Linking leaf and root trait syndromes among 39 grassland and savannah species

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\section*{Summary}

- Here, we tested hypothesized relationships among leaf and fine root traits of grass, forb, legume, and woody plant species of a savannah community.
- \(	ext{CO}_2\) exchange rates, structural traits, chemistry, and longevity were measured in tissues of 39 species grown in long-term monocultures.
- Across species, respiration rates of leaves and fine roots exhibited a common regression relationship with tissue nitrogen (N) concentration, although legumes had lower rates at comparable N concentrations. Respiration rates and N concentration declined with increasing longevity of leaves and roots. Species rankings of leaf and fine-root N and longevity were correlated, but not specific leaf area and specific root length. The C\textsubscript{3} and C\textsubscript{4} grasses had lower N concentrations than forbs and legumes, but higher photosynthesis rates across a similar range of leaf N.
- Despite contrasting photosynthetic pathways and \textsubscript{N}2-fixing ability among these species, concordance in above- and below-ground traits was evident in comparable rankings in leaf and root longevity, N and respiration rates, which is evidence of a common leaf and root trait syndrome linking traits to effects on plant and ecosystem processes.

\textbf{Key words:} functional groups, leaf lifespan, nitrogen-use efficiency, photosynthesis, respiration, root turnover, specific leaf area, specific root length.


\section*{Introduction}

Despite considerable variation in leaf traits among plant species, common patterns of trait covariation are observed across diverse species and biomes (Diemer \textit{et al.}, 1992; Reich \textit{et al.}, 1997, 1998a, 1999; Niinemets, 1999; Craine & Lee, 2003; Wright \textit{et al.}, 2004). High specific rates of \textsubscript{CO}_2 exchange are associated with high leaf nitrogen (N) concentrations, high specific leaf area (SLA, leaf area per unit leaf dry mass) and short leaf lifespan. Consistent correlations among leaf traits are thought to reflect fundamental trade-offs in leaf morphology, metabolic rates, and longevity and may be useful in categorizing species based on their ecological attributes and defining the linkages of traits to species effects on ecosystem functioning (Grime \textit{et al.}, 1997; Lavorel & Garnier, 2002; Eviner & Chapin, 2003; Diaz \textit{et al.}, 2004).

Compared with leaf traits, far less is known about interspecific variation in root traits or their correspondence with above-ground trait syndromes (Eissenstat & Yanai, 1997; Eissenstat \textit{et al.}, 2000; Bouma \textit{et al.}, 2001; Craine & Lee, 2003). Are there consistent correlations of leaf and fine root structure, function and longevity among species? For example, as in leaves, are high specific respiration rates in fine roots correlated with high N, high specific root length (SRL, root length per unit root dry mass) and short lifespan? There is evidence that fine roots of species differ widely in SRL and N concentration (Comas \textit{et al.}, 2002; Pregitzer \textit{et al.}, 2002) and that specific respiration rates of fine roots increase with higher
N concentration and SRL (Pregitzer et al., 1998; Reich et al., 1998b; Comas et al., 2002), likely reflecting metabolic activity associated with nutrient uptake and assimilation, although species may differ in their specific costs of ion uptake (Scheurwater et al., 1998). In some studies, root longevity among species is negatively correlated with N concentration, specific respiration rates, and SRL (Eissenstat et al., 2000) or root tissue density (Ryser, 1996; Craine et al., 2001), a pattern analogous to leaf trait correlations. Nitrogen concentration and tissue density of leaves were correlated with those of fine roots among 24 grass species sampled along an altitudinal transect (Craine & Lee, 2003). The correspondence of leaf and root traits raises the possibility that root traits may, in part, be predicted from more readily observed leaf traits. However, the generality of leaf and root trait syndromes, particularly in relation to physiological function remains largely untested.

