits are of considerable interest, and it is important to ascertain whether these traits are associated with functional group membership. In this study, we grew seedlings of 34 grassland and savanna species from five major functional groups from eastern Minnesota, USA, under contrasting N availability (unfertilized soil vs the same soil with added N) to address the following series of hypotheses.

1 RGR and related traits (such as leaf area ratio, net photosynthetic rate) will be significantly different on average for species from the five functional groups.

2 The differences in RGR among species and functional groups will be a result of differences in determinants of RGR such as specific leaf area (SLA), leaf area ratio (LAR), leaf mass fraction (LMF) and net photosynthetic rates; and RGR should correlate well with traits such as SLA, LAR and photosynthetic rates (Lambers & Poorter, 1992), both within functional types and pooling among all species and treatments.

We base these two hypotheses on results of prior studies (Poorter *et al.*, 1990; Garnier, 1992; Walters *et al.*, 1993; Cornelissen *et al.*, 1996; Hunt & Cornelissen, 1997; Reich *et al.*, 1998a,c) that found that differences in seedling RGR among species can usually be explained by their differences in a small number of key traits. Moreover, since not only average differences are important, but also differences in the heterogeneity of traits within groups, we argue that for functional types to be useful, most members of groups should differ from most members of other groups in most traits. We predict that will also be the case in this study.

3 Given the differential responses of these and related species and functional groups to N addition experiments in Minnesota (Tilman, 1988; Wedin & Tilman, 1993; Davis *et al.*, 1999; Reich *et al.*, 2001b) and elsewhere (Muller & Garnier, 1990; Hunt & Cornelissen, 1997; van der Werf *et al.*, 1998), we hypothesize that responsiveness to N will differ, with C_3 grasses, forbs, and oaks showing the greatest stimulation of RGR, in that order, followed by C_4 grasses (weak response) and legumes (weak or no response). Our hypothesis for this latter group involves the idea that these species are likely to be carbon, rather than N, limited. As a corollary, species rankings in RGR should shift substantially between unfertilized soil and the N fertilized treatment (with N-fixers moving downward the most, C_4 grasses moving downward as well, and C_3 grasses and forbs moving up).

Materials and Methods

Species and experimental procedures

The species were chosen to represent common species and functional types growing in outwash sand plain grassland and savanna communities of central North America, specifically those of eastern Minnesota, USA. Thirty-four native or naturalized plant species found at the Cedar Creek Natural History Area (CCNHA), University of Minnesota, East Bethel MN, USA were used in this study. Seeds were purchased from either Prairie Restoration (Princeton, MN, USA) or Prairie Moon (Winona, MN, USA). See Table 1 for the species list, functional type, and initial seed mass.

Table 1 Functional type, species, seed mass, relative growth rate (RGR), root mass fraction (RMF), specific leaf area (SLA), leaf area ratio (LAR), specific root length (SRL) and net assimilation rate (NAR) on mass and area bases for 34 species grown in unamended sandy soil (u) or the same soil fertilized with additional nitrogen (f). SD of RGR among blocks 5–10% of RGR.

Functional type	Species	Seed mass (mg)	N trt	RGR ($g\ g^{-1}\ day^{-1}$)	RMF (g root/g plant)	SLA ($cm^2\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	SRL ($m\ g^{-1}$)	NAR_{area} ($g\ m^{-2}\ d^{-1}$)	NAR_{mass} ($g\ g^{-1}\ d^{-1}$)
Forb	*Achillea millefolium*	0.0529	f	0.151	0.444	324	160	123	9.42	0.34
			u	0.101	0.548	274	105	138	9.63	0.18
	Anemone cylindrica	1.0800	f	0.103	0.333	189	87	135	11.83	0.31
			u	0.093	0.462	212	88	131	10.48	0.20
	Asclepias tuberosa	5.2700	f	0.100	0.445	287	122	35	–	–
			u	–	0.615	290	86	21	–	–
	Aster azureus	0.1710	f	0.190	0.351	–	–	95	–	0.54
			u	0.162	0.500	–	–	86	–	0.32
	Coreopsis palmata	1.4100	f	0.141	0.362	311	117	71	12.05	0.39
			u	0.105	0.463	320	107	117	9.80	0.23
	Liatris aspera	2.4000	f	0.096	0.215	230	174	297	5.53	0.45
			u	0.088	0.213	218	165	598	5.32	0.41
	Monarda fistulosa	0.3300	f	0.144	0.420	441	192	84	7.51	0.34
			u	0.071	0.572	428	135	61	5.25	0.12
	Rudbeckia hirta	0.2370	f	0.140	0.466	302	130	142	10.77	0.30
			u	0.104	0.478	325	166	279	6.23	0.22
	Solidago namoralis	0.0620	f	0.160	0.189	303	221	180	7.24	0.85
			u	0.130	0.254	253	149	376	8.69	0.51
C3 grass	*Agropyron repens*	8.8380	f	0.094	0.362	357	103	106	9.13	0.26
			u	0.086	0.549	384	93	38	9.26	0.16
	Agropyron smithii	0.4172	f	0.104	0.346	232	72	55	14.39	0.30
			u	0.093	0.381	189	66	92	14.25	0.25
	Bromus inermis	2.8010	f	0.098	0.583	262	76	55	13.06	0.17
			u	0.083	0.722	299	58	72	14.20	0.12
	Calamagrostis canadensis	0.0833	f	0.139	0.375	446	154	52	9.04	0.37
			u	0.137	0.484	336	121	95	11.28	0.28
	Elymus canadensis	5.4600	f	0.106	0.435	307	107	31	9.97	0.24
			u	0.097	0.620	350	71	72	10.80	0.12
	Koeleria cristata	0.0900	f	0.151	0.351	378	191	108	7.81	0.43
			u	0.123	0.507	226	96	52	12.87	0.24
	Leersia oryzoides	1.7400	f	0.120	0.360	391	127	43	9.47	0.33
			u	0.091	0.493	352	94	101	9.64	0.19
	Stipa comata	3.9320	f	0.082	0.399	184	88	49	9.40	0.21
			u	0.080	0.550	155	47	47	16.99	0.15

Table 1 *Continued*

Functional type	Species	Seed mass (mg)	N trt	RGR ($g\ g^{-1}\ day^{-1}$)	RMF (g root/g plant)	SLA ($cm^2\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	SRL ($m\ g^{-1}$)	NAR_{area} ($g\ m^{-2}\ d^{-1}$)	NAR_{mass} ($g\ g^{-1}\ d^{-1}$)
Legume	*Amorpha canescens*	2.2600	f	0.086	0.294	377	217	34	–	–
			u	–	0.366	–	–	56	–	–
	Astragalus canadensis	1.6000	f	0.073	0.230	343	203	39	3.58	0.32
			u	0.062	0.312	251	129	39	4.81	0.20
	Baptisia leucantha	14.8000	f	0.072	0.331	243	133	76	5.38	0.22
			u	0.061	0.348	298	157	68	3.88	0.18
	Lespedeza capitata	2.4200	f	0.044	0.216	416	232	115	1.89	0.20
			u	0.048	0.241	318	202	206	2.37	0.20
	Lupinus perennis	24.6000	f	0.102	0.422	233	107	38	9.53	0.24
			u	0.124	0.500	232	97	45	12.83	0.25
	Petalostemum candidum	1.4800	f	0.084	0.267	239	114	26	7.35	0.31
			u	0.100	0.378	238	108	82	9.24	0.27
	Vicia villosa	24.2000	f	0.081	0.459	448	138	108	5.86	0.18
			u	0.082	0.486	444	135	113	6.10	0.17
C4 grass	*Andropogon gerardi*	2.8900	f	0.114	0.433	268	89	71	12.83	0.26
			u	0.079	0.611	287	76	103	10.41	0.13
	Bouteloua curtipendula	4.1800	f	0.136	0.367	202	83	50	16.30	0.37
			u	0.105	0.524	223	76	146	13.93	0.20
	Bouteloua gracilis	0.4210	f	0.120	0.236	190	113	87	10.69	0.51
			u	0.119	0.332	204	85	78	13.94	0.36
	Buchloe dactyloides	16.6930	f	0.098	0.457	318	134	73	7.30	0.21
			u	0.111	0.394	221	97	148	11.37	0.28
	Panicum virgatum	0.8950	f	0.096	0.330	461	203	103	4.73	0.29
			u	0.149	0.492	352	129	150	11.56	0.30
	Schizachyrium scoparium	1.8000	f	0.119	0.290	312	142	45	8.39	0.41
			u	0.085	0.435	394	152	46	5.59	0.20
	Sorghastrum nutans	2.2840	f	0.098	0.385	326	123	71	7.97	0.26
			u	0.101	0.534	262	75	122	13.39	0.19
	Sporobolus cryptandrus	0.0867	f	0.124	0.257	312	138	126	9.02	0.48
			u	0.114	0.389	284	127	100	8.98	0.29
Oaks	*Quercus ellipsoidalis*	1960	f	0.031	0.363	179	82	–	3.75	0.09
			u	0.022	0.461	200	45	–	4.87	0.05
	Quercus macrocarpa	3080	f	0.026	0.429	209	67	–	3.93	0.06
			u	0.028	0.451	210	55	–	5.11	0.06

Growth conditions

Soil was collected from a secondary successional postagricultural field at CCNHA. The soil is classified as Nymore series (Grigal *et al.*, 1974), which is an excessively drained, infertile, medium texture sand (94% sand, 5% silt and 1% clay, pH_{water} of 6.6; in the 0–23 cm horizon). Sowing rates depended on previously determined germination rates, and 4–10 seeds were sown per pot into 6.25 cm diameter × 25 cm containers (D-40 'deepot' containers, Stuewe and Sons, Inc. Corvallis, OR, USA). Acorns of the two oak species were pregerminated before planting. The plants were grown in a temperature controlled glasshouse at the University of Minnesota, St. Paul. Day/night temperatures averaged 25/20°C for the duration of the experiment. Seedlings began to emerge 5–33 d after planting. Germination dates were recorded for each seedling. Seedlings were eventually thinned to one per pot, for a total of 20 plants of each species per treatment – fertilized and unfertilized. Beginning 2 wk after sowing, fertilizer treated pots received 30 ml of half strength Hoagland's solution three times per week. Unfertilized pots received 30 ml water at the same intervals. All pots were watered as needed between treatment applications to maintain soils near field capacity. Supplemental lighting provided an additional 130–170 μmol m^{-2} s^{-1} above ambient light levels during a 14-h photoperiod, for the duration of the study 2 wk after sowing. Daily maximum light levels (on sunny days) at plant height ranged from 1000 to 1200 μmol m^{-2} s^{-1}, and overall, plants received roughly 16–20 mol m^{-2} d^{-1} of light during the course of the study.

Experimental design and growth measurements

The pots fit into 20-cell sleeves, and following germination, each 34 pot group (two sleeves kept together) consisted of a single individual of every species, either all fertilized or all unfertilized for ease of treatment application. The 40 34-pot groups (20 per N treatment) were randomly distributed on the same glasshouse bench, but then assigned into three blocks (each with 6–7 groups) by position. The pots were randomly rearranged within sleeves and the blocks moved approximately weekly throughout the study. Three staggered harvests of roughly equal plant numbers (or groups) per block were done at approximately 3, 6, and 9 wk after germination. At each harvest, plants were carefully washed from their pots keeping all roots and shoots intact as much as possible. Plants were separated into roots and shoot prior to image analysis. Root length and areas and leaf areas were obtained with an AgVision instrument (Decagon Devices, Inc., Pullman, WA, USA), so specific leaf area (SLA) and specific root length (SLR) could be calculated. Dry weights (70C in a forced air oven) were also determined. At the first harvest, most plants were extremely small so no photosynthesis or respiration data and few data on SLA and LAR were collected, and limited plant partitioning data were obtained.

Gas exchange rates and tissue N and carbon concentrations

Photosynthesis, respiration, SLA and partitioning data were collected on almost all species at the second and third harvests. For consistency in comparison, for these traits we use data averaged from these latter two harvests in this paper. Photosynthesis and respiration measurements were made for plants in growth chambers (Conviron E15, Controlled Environments, Inc., Winnipeg, Manitoba, Canada) at standard temperature (25.1 ± 0.5, SD oC), 65%RH, and CO_2 concentration (378 ± 15 μmol mol^{-1} CO_2). Plants were removed from the glasshouse and placed in a growth chamber the night before photosynthesis measurements were taken. Growth chamber lights came on 1 h before photosynthesis measurements. ADC LCA-2 and LCA-3 portable gas-exchange systems were used to determine instantaneous rates of net photosynthesis on individual leaves and tissue respiration under chamber conditions. Young fully expanded leaves (forbs and oaks) or blade sections (grasses) from the upper canopy of each plant were selected for photosynthesis measurement. Net photosynthesis was measured at approximately 1000 μmol m^{-2} s^{-1} PPFD, similar to the highest PPFD experienced by plants in the glasshouse growth conditions.

For leaf respiration, depending on leaf morphology and size, we measured either single leaves or multiple leaves with attached stems, selecting leaves with the same criteria used in photosynthesis measurements. Measurements were made for plants that had been at least several hours in the dark before being placed in the gas exchange system cuvette. Following gas exchange measurement, we removed the sampled tissue and stored it in a cooler before measuring leaf area (one-sided, projected). Samples were oven dried (65°C) to determine dry mass. Light-saturated photosynthesis rates and leaf respiration rates were calculated on both area and mass bases.

Roots were separated during plant harvest and gently washed with water. The root systems or subsystems were kept moist and placed in the dark in the growth chamber for at least 1 h before measurement in a gas exchange cuvette, typically within 2.5 h of harvest. We determined the net CO_2 efflux of the root tissue using infrared gas analyzers under conditions as described above.

Percent N and carbon were determined on dried, ground leaf and root samples (20 mesh in a Wiley Mill) using a Carlo Erba NA1500 CHN analyzer (Carlo Erba Instruments, Milan, Italy). Percent carbon: N ratios were closely, inversely coupled to %N among species and treatments and hence these data will not be presented.