The evidence to date suggests that a priori plant functional groups based on taxonomy (e.g. monocot, dicot) or functional categories (N₂-fixing, photosynthetic pathway) may be useful in categorizing species traits and their effects on ecosystem processes (Aerts & Chapin, 2000; Lavello & Garnier, 2002). However, species effects on ecosystem function often arise from multiple, often continuously distributed traits and their potentially additive or interactive effects (Eviner & Chapin, 2003). Elucidating structural (tissue morphology and chemistry) and functional trait correlations (e.g. CO₂ exchange) and their correspondence in leaves and roots will aid in identifying underlying mechanisms and scaling relationships that link traits to plant and ecosystem function. Whether or not trait correlations differ among a priori plant functional groups in predictable ways is not well understood. For example, among grass species, high SLA, low tissue density leaves and corresponding low tissue density roots is associated with faster plant relative growth rates (van der Werf et al., 1993; Ryser & Lambers, 1995; Wahl & Ryser, 2000), whereas among tree species, a faster growth rate is associated with higher SRL and smaller root diameters and not root tissue density (Comas et al., 2002; Comas & Eissenstat, 2004). This suggests that grasses and trees may, in part, differ in trait correlations. In addition, woody and herbaceous plant growth forms may differ in traits underpinning leaf and plant-level nutrient-use efficiency (Aerts & Chapin, 2000).

The temperate grassland–savannah ecotone in the northern great plains of North America offers a diverse array of taxa with which to examine hypothesized trait correlations for leaves and fine roots in terms of morphology, longevity and rates of CO₂ exchange. Do species with leaves exhibiting high rates of CO₂ exchange and associated high SLA, N contents and short lifespan also exhibit a parallel syndrome of traits in fine roots? Do trait correlations differ among a priori functional groups (i.e. C₄ vs C₃, photosynthetic pathway)? Linking leaf and root trait relationships among species and functional groups may aid in predicting trait combinations and their ecological effects, including composition and productivity at regional scales (Paruelo et al., 1998; Lavello & Garnier, 2002), as well as species response to global change factors (Reich et al., 2001b; Lee et al., 2001; Poorter & Navas, 2003).

We tested several predictions of correlated leaf and fine root trait relationships among species and functional groups as follows: (1) rates of net photosynthesis and respiration of leaves and fine roots are correlated with tissue N (Ryan, 1991; Reich et al., 1997, 1998a, 1999; Tjoelker et al., 1999); (2) leaf and fine root N concentration increases with increasing SLA and SRL and N concentrations decrease with increasing tissue longevity; and (3) species exhibit similar rankings in leaf and fine root traits, namely, N concentration, SLA and SRL, longevity and specific respiration rates. Given hypothesized functional group contrasts, to differing photosynthetic pathways, we tested the predictions that: (4) C₄ vs C₃ groups differed in bivariate leaf trait-N relationships, reflecting expected higher leaf-level water- and photosynthetic N-use efficiencies for C₄ than for C₃ species (Sage & Pearcy, 1987; Ehleringer & Monson, 1993); and (5) that owing to higher N concentrations in legumes, leaf and root traits correlated with increased tissue N concentrations are higher in legumes compared with non-N₂-fixing forbs and grasses.

Materials and Methods

Site

The study site is at the Cedar Creek Natural History Area, a US National Science Foundation long-term ecological research site near Bethel, in east central Minnesota, USA (http://www.lettersmn.edu). The uplands at this site are
dominated by oak savannah, prairie, hardwood forest, pine forests and abandoned agricultural fields. The soils are entisols derived from a glacial outwash sand plain and are sandy and N-poor ( Tilman, 1988 ). The region has a continental climate with cold winters, hot summers (mean January and July temperatures of −10 °C and 25 °C, respectively), and a mean annual precipitation of 660 mm.