Growth analyses

Growth analyses were conducted using the classical approach, involving harvest-interval calculations (Evans, 1972),

described as ((ln plant mass) at time 2 – (ln plant mass at time 1))/time interval in days between time 1 and 2. To limit the effect of seed reserves, the RGR was derived across the interval between the first and third harvests, a 6-wk period from the third to ninth week of growth postgermination. Leaf area ratio (LAR), SLA, root mass fraction (RMF), tissue %N and net CO_2 exchange rates were derived from the means of these values for harvests two and three, as available. Additionally, using RGR calculated for the interval between harvests 1 and 3 to calculate RGR provided the best temporal match with the physiological variables measured at harvests 2 and 3. Results differed only in minor ways if RGR from harvest 1–2 was used instead. Net assimilation rate (NAR) on both mass and area bases was calculated for the same six week period as RGR based on the mean RGR and the mean LAR and SMF described above.

We tested for block effects, treatment effects, functional group differences and species differences, and all interactions with ANOVA. Blocking had no significant effect on or interaction with any measured variable and is not mentioned hereafter. The ANOVA model included the following effects: nutrient level, functional group, species nested within functional group, nutrient level × functional group, and nutrient level × species within functional groups. The oaks were omitted from these analyses because of the low number of species included. Multivariate AVONVA (MANOVA) was also performed, with indistinguishable results using either the identity or contrast response design and using both Pillai's Trace and Wilk's Lambda tests. Preplanned comparisons among the functional groups included C_3 vs C_4 photosynthetic pathways for the grasses, nitrogen-fixing forb (legumes) vs nonfixing forbs, and nonfixing C_3 grasses vs forbs. Given the explicit goal of the study to test among functional groups, species (and treatments) were considered the experimental units in correlation and regression of pairs of traits and in principal components analysis (PCA). We also used separate and same slopes analyses to compare whether slopes and intercepts of allometric relations varied by treatment. All analyses were performed using JMP software (JMP 3.2.2 and JMP 4.0, SAS Institute, Cary, NC, USA).

Results

Variation among treatments, species, and functional types in RGR

Forbs, C_4 grasses and C_3 grasses had the highest RGR (but did not differ on average) (Tables 1–3), with lower RGR for legumes and the lowest RGR for oaks. In the ANOVA for RGR, functional groups, species (within functional groups) and N treatments differed significantly ($P < 0.001$), and the interactions terms were significant ($P < 0.05$), indicating that functional groups and species responded differently to N supply. On average, species had higher RGR ($P < 0.001$, +16%) at higher N supply (Tables 1–3). Of the 32 species with complete RGR data, 20 species increased RGR by 10–100% with N fertilization, 4 species decreased RGR by 10% to 35%, and in the remainder RGR differed by < 10% across N treatments. All forbs increased RGR (Table 1). The four species which reduced RGR with N fertilization included two legumes and two C_4 grasses. The forbs increased RGR by 32% on average under high N supply; whereas RGR in the legumes was 5% reduced under high N (Table 2). The other functional groups had intermediate responses, with N-induced increases in RGR of 17%, 16%, and 5%, respectively, for the C_3 grasses, oaks, and C_4 grasses. These responses partially support our hypotheses – legumes were unresponsive to N, and forbs and oaks were positively responsive, and the C_3 grasses were more responsive to N than the C_4 grasses. However, C_3 grasses were not more responsive than forbs or oaks, contradicting our hypothesis. Responsiveness of RGR to N supply was not related to the RGR; that is, neither slow-growing nor fast growing species were more likely to respond with enhanced RGR in response to added N.

Species varied widely in RGR. Forbs had, on average, the smallest seed mass and there were significant inverse relationships between RGR and seed mass (data not shown). Rankings of RGR among all species were modestly consistent with mean functional group differences. The two oaks had lowest RGR of all species. Among the herbaceous species, under unfertilized and fertilized conditions, respectively, the four and five slowest growing species were legumes. In fertilized plants, forb species represented five of the six fastest growing. However, among the 7–9 species within each herbaceous functional group, there were very large differences in overall RGR rankings; for example, in the unfertilized treatment (of 30 herbs), rankings ranged from 1st to 27th for forbs, 2nd to 25th for C_4 grasses, 3rd to 26th for C_3 grasses, and 5th to 30th for legumes.

Across N treatments, shifts in the RGR rankings of the 34 species also reflected both functional group differences and intragroup heterogeneity. Several forb species increased their RGR ranking markedly from unfertilized to fertilized N treatment; moving from 27th to 5th, 13th to 4th, and 11th to 6th position, respectively. However, the other forb species, which varied widely in growth ranking (from 1st to 19th when unfertilized), showed little shift in relative ranking across N treatments. For both C_3 and C_4 grass groups, individual species moved up, moved down, or had little shift in rankings comparing fertilized to unfertilized treatments, with no overall pattern.

Biomass distribution and tissue morphology

In every functional type, species on average had a greater fraction of their biomass in roots ($P < 0.0001$) in the low than the high N supply treatment (Tables 2,3) and there were no significant differences (interaction term was not significant)

Table 2 Species means (and standard error) averaged by functional groups and N treatment, for relative growth rate (RGR), root mass fraction (RMF), specific leaf area (SLA), leaf area ratio (LAR), specific root length (SRL), and net assimilation rate (NAR) on mass and area bases. For each variable, data used only for species with data in both N supply treatments

Functional group	N trt	RGR ($g\ g^{-1}\ d^{-1}$)	RMF	SLA ($cm^2\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	SRL ($m\ g^{-1}$)	NAR_{mass} ($g\ g^{-1}\ d^{-1}$)	NAR_{area} ($g\ m^{-2}\ d^{-1}$)
C_3 grass	f	0.132 ± 0.008	0.40 ± 0.028	320 ± 31	115 ± 15	62 ± 10	0.289 ± 0.031	10.29 ± 0.78
	u	0.125 ± 0.009	0.54 ± 0.036	287 ± 30	81 ± 9	71 ± 8	0.187 ± 0.022	12.41 ± 0.94
C_4 grass	f	0.113 ± 0.005	0.34 ± 0.028	299 ± 30	128 ± 13	78 ± 9	0.349 ± 0.039	9.65 ± 1.27
	u	0.108 ± 0.007	0.46 ± 0.033	278 ± 24	102 ± 11	112 ± 13	0.243 ± 0.027	11.15 ± 1.01
Forb	f	0.141 ± 0.011	0.36 ± 0.033	298 ± 26	150 ± 16	129 ± 25	0.439 ± 0.065	9.19 ± 0.95
	u	0.107 ± 0.010	0.46 ± 0.046	290 ± 25	125 ± 12	201 ± 62	0.275 ± 0.046	7.92 ± 0.85
Legume	f	0.076 ± 0.008	0.32 ± 0.035	329 ± 34	155 ± 20	62 ± 14	0.244 ± 0.024	5.60 ± 1.10
	u	0.079 ± 0.011	0.38 ± 0.035	297 ± 33	138 ± 15	87 ± 22	0.209 ± 0.016	6.54 ± 1.58
Oaks	f	0.029 ± 0.002	0.40 ± 0.033	194 ± 15	75 ± 8		0.073 ± 0.012	3.84 ± 0.09
	u	0.025 ± 0.003	0.46 ± 0.005	205 ± 5	50 ± 5		0.055 ± 0.008	4.99 ± 0.12

Table 3 Summary of the results of analyses of variance. These were conducted without the oaks, due to their low number

		P-values				
Variable	Whole model R^2	Nutrient level	Functional groups	Species within functional groups	Nutrient level – functional groups	Nutrient level – species within functional groups
RGR	0.70	0.0003	< 0.0001	< 0.0001	< 0.0001	0.03
RMF	0.77	< 0.0001	< 0.0001	< 0.0001	NS	NS
SLA	0.38	0.10	NS	0.05	NS	NS
LAR	0.33	0.009	0.01	0.04	NS	NS
SRL	0.32	0.08	0.04	0.03	NS	NS
NAR_{area}	0.38	NS	< 0.0001	0.02	NS	NS
NAR_{mass}	0.38	0.0005	0.05	0.01	NS	NS
Root %N	0.67	< 0.0001	< 0.0001	0.01	NS	NS
Leaf %N	0.81	< 0.0001	< 0.0001	0.004	NS	NS
Root respiration	0.33	0.02	0.03	0.004	NS	NS
Leaf respiration	0.42	< 0.0001	< 0.0001	0.002	NS	NS
A_{mass}	0.53	0.001	< 0.0001	< 0.0001	< 0.0001	NS
A_{area}	0.63	0.0002	< 0.0001	< 0.0001	< 0.0001	NS

NS, not significant.

among functional types in the degree of responsiveness. Functional types differed in fractional distribution of biomass to roots ($P < 0.0001$), in the following ranking from high to low: C_3 grasses > oaks > C_4 grasses ≈ forbs > legumes.

An alternative and perhaps better way of examining these patterns (Evans, 1972; Farrar & Gunn, 1998; McConnaughay & Coleman, 1999) involves examining the allometric relationship between root mass and plant mass over the range of plant mass (using all harvested seedlings at all times during the study). Using this allometric approach, the differences between N treatments are still extant (Fig. 1). The rate of increase in root biomass with increasing plant mass (i.e. the slope) did not vary ($P > 0.10$) with N supply in any functional group, but the intercept was always slightly lower for plants grown at higher N supply ($P < 0.05$). Given similar slopes among treatments, the intercept difference is a test of the difference in position or elevation of the entire line. Plants, very early during their life, apparently allocated more to roots under low N conditions. Thereafter, relative allocation to roots vs leaves did not differ with N treatment, leading to a consistently greater RMF under low N, despite no tendency towards enhanced allocation to roots during the latter stages of the study. Thus, in all functional groups, for plants of comparable size, those growing at higher N had greater fractional distribution of biomass in roots, despite no difference in the observed allometric coefficient.

The slopes of these allometric relationships differed significantly ($P < 0.05$) among functional types, such that with increasing plant mass, the slope was steepest in oaks followed by legumes > forbs > C_3 grasses > C_4 grasses, that is, oaks

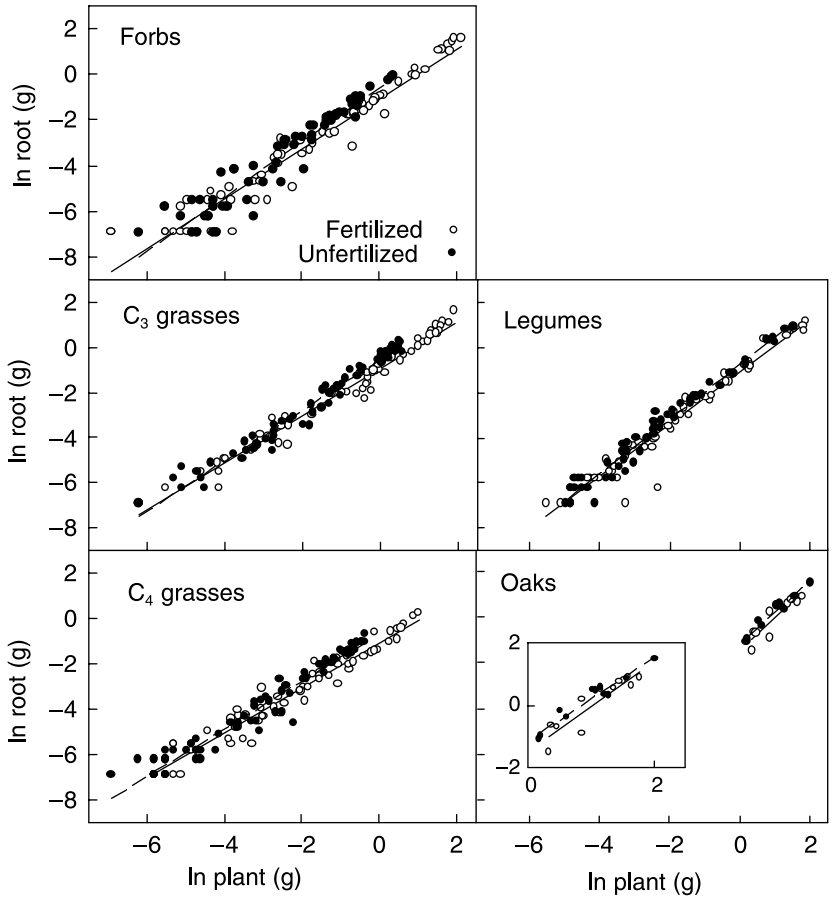

Fig. 1 Relationship between root mass and whole plant mass for species from five functional groups. In all cases, there was no significant difference in slope between control (closed circles) and fertilized (open circles) treatments ($P > 0.10$) and the intercepts differed significantly ($P < 0.05$). There were significantly different slopes among the functional groups for both unfertilized ($P < 0.08$ for all five groups, $P < 0.05$ for the four herbaceous groups) and fertilized treatments ($P < 0.01$ for all five groups, $P < 0.02$ for the four herbaceous groups) considered separately. For improved clarity, the data for oaks are shown in expanded axes in the inset.

became increasingly more rooty with increasing plant size at a rate greater than for all other functional groups, with C_4 grasses showing the least such tendency. However, C_4 grasses had proportionally higher root mass than other groups for very small plants.

Variation among functional groups in SRL did not follow the same, or the opposite pattern, as for RMF, ranking from high to low: forbs > C_4 grasses > legumes > C_3 grasses (Table 2). There were no significant differences among herbaceous functional types in SLA (Table 3), although all had greater SLA than the oaks (Table 2). Leaf area ratio (LAR) differences existed ($P < 0.01$) among groups and followed the reverse ranking of RMF. Fertilized herbaceous plants, regardless of functional type, had slightly higher SLA, lower SRL, lower RMF, and higher LAR and NARmass than unfertilized plants (Tables 2,3). For all of these variables, all functional types responded similarly to N supply (no significant interaction). Functional types differed in NARmass, in a ranking similar to that for RGR: Forbs > C_3 grasses > C_4 grasses > legumes.