The 39 species we examined in this long-term monoculture study include grasses, forbs, and woody plants common to remnant native oak savannah–prairie grasslands in the Midwestern USA. Species binomials are reported in the Appendices. Names and authorities follow those of Moore (1973) and B. C. Delaney (University of Minnesota, unpublished; http://www.lter.umn.edu). In the autumn of 1992, an old field was prepared by removing the topsoil, creating a sandy substrate (93%) low in organic matter. Replicated monoculture plots of the species were seeded in a fenced area and maintained by annual weeding. Plots were 2.2 × 1.5 m for most species, 1.1 × 1.5 m for the others. Sheet metal barriers that extended 25 cm below the ground separated the plots. We watered the plots weekly during the growing season in 1997 in the 5-yr-old plots as needed to ensure an equivalent of at least 2.5 cm of weekly precipitation to minimize water stress. Further details are provided in Craine et al. (2002).

Leaf gas exchange

We determined light-saturated rates of leaf gas exchange of 36 species in the field sampled across four dates (25 June, and 7, 21 and 28 August, 1997). A portable photosynthesis system (CIRAS-1; PP Systems, Hitchin, UK) measured rates of net CO₂ and water vapor exchange. We conducted measurements on clear sunny days at light-saturating conditions between 10:30 and 14:00 hours Central Daylight Time (CDT). Mean photosynthetic photon flux density (PPFD, 400–700 nm) ranged from 1440 to 1560 µmol m⁻² s⁻¹ and mean air temperatures ranged from 26.3 to 29.3 °C on the four dates. We measured two to four mature leaves from the top of the canopy in each of two to four replicate plots for a species. Following gas exchange measurement, we removed the sampled leaf and stored it in a cooler before measuring leaf area (one-sided, projected). Samples were oven dried at 65 °C before determining dry mass. We calculated light-saturated photosynthesis rates on the basis of leaf area (A area, µmol m⁻² s⁻¹) and dry mass (A mass, nmol g⁻¹ s⁻¹).

Leaf respiration

We harvested several intact shoot samples (two or three) from individual plots on the mornings (between 08:30 and 11:00 hours CDT) of 17 and 18 June, placed the samples in plastic bags in a cooler (c. 11 °C), and transported them to the laboratory. Samples were immediately transferred to a controlled-environment chamber (Convivon E15; Controlled Environments, Inc., Winnipeg, Manitoba, Canada) to measure dark respiration at a standard temperature (26.1 ± 0.6 °C SD) and CO₂ concentration (381 ± 15 µmol mol⁻¹ CO₂). We determined rates of net CO₂ efflux using infrared gas analysers and cuvettes (LCA-3 and PLC-C; Analytical Development Co. Ltd, Hoddesdon, UK), operated in an open configuration. Columns of magnesium perchlorate removed water vapor from the analyser air stream. Depending on leaf morphology and size, we measured either single leaves or multiple leaves with attached stems to provide adequate differential measurements, one per replicate plot. We dried the samples in an oven (65 °C) and determined dry masses for calculation of respiration rates based on leaf mass (R mass, nmol g⁻¹ s⁻¹) or area (R area, µmol m⁻² s⁻¹).

Fine root respiration

We collected aggregate root samples from soil cores (5 cm diameter, 20 cm deep) for each of up to four plots per species (minimum of two plots for three species) of 36 species on 18, 20, and 21 August. Roots were washed from soil cores with water over a 1.3 mm screen and separated into fine (< 2 mm diameter) and coarse fractions. Based on morphological measures described later, the mean diameter of the fine root sample among species was 0.30 mm with mean species values ranging from 0.13 to 0.44 mm. On average across species, 84% (± 0.08 SD) of the sampled root length was distributed in diameter classes < 0.5 mm and 92% (± 0.03 SD) was < 1.0 mm. An inspection of the diameter distributions and mean values suggested that we sampled only the finest two or three root orders, comparable to those reported for grass (Wahl & Ryser, 2000) and tree species (Pregitzer et al., 2002; Comas & Eissenstat, 2004).