Photosynthesis, respiration and tissue nitrogen

Rates of leaf level gas exchange varied widely ($P < 0.05$) among species, treatments and functional types (Tables 3–5), and there were significant differences ($P < 0.0001$) among groups in response of net photosynthesis to N treatment. Except for legumes, plants of all other functional types had significantly greater (by 35–55%) rates of net photosynthesis when fertilized (Table 5). Legumes, however, had 24% lower rates of photosynthesis on average, in fertilized treatments. These responses were consistent within functional groups: none of the legume species had significantly higher photosynthetic rates when fertilized. By contrast, in all other functional groups, most species had higher photosynthetic rates when fertilized. Leaf and root respiration rates were greater ($P < 0.02$) in the high N treatment and all functional groups showed this pattern (i.e. no significant interaction term). However, the proportional increase in root and leaf respiration rates with increased N treatment was less in legumes (3% and 20%, respectively) than in species in all other groups (which increased both by roughly 30% on average).

In unfertilized plants, rates of photosynthesis were higher in C_4 grasses and legumes than in the other functional types, with oaks having the lowest rates (Table 5). In fertilized plants, the ranking of legumes vs all other groups declined, since photosynthesis declined with fertilization in legumes but increased in all other groups. Rates of leaf respiration followed a similar ranking, being greatest in C_4 grasses and

Table 4 Functional type, species, shoot and root respiration (measured at 25°C), shoot %N, root %N, net photosynthesis (A) based on mass (A_{mass}), and on area (A_{area}), for 34 species grown in unamended sandy soil (u) or the same soil fertilized with additional nitrogen (f)

Functional type	Species	N trt	Respiration (nmol g^{-1} s^{-1})		Shoot %N	Root %N	A_{mass} (nmol g^{-1} s^{-1})	A_{area} (μmol m^{-2} s^{-1})
			Shoot	Root				
Forb	*Achillea millefolium*	f	30.3	32.4	4.80	2.61	323	10.4
		u	19.1	25.1	2.63	1.83	262	9.4
	Anemone cylindrica	f	26.1	53.0	3.83	3.45	217	11.5
		u	27.8	39.7	2.92	1.80	147	6.8
	Asclepias tuberosa	f	30.9	28.9	4.35	2.92	366	12.8
		u	22.4	22.9	4.53	1.58	332	11.4
	Aster azureus	f	33.1	49.1	5.19	3.51	–	–
		u	21.9	32.2	3.81	2.45	–	–
	Coreopsis palmata	f	37.0	73.4	3.82	3.14	521	16.3
		u	28.6	32.9	3.23	2.30	434	13.8
	Liatris aspera	f	25.3	79.5	4.68	3.81	378	16.8
		u	20.2	79.5	3.58	3.44	327	14.7
	Monarda fistulosa	f	37.3	35.2	4.47	2.76	607	13.7
		u	25.3	17.3	2.52	1.71	305	6.8
	Rudbeckia hirta	f	31.7	31.1	4.71	2.53	332	10.6
		u	32.5	45.1	2.63	1.80	178	4.8
	Solidago nemoralis	f	35.2	58.9	4.83	4.47	471	15.5
		u	24.1	79.5	3.80	2.53	397	15.3
C_3 grass	*Agropyron repens*	f	29.8	43.6	3.81	2.20	343	9.9
		u	20.3	14.7	2.25	1.73	293	7.7
	Agropyron smithii	f	30.6	23.3	3.33	2.32	440	21.4
		u	23.9	37.9	2.70	2.30	255	13.7
	Bromus inermis	f	31.7	13.2	4.18	1.78	220	8.3
		u	29.5	12.9	2.43	1.43	177	5.8
	Calamagrostis canadensis	f	28.2	32.7	3.22	3.04	344	8.0
		u	36.2	39.5	3.52	1.65	255	7.7
	Elymus canadensis	f	28.4	22.6	4.41	2.05	389	12.3
		u	22.4	21.3	2.18	1.29	235	7.0
	Koeleria cristata	f	41.0	50.6	4.20	2.96	457	13.2
		u	27.2	29.9	2.63	1.48	214	9.2
	Leersia oryzoides	f	62.4	75.3	3.41	2.31	461	11.3
		u	24.7	34.7	1.82	1.64	275	8.3
	Stipa comata	f	24.8	25.3	4.38	2.52	372	22.6
		u	18.9	14.3	4.00	1.79	233	15.4
Legume	*Amorpha canescens*	f	27.2	41.9	3.94	3.34	240	6.3
		u	–	–	4.35	3.04	–	–
	Astragalus canadensis	f	30.2	66.2	5.13	3.81	247	7.7
		u	25.4	33.9	2.39	2.32	282	11.3
	Baptisia leucantha	f	26.5	37.8	4.44	3.68	286	11.5
		u	23.2	35.8	3.57	2.87	295	9.8
	Lespedeza capitata	f	23.4	72.2	4.39	4.16	–	–
		u	25.6	86.6	3.56	3.72	153	4.5
	Lupinus perennis	f	21.7	31.7	3.94	3.70	437	14.9
		u	26.4	34.5	3.70	3.93	593	19.7
	Petalostemum candidum	f	27.2	39.1	4.54	3.46	475	19.8
		u	18.9	33.1	3.56	2.91	481	20.6
	Vicia villosa	f	36.2	24.8	4.16	3.26	214	5.3
		u	28.0	40.5	3.53	2.77	522	12.0
C_4 grass	*Andropogon gerardi*	f	34.5	27.1	3.10	2.40	514	19.1
		u	30.8	24.9	1.56	1.20	362	12.9
	Bouteloua curtipendula	f	38.3	32.8	3.39	3.11	519	25.6
		u	34.1	17.5	2.39	2.24	420	16.4
	Bouteloua gracilis	f	28.9	66.2	3.78	2.82	330	18.3
		u	25.6	46.7	3.48	2.10	340	17.5
	Buchloe dactyloides	f	48.3	35.3	3.89	2.22	587	19.2
		u	37.8	31.8	2.87	1.78	433	21.1

Table 4 *Continued*

Functional type	Species	N trt	Respiration (nmol g^{-1} s^{-1}) Shoot	Root	Shoot %N	Root %N	A_{mass} (nmol g^{-1} s^{-1})	A_{area} (μmol m^{-2} s^{-1})
	Panicum virgatum	f	37.3	53.3	3.66	3.57	822	17.7
		u	29.2	18.1	2.65	1.98	478	12.8
	Schizachyrium scoparium	f	38.2	25.4	2.92	2.67	538	17.6
		u	20.3	27.3	2.27	1.81	371	10.8
	Sorghastrum nutans	f	54.7	32.4	3.27	2.63	862	27.5
		u	26.3	18.1	2.44	1.45	504	19.0
	Sporobolus cryptandrus	f	44.1	59.0	4.10	3.00	909	27.8
		u	37.7	51.9	3.36	2.57	697	23.7
Oaks	*Quercus ellipsoidalis*	f	15.7	12.8	2.59	1.98	123	5.9
		u	11.2	10.8	1.20	0.90	67	3.3
	Quercus macrocarpa	f	13.7	14.2	2.39	1.21	115	5.3
		u	11.0	12.3	1.42	0.90	92	4.4

Table 5 Species means (and standard error) by functional groups and N treatment, for root %N, shoot %N, root and shoot respiration (measured at 25°C), and net photosynthetic rates on mass (A_{mass}), and area bases (A_{area}). For each variable, data used only for species with data in both N supply treatments

Functional group	N trt	Root %N	Shoot %N	Root respiration (nmol g^{-1} s^{-1})	Shoot respiration (nmol g^{-1} s^{-1})	A_{mass} (nmol g^{-1} s^{-1})	A_{area} (μmol m^{-2} s^{-1})
C_3 grass	f	2.40 ± 0.15	3.87 ± 0.17	35.8 ± 7.1	34.6 ± 4.30	378 ± 28	13.4 ± 2.0
	u	1.66 ± 0.11	2.69 ± 0.26	25.6 ± 3.9	25.4 ± 1.96	242 ± 13	9.4 ± 1.2
C_4 grass	f	2.80 ± 0.15	3.51 ± 0.14	41.4 ± 5.5	40.5 ± 2.89	635 ± 73	21.6 ± 1.6
	u	1.89 ± 0.15	2.63 ± 0.22	29.5 ± 4.7	30.2 ± 2.18	451 ± 41	16.8 ± 1.6
Forb	f	3.24 ± 0.21	4.52 ± 0.15	49.0 ± 6.3	31.9 ± 1.46	402 ± 44	13.5 ± 0.9
	u	2.16 ± 0.20	3.29 ± 0.23	41.6 ± 7.7	24.7 ± 1.45	298 ± 35	10.4 ± 1.4
Legume	f	3.63 ± 0.12	4.36 ± 0.16	45.3 ± 6.7	27.5 ± 1.79	332 ± 52	11.8 ± 2.5
	u	3.08 ± 0.21	3.52 ± 0.22	44.1 ± 8.6	22.9 ± 1.98	435 ± 69	14.7 ± 2.3
Oaks	f	1.60 ± 0.39	2.49 ± 0.10	13.5 ± 0.7	14.7 ± 0.98	119 ± 4	5.6 ± 0.3
	u	0.90 ± 0.00	1.31 ± 0.11	11.6 ± 0.7	11.1 ± 0.07	80 ± 13	3.9 ± 0.6

least in oak. However, rates of root respiration did not parallel leaf respiration rankings among herbaceous functional types: the forbs and legumes had slightly higher root respiration than C_4 grasses, with C_3 grasses slightly lower.

Leaf and root %N were significantly different ($P < 0.0001$) among functional groups (Table 3) and, not surprisingly, were greater in fertilized than control treatments (Table 5). All functional groups responded similarly in terms of tissue %N to fertilization (there were no functional group–treatment interactions). On average, %N in roots and leaves was 35–40% greater in the fertilized plants. The ranking among functional types for tissue %N was as follows: legumes > forbs > C_4 grasses C_3 grasses > oaks – in unfertilized plants, whole plant %N averaged 3.35, 2.73, 2.31, 2.13 and 1.11%, respectively, among these groups.

We also conducted MANOVA using the same effects as in the ANOVA. For this MANOVA, we used all 13 variables in Table 3. Nitrogen treatment, functional group, and species within functional groups were all significant ($P < 0.0001$), but there were no differences among groups or species in response to N (i.e. interaction term not significant).

Correlations among plant traits

RGR was generally weakly correlated with most other measured traits, and there were generally modest correlations among these traits as well. RGR was significantly positively related ($P < 0.005$) to leaf respiration rate and both mass- and area-based photosynthetic rates (Fig. 2). These relationships did not differ significantly (in either slope or intercept) for the two N treatments. Thus the relationship between RGR and leaf metabolism was unaffected by N treatment, but N fertilized plants moved towards higher levels along the same general slope. RGR was also significantly positively related to

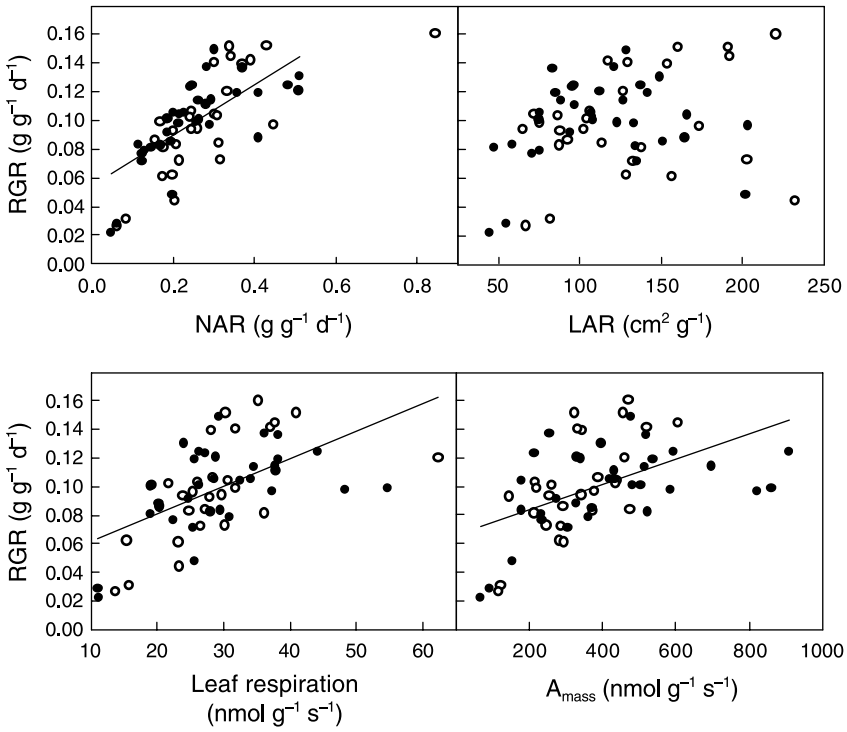

Fig. 2 Relationship between relative growth rates (RGR) and leaf respiration ($r^2 = 0.32$, $P < 0.001$), net photosynthesis (mass basis) ($r^2 = 0.26$, $P < 0.001$), net assimilation rate (NAR) (mass basis) ($r^2 = 0.51$, $P < 0.001$), and leaf area ratio (LAR) (not significant). The relationships did not significantly differ among N treatments (controls are closed circles and fertilized are open circles) and hence one common regression line is shown for each relationship.