The root samples were kept moist at room temperature (c. 26 °C) before measurement of respiration, typically within 2.5 h of harvest. We determined the net CO₂ efflux on each fine root sample of each plot at a standard temperature (25.7 ± 0.4 °C) and atmospheric CO₂ concentration (366 ± 13 SD µmol mol⁻¹ CO₂) using infrared gas analysers and cuvettes, as described above. Checks revealed no direct effect of measurement CO₂ concentration on rates of root respiration in agreement with recent findings (Amthor et al., 2001; Tjoelker et al., 2001; Burton & Pregitzer, 2002). Use of detached roots for measures of respiration followed standard protocols (Burton & Pregitzer, 2002; Comas et al., 2002) and no correlation was observed between respiration rate and length of measurement for each of three sampling dates. Root length (see next section) and oven-dry mass measures were used to calculate respiration rates on the basis of root mass (R mass, nmol g⁻¹ s⁻¹) and root length (R length, nmol m⁻¹ s⁻¹).

Tissue morphology, chemistry, and longevity

We measured leaf area (one-sided projected) using a video image analysis system (AgVision; Decagon Devices, Inc.,
Root lengths and diameters were determined using a scanner-based, digital image analysis system (WinRhizo; Régent Instruments, Inc. Quebec City, Quebec, Canada). Following area or length determinations, the tissues were oven dried (65°C) and masses determined to calculate specific leaf areas (SLA, cm² leaf g⁻¹ leaf) and specific root lengths (SRL, m root g⁻¹ root). Nitrogen and carbon (C) concentrations of dried and ground leaf and root samples were measured using a CHN analyser (NA1500; Carlo Erba Instruments, Milan, Italy). Leaves sampled for the photosynthesis measures were pooled by plot before C and N analysis. Leaf N concentrations were used to calculate instantaneous photosynthetic nitrogen-use efficiency (PNUE, µmol CO₂ g⁻¹ N⁻¹ s⁻¹). We obtained leaf longevity data of 14 species determined the same year in the same study (Craine et al., 1999). Root longevity was estimated based on measured root turnover determined by in-growth and in situ root sampling in the plots. The standing crop of root biomass was determined in mid-August and root production estimated from ingrowth cores collected in July, August and October (Craine et al., 2002). Root longevity was calculated as standing crop divided by total ingrowth, the inverse of root turnover. We assumed that annual root production and turnover approximated steady-state levels in these long-term (5-yr-old) monoculture plots, based on observed plant density and dry matter production values. With the exception of one annual and two biennial species, all species are perennial. Poorly stocked plots were subsequently grouped and separate Type II linear regressions fitted to illustrate the a posteriori group relationships. Incomplete sampling resulted in differing numbers of species in the various trait comparisons (n = 31–36). Spearman's nonparametric rank correlation analysis was used to compare leaf vs root traits among species. All analyses were conducted using statistical analysis software (JMP 4.0; SAS Institute, Cary, NC, USA). Unless otherwise stated, α = 0.05.

Results

Comparison of leaf traits among functional groups

The a priori functional groups differed in mean rates of leaf net photosynthesis whether expressed on the basis of leaf area (A_area, µmol m⁻² s⁻¹) or leaf dry mass (A_mass, nmol g⁻¹ s⁻¹). Overall, the C₃ and C₄ grasses had the highest mean rates of A_area or A_mass followed by the legumes and forbs (Table 1). Since specific leaf area (SLA, cm² g⁻¹) did not differ significantly among the functional groups (Table 1), group rankings of mean A_area and A_mass were comparable. Mean leaf longevity of a subset of the species (n = 14) ranged from 29 to 94 d (Appendix 1; see also Craine et al., 1999).

As predicted, light-saturated rates of net photosynthesis on mass and area bases were positively correlated with leaf N_mass (%) and N_area (g N m⁻² leaf area), respectively (Fig. 1a,b). A_area was positively correlated with SLA (r = 0.52, P = 0.002, n = 32, not shown). Mean leaf N_max ranged from 1.0 to 4.1% among species and differed among functional groups (Table 1),
Table 1 Least squares mean (lower and upper 95% confidence intervals) leaf and fine root traits for functional groups of 39 grassland and savannah species grown in a common garden

<table>
<thead>
<tr>
<th></th>
<th>C₃ grasses</th>
<th>C₄ grasses</th>
<th>Forbs</th>
<th>Legumes</th>
<th>ANOVA P &gt; F</th>
<th>Contrast P &gt; ltl</th>
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<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>n</td>
<td>Mean</td>
<td>95% CI</td>
<td>n</td>
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<td><strong>Leaf traits</strong></td>
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<tr>
<td>Aarea (µmol m⁻² s⁻¹)</td>
<td>29.2</td>
<td>23.0–37.1</td>
<td>5</td>
<td>27.4</td>
<td>22.0–34.1</td>
<td>7</td>
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<tr>
<td>Amass (nmol g⁻¹ s⁻¹)</td>
<td>283</td>
<td>207–386</td>
<td>5</td>
<td>263</td>
<td>202–342</td>
<td>7</td>
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<tr>
<td>gₙ (mmol m⁻² s⁻¹)</td>
<td>588</td>
<td>407–849</td>
<td>5</td>
<td>273</td>
<td>195–382</td>
<td>7</td>
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<tr>
<td>SLA (cm² g⁻¹)</td>
<td>99</td>
<td>78–127</td>
<td>5</td>
<td>102</td>
<td>82–128</td>
<td>7</td>
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<tr>
<td>Nmass (g m⁻²)</td>
<td>1.79</td>
<td>1.43–2.23</td>
<td>5</td>
<td>1.34</td>
<td>1.09–1.64</td>
<td>7</td>
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<tr>
<td>Narea (%)</td>
<td>1.90</td>
<td>1.47–2.45</td>
<td>5</td>
<td>1.38</td>
<td>1.09–1.74</td>
<td>7</td>
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<tr>
<td>C:N</td>
<td>25.1</td>
<td>19.9–31.6</td>
<td>5</td>
<td>33.3</td>
<td>26.9–41.1</td>
<td>7</td>
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<tr>
<td>WUE (mmol CO₂ mol⁻¹ H₂O)</td>
<td>16.3</td>
<td>13.0–20.4</td>
<td>5</td>
<td>20.3</td>
<td>16.5–24.9</td>
<td>7</td>
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<tr>
<td>PNUE (µmol CO₂ g⁻¹ N s⁻¹)</td>
<td>17.5</td>
<td>13.7–22.3</td>
<td>5</td>
<td>13.0</td>
<td>10.4–16.2</td>
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<tr>
<td>Rmass (nmol g⁻¹ s⁻¹)</td>
<td>2.1</td>
<td>1.5–3.0</td>
<td>5</td>
<td>1.0</td>
<td>0.7–1.4</td>
<td>6</td>
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<tr>
<td>Rarea (µmol m⁻² s⁻¹)</td>
<td>13.3</td>
<td>9.7–18.3</td>
<td>5</td>
<td>28.1</td>
<td>21.1–37.6</td>
<td>14</td>
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<tr>
<td>Longevity (d)</td>
<td>77</td>
<td>50–118</td>
<td>3</td>
<td>68</td>
<td>44–104</td>
<td>3</td>
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<td><strong>Fine root traits</strong></td>
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<td>Rmass (nmol g⁻¹ s⁻¹)</td>
<td>11.4</td>
<td>7.7–16.6</td>
<td>5</td>
<td>5.7</td>
<td>4.0–8.1</td>
<td>6</td>
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<tr>
<td>Nmass (µmol g⁻¹)</td>
<td>1.08</td>
<td>0.81–1.44</td>
<td>5</td>
<td>0.52</td>
<td>0.40–0.67</td>
<td>6</td>
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<tr>
<td>Nlength (mg m⁻³)</td>
<td>0.32</td>
<td>0.10–1.03</td>
<td>5</td>
<td>0.18</td>
<td>0.06–0.52</td>
<td>6</td>
</tr>
<tr>
<td>SRL (m g⁻¹)</td>
<td>76.5</td>
<td>50.6–115.6</td>
<td>5</td>
<td>56.2</td>
<td>38.5–81.9</td>
<td>6</td>
</tr>
<tr>
<td>C : N</td>
<td>37.8</td>
<td>28.4–50.2</td>
<td>5</td>
<td>79.5</td>
<td>61.3–103.1</td>
<td>15</td>
</tr>
<tr>
<td>Longevity (d)</td>
<td>504</td>
<td>243–1044</td>
<td>5</td>
<td>791</td>
<td>407–1540</td>
<td>6</td>
</tr>
</tbody>
</table>