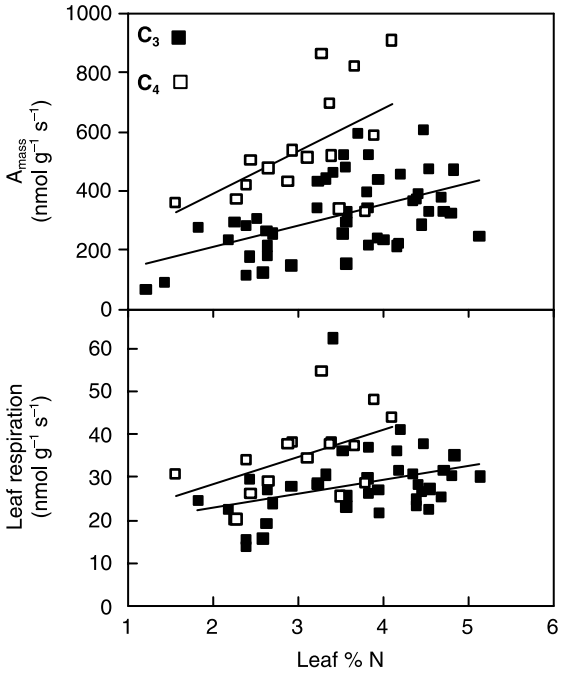

Fig. 3 Relationship between net photosynthesis (mass basis) and leaf %N for all C_3 species pooled (closed squares) ($r^2 = 0.28$, $P < 0.001$) and for the C_4 grasses (open squares) ($r^2 = 0.28$, $P < 0.04$); and between leaf respiration and leaf %N for all C_3 species pooled ($r^2 = 0.33$, $P < 0.001$) and for the C_4 grasses ($r^2 = 0.25$, $P < 0.05$). The relationships did not significantly differ among C_3 groups and hence they are shown pooled. The relationships did not significantly differ among N treatments within these groupings and hence one common regression line is shown for each relationship.

NAR on both mass and area bases, but not related to LAR (Fig. 2).

Leaf respiration rates and net photosynthetic rates were positively correlated with leaf %N (Fig. 3). These relationships were similar for the four groups with C_3 photosynthetic machinery (no differences based on analyses of covariance), but had greater intercepts and steeper slopes for the C_4 grasses. The relationship was unchanged for plants grown in the contrasting N treatments; hence the N fertilized plants generally occupied positions further up the similar relation.

Among all species and treatments, RMF, root %N, and root respiration rate were generally well correlated: species with high biomass fraction in roots had low root %N and low root respiration rates (Fig. 4). The relationships between RMF, root %N and root respiration held across treatments and functional groups, but were not significantly altered by functional types (there were no interactions involving functional types). Thus, these relationships were not a result of functional groups differences (i.e. not due to one functional group having low RMF, high N, and high respiration, and another the reverse set of traits).

An important question is whether these relationships indicate a trade-off between root/shoot distribution on the one hand and tissue %N and respiration on the other, or result from species with high RMF tending to have larger root sizes (which would likely contain a greater fraction of coarser roots with lower tissue %N). Analysis of covariance including root size indicates the former is likely, since the relationships (of both root %N and root respiration to RMF) remained significant

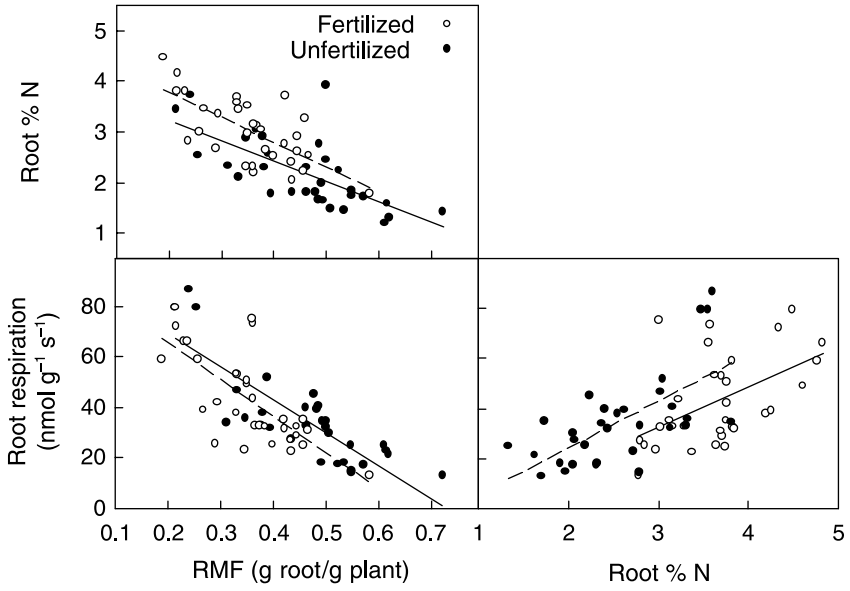

Fig. 4 Relationship between root %N, root respiration and root mass fraction (RMF) for species from all functional groups and treatments. For unfertilized plants, root %N was correlated with RMF ($r^2 = 0.46$, $P < 0.001$), root respiration was correlated with RMF ($r^2 = 0.68$, $P < 0.001$), and root respiration rate (nmol g^{-1} s^{-1}) was correlated with root %N ($r^2 = 0.41$, $P < 0.001$). For fertilized plants, root %N was correlated with RMF ($r^2 = 0.47$, $P < 0.001$), root respiration was correlated with RMF ($r^2 = 0.52$, $P < 0.001$), and root respiration rate (nmol g^{-1} s^{-1}) was correlated with root %N ($r^2 = 0.29$, $P < 0.001$).

even after variation related to root size was removed (it was not a significant covariate for either, in any case).

The significant negative relationship of root respiration to RMF was likely due to the relationship between root %N and RMF, since root respiration was significantly related positively to root %N and it is well known that %N is mechanistically related to respiration (Lambers & Poorter, 1992). In this case, root biomass was a significant covariate (respiration decreases with increasing root mass), but the relationship between respiration and root %N remained highly significant even once the effect of root size was taken into account. Of interest, legumes had lower respiration rates at a given root or leaf %N than other nonwoody species, which were similar in this respect. Fertilized plants also had a lower respiration rate at any given tissue %N than control plants.

Across all treatment and functional groups, RMF, %N, and respiration were all weakly correlated with SRL, but functional groups differed widely in this respect. For forbs, SRL declined with RMF (in both treatments), and both root %N and root respiration were correlated with SRL, again both within and across N treatments. In all other functional groups, there were no relationships among this set of root traits.

PCA was used on all species together, but performed separately on fertilized and control soil treatments (Fig. 5). Thirteen variables (all traits listed in Table 3) were included in the PCA. For both N treatments, the first three axes included 69% to 73% of the variation, and the first four axes 80–82%. In both the fertilized and unfertilized data sets, the species were separated by similar combinations of traits. The largest amount of variation (Axis 1, ≈ 35%) was associated with species that varied in a set of root traits (either high RMF, low