Means and 95% confidence intervals were back-transformed from log₁₀; n is the number of species (see Appendices). Aarea, area-based photosynthesis; Amass, mass-based photosynthesis; gs, stomatal conductance; SLA, specific leaf area; Nmass, nitrogen concentration; Narea, area-based nitrogen concentration; C : N, carbon–nitrigen ratio; WUE, water use efficiency; PNUE, photosynthetic N-use efficiency; Rmass, mass-based respiration; Rarea, area-based respiration; A : R, ratio of net photosynthesis to respiration; Nlength, root length-based nitrogen concentration; SRL, specific root length.
being greatest in legumes, followed in decreasing order by forbs, C3 grasses, and C4 grasses. However, for a given leaf Nmass or Narea the two grass groups (regardless of photosynthetic pathway) had higher A mass and Aarea compared with the forbs and legumes. Among the four a priori groups, the C3 and C4 grasses differed from forbs and legumes in terms of intercept (P < 0.001) but not slopes (P ≥ 0.32) of the Type II regression between A mass and Nmass as well as Aarea and Narea, providing evidence of separate trait correlations for the combined grass and combined forb and legume species (Fig. 1a,b).

Expressing rates of photosynthesis on a leaf nitrogen basis provided an estimate of leaf photosynthetic N-use efficiency (PNUE, µmol CO2 g⁻¹ N s⁻¹). The two grass groups, regardless of photosynthetic pathway, had roughly double the PNUE of the legume and forb dicot groups (Table 1). The C4 grasses also had higher PNUE than the C3 grasses (Table 1), but this difference was modest compared with the monocot–dicot distinction. In addition, mean leaf conductance to water vapor (gs) was lower in the C4 grasses than the remaining C3 groups. Consequently, measures of instantaneous leaf WUE were about 60% higher among the C4 grasses than in the other functional groups (Table 1).

Rates of leaf dark respiration differed three- to four-fold among species and increased with increasing leaf Nmass and Narea (Fig. 1c,d). Slopes of leaf respiration–N regression relationships did not differ from a common slope among the four functional groups on either a leaf mass (P = 0.22) or area basis (P = 0.85). However, legumes differed from the forb and two
grass groups by exhibiting lower elevations of the leaf $R_{\text{mass}} - N_{\text{mass}}$ ($P = 0.002$) and $R_{\text{area}} - N_{\text{area}}$ ($P = 0.003$) relationships, suggesting an a posteriori contrast between the legumes and the nonlegume species (Fig. 1c,d). As a group, the $C_4$ grasses had lower mean leaf $N_{\text{area}}$ and lower rates of leaf respiration than the $C_3$ grass, forb, and legume groups on a leaf area basis (Table 1). As a result of comparatively lower respiration and higher rates of net photosynthesis, $C_4$ grasses had roughly doubled ratios of net photosynthesis to respiration ($A : R$, C-use efficiency) compared with the $C_3$ grass, forb, and legume groups, which were similar in this regard (Table 1). Unlike the photosynthesis–leaf trait correlations in which $C_3$ and $C_4$ grasses differed from forbs and legumes, respiration-leaf trait correlations differed between legumes and the three nonlegume groups.

Comparison of fine root traits among functional groups

Overall, fine root $R_{\text{mass}}$ was highest for the legume group and lowest for $C_4$ grasses compared with the other functional groups, largely paralleling functional group differences in fine root $N_{\text{mass}}$ (Table 1). Specific rates of fine root respiration ($R_{\text{mass}}$) increased with increasing fine root $N_{\text{mass}}$ (%) among the species (Fig. 2a). The regression slopes did not differ among the four functional groups ($P = 0.53$), but legumes differed from the remaining three groups in terms of the intercept ($P < 0.001$) of the relationships, providing evidence of differing correlations for legumes and nonlegume species (Fig. 2a). Legumes had a lower fine root $R_{\text{mass}}$ at a given $N_{\text{mass}}$ than the forb and two grass groups, similar to the leaf respiration–$N$ relationships (Fig. 1c,d).

Specific root length (SRL, m root length g$^{-1}$ root dry mass) and $N_{\text{length}}$ (mg N m$^{-1}$ root length) of fine roots did not differ among the functional groups, although legumes had a lower SRL than the other groups (Table 1). Yet, fine root $R_{\text{mass}}$ was positively correlated with SRL among species, which were differentiated by Type II regression relationships of common slope ($P = 0.16$) but lower elevation ($P < 0.001$) for $C_4$ grasses compared with the $C_3$ grasses, forbs and legumes. For a given SRL, the $C_4$ grasses had lower fine root $R_{\text{mass}}$ (Fig. 2b). However, across species, fine root respiration rates expressed on the basis of root length ($R_{\text{length}}$, nmol m$^{-1}$ s$^{-1}$) were positively correlated with $N_{\text{length}}$. Neither regression slopes ($P = 0.32$) nor intercepts ($P = 0.40$) differed among the four a priori functional groups, providing evidence that all species groups exhibited the same regression relationship (Fig. 2c).

Estimated mean root longevity, based on root turnover calculations, ranged from 32 to 1409 d among the species and differed among species groups (Table 1). Mean root longevity ranged from 136 d in legumes to 791 d in $C_4$ grasses.
Leaf and fine root longevity relationships

When comparing trait correlations with leaf and root longevity, \( R_{\text{mass}} \) and \( N_{\text{mass}} \) of leaves and fine roots declined with increased tissue longevity (Fig. 3a–d). Although Leaf \( R_{\text{mass}} \) and longevity were weakly correlated for a subset of 14 species for which leaf longevity data were available (Fig. 3a), the trend follows well-established relationships. Neither the regression slopes of the fine root \( R_{\text{mass}} \)–root longevity relationship (\( P = 0.21 \)) nor the intercepts (\( P = 0.39 \)) differed among functional groups, suggesting a common relationship among species (Fig. 3b). Similarly, slopes of the fine root \( N_{\text{mass}} \)–root longevity relationship did not differ among the a priori functional groups (\( P = 0.66 \)). The slope of the major axis for the \( C_4 \) grasses had a lower intercept compared with the legumes (\( P = 0.009 \)); however, the individual group correlations were not statistically significant and thus the overall linear relationship is shown (Fig. 3d). Although SLA declined with increasing leaf longevity (Fig. 3e), root longevity was unrelated to SRL (Fig. 3f), \( R_{\text{length}} \) or \( R_{\text{mass}} \) among species (\( P \geq 0.28 \), not shown). Leaf \( N_{\text{mass}} \) and SLA were positively correlated among the species (\( r = 0.38, P = 0.02, n = 36, \) not shown). By contrast, fine root \( N_{\text{mass}} \) was unrelated to SRL (not shown). Therefore, for leaves, and not for roots, an increased ratio of area or length per unit dry mass was associated with higher tissue \( N_{\text{mass}} \).

Comparing leaf and fine root trait syndromes

Rankings of leaf and fine root \( N_{\text{mass}} \) were correlated among the species (Spearman rank correlation, Table 2), as was the bivariate trait correlation (\( r = 0.77, P < 0.001 \)). Similarly, species rankings of leaf \( R_{\text{mass}} \) and fine root \( R_{\text{mass}} \) were positively correlated. Overall, specific rates of respiration of leaves and fine roots were positively correlated with tissue \( N_{\text{mass}} \) and the regression slopes did not differ among the four a priori functional groups (\( P = 0.21 \), Fig. 4). However, the intercept was lower in the legumes compared with the other species groups (\( P < 0.001 \)), suggesting differing correlations between legumes and nonlegume species (Fig. 4). By contrast, neither the rankings of SLA and SRL nor leaf \( N_{\text{area}} \) and fine root \( N_{\text{length}} \) were related among species (Table 2). Root and leaf longevity were positively correlated (\( r = 0.67, P = 0.009 \)), as was the rank correlation (Table 2).