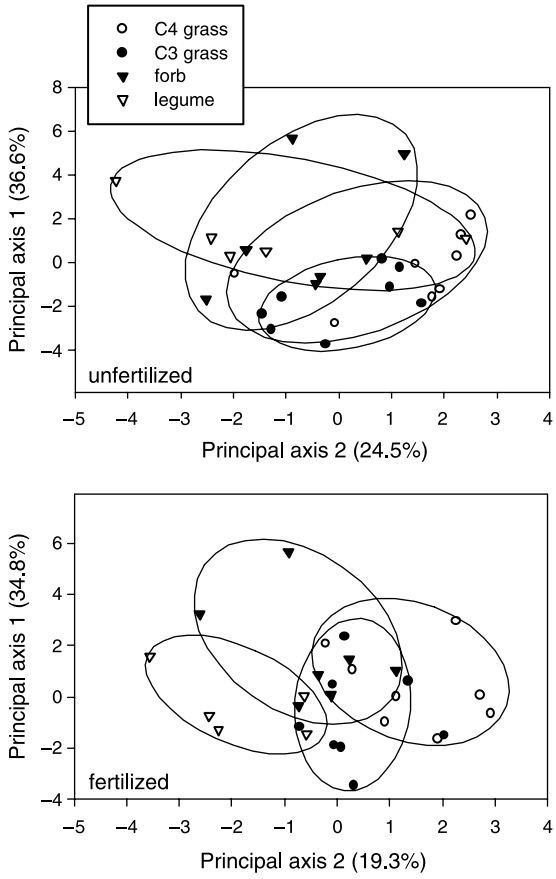

Fig. 5 Principal components analysis of 13 variables (those given in Table 3), performed on all herbaceous species together, but separately on fertilized and control N treatments. Closed circles are C_3 grasses, open circles are C_4 grasses, closed triangles are forbs, and open triangles are legumes.

root %N, low respiration rate and low SRL; or the reverse). The second axis was associated with species with either high rates of net photosynthesis and NAR, or the reverse. Of the third and fourth axes, one pointed towards species variation in RGR and seed size (high RGR and small seeds or the reverse) and the other towards species variation in SLA and leaf respiration. There was substantial spread among species within groups and overlap among all pairs of groups in both PCA analyses, except for legumes and C_4 grasses in the fertilized treatment.

Cluster analysis by herbaceous species had poor results in terms of the segregation of the functional groups (data not shown). If functional group membership did separate species by their collective traits and responses to variation in N supply, one would expect to see cluster groups containing members of the same functional group under a given N treatment. This was not typically the case. Cluster analyses for each N treatment separately also failed to separate species by their classically defined *a priori* group membership.

Discussion

The results of this study suggest that functional types based on important biological features of sympatric herbaceous plants, such as photosynthetic pathway or N-fixing capacity, serve modestly well, at best, to distinguish groups from a holistic perspective of a constellation of seedling traits. This is consistent with field studies of 5-yr-old assemblages based on a related but different set of traits (Craine *et al.*, 2002). For many specific traits (e.g. RMF, photosynthetic capacity, %N) and for all traits using MANOVA, the functional types did differ relatively consistently from each other, but functional groups could not be clearly differentiated by their broader sets of traits using PCA or cluster analysis.

Moreover, although on average, functional types did differ in mean RGR and other traits, there was large variance in each trait among species within each group. Thus, we partially reject the first part of the first hypothesis – that functional types differ consistently in seedling RGR. Even for contrasts that were observed among groups (e.g. the lower RGR of legumes than forbs), the second hypothesis was rejected – differences in mean RGR among functional groups could not be ascribed to simple and consistent differences in any single trait or set of traits. By contrast to the failure of functional typing to distinguish among herbaceous species, the two woody species had clearly much lower RGR, which was likely in part due to the combination of low LAR and low photosynthetic rates. Although many other woody angiosperm species from the nearby woodlands of central North America have higher RGR on average than oaks (Walters *et al.*, 1993; Reich *et al.*, 1998a), these woody species as a group still tend to have lower RGR than herbaceous species, consistent with data from Hunt & Cornelissen (1997).

Our hypotheses about the linkage between traits (such as SLA, %N, and net photosynthetic rates) and RGR (Hypothesis 2) were also weakly supported, with much stronger linkage of metabolic rate (photosynthesis, NAR, leaf respiration) with RGR than LAR with RGR. This contrasts with prior studies showing strong RGR-LAR relationships under both so-called 'optimum' conditions and low resource supply conditions (Poorter *et al.*, 1990; Garnier, 1992; Cornelissen *et al.*, 1996; Reich *et al.*, 1998a). Net CO_2 exchange rates of both leaves and roots were related to the tissue %N, and these relationships were affected by N treatment for roots but not for leaves. As seen previously (Field & Mooney, 1986), the relationship of photosynthesis to N differed for C_4 and C_3 species, and our study provides evidence for a similar contrast of leaf respiration-N relations. Moreover, regardless of functional type, species with high fractional distribution of biomass in roots also had low SRL, %root N and root respiration rate.

The functional types varied in their responses of RGR to variation in N supply (supporting Hypothesis 3 in part). However, our patterns vary from previous work. Muller & Garnier (1990) and Hunt & Cornelissen (1997) found that faster growing species showed large downward shifts in rank when grown at lower nutrient supply rates, but that was not seen here. Van der Werf *et al.* (1998) reported that in chamber studies with European herbs, RGR in monocots was mainly associated with variation in SLA and LAR, whereas in dicots it was mainly associated with variation in NAR. We did not find this pattern in our study-in both groups RGR had positive, linear and similar relations with NAR (mass basis) and no relationship whatsoever with SLA or LAR. Perhaps different patterns were observed in different studies because of differences in growth conditions. Reich *et al.* (2001b) found that that forbs and legumes in the field had a much less positive response to N fertilization compared to both C_3 and C_4 grasses, but in the present study this was true for legumes but false for forbs. In the present study, the legumes showed several similar morphological and chemical responses to high N supply, such as a lower RMF, higher SLA and LAR, as all other functional groups (see Table 3). However, the legumes showed little increase in root respiration with higher N (all other functional types did) and had lower photosynthetic rates, rather than higher, at high N treatment.

In this study, seedlings of herbaceous functional types differ in the factors contributing to LAR in a different fashion than seedlings of differing woody functional types common to central North America or to European herbaceous species (Poorter *et al.*, 1990). For North American tree seedlings or European herbs, by far the greatest contribution to variation in LAR was from differences in SLA, with RMF varying minimally. By contrast, among the herbaceous functional types in this study, variation in RMF, but not SLA, was the key driver of variation in LAR. Herbaceous species also differed from tree species in the relationships between leaf and root traits. For trees, species with high SRL had high SLA, and those with high leaf respiration had high root respiration, i.e. there was parallel variation among species in both root and leaf traits

(Reich *et al.*, 1998a; Wright & Westoby, 1999). This was not the case for the herbs in this study: there was little correspondence in ranking of root vs leaf traits.

In conclusion, when examining by PCA or cluster analysis the full set of traits measured in this study, *a priori* trait-based functional groupings did not effectively discriminate, segregate or sort among herbaceous species. Instead, species could be grouped more effectively by trait combinations including: a set of root traits involving morphology, tissue chemistry and metabolism (as also seen by Craine *et al.*, 2002); a set involving growth rate and seed size; a set involving photosynthetic rates and NAR; and a set involving SLA and leaf respiration. Additionally, for only three of the 13 variables, did functional groups respond differently to N treatments (Table 3). However, in ANOVA for each response variable, and in MANOVA for all variables combined, functional groups were always significantly different than each other. Therefore, the 'glass' is either half full or half empty depending on one's prospective. Clearly, trait-based groupings do provide meaningful information about every growth, morphometric, metabolic and chemical attribute measured in this study. Differences were pronounced enough to be considered representative of the central tendencies of groups, but likely not pronounced enough to be of major predictive value.

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