Discussion

Are there parallel leaf and root trait syndromes?

We tested several specific predictions of parallel leaf and fine root trait relationships among species and functional groups of the grassland savannah taxa. First, rates of \( CO_2 \) exchange of leaves and fine roots were positively correlated with tissue \( N \), as expected (prediction 1) based on well-documented leaf trait relationships (Evans, 1989; Ryan, 1991; Reich et al., 1997, 1998a). However, our findings suggest that these relationships also hold among sympatric taxa within a regional flora. A striking pattern was that specific respiration rates of both leaves and fine roots shared a common regression relationship with \( N \), with the exception of the legumes which exhibited somewhat lower respiration rates for a given \( N \) concentration. Thus, species not only exhibited concordant rankings in leaf and fine root \( N \) concentrations and leaf and fine root specific respiration rates, but also exhibited a common respiration–\( N \) regression relationship across combined roots and leaves of \( C_3 \) and \( C_4 \) grasses and forbs. By contrast, for young seedlings of nine woody plant species during a rapid growth phase, both leaf and fine root specific respiration rates were linearly related to leaf and fine root \( N_{\text{mass}} \), respectively, but fine roots had much higher respiration rates than leaves at any given tissue \( N \) concentration (Reich et al., 1998b). The relative contribution of growth vs maintenance respiration was likely much higher in the study of young woody plant seedlings than in our current study of older field-grown plants late in the growing season. We are unaware of other direct comparisons of leaf and root respiration–\( N \) relationships. Our findings suggest that the respiration–\( N \) relationship may be robust across species and tissue type and thus a useful predictor in linking species traits to their effects on ecosystem function (Lavorel & Garnier, 2002; Eviner & Chapin, 2003). In this regard, tissue \( N \) contents may be used to model autotrophic respiration (Ryan, 1991), a critical component of net ecosystem exchange and production.

Second, we tested predicted leaf and root trait correlations linking structural traits to tissue longevity (prediction 2). Although root longevity (estimated as the inverse of root turnover) was much greater than that of leaves, species rankings in leaf and root longevity were correlated, suggesting that tissue longevity constitutes a consistent leaf and root trait syndrome among these species. In leaves, respiration rates, \( N \) concentration, and SLA decreased with increasing longevity in agreement with well-documented leaf trait syndromes in plants (Reich et al., 1997, 1998a,b, 1999; Garnier et al., 1999; Wright et al., 2001, 2004). Similarly in roots, \( N \) concentration and respiration rates declined with increasing longevity, as noted in other studies (Eissenstat et al., 2000), but demonstrated here among a large set of species. We are unaware of other studies that directly compare leaf and root longevity among species grown in a common environment as there are few datasets available to compare leaf and root traits on the same sets of plants. Nitrogen concentration and tissue density of leaves are correlated with those of fine roots among 24 grass species along an altitudinal transect (Craine & Lee, 2003). Our findings suggest that tissue longevity, \( N \) concentration and metabolic activity (respiration) traits are linked above and below ground, constituting a consistent leaf–root trait syndrome among this set of sympatric grassland and savannah species, and potentially linking above- and below-ground processes.
Fig. 3 Comparison of leaf and fine root traits of savannah species in relation to tissue longevity (○, forbs; ●, legumes; ■, C₃ grasses; □, C₄ grasses). Type II regression lines illustrate a posteriori group contrasts among species. Leaf longevity was determined on a subset of the species (Appendix 1; see also Craine et al., 1999). (a) Leaf R\text{mass}–leaf longevity (r = −0.43, P = 0.12, n = 14); (b) fine root R\text{mass}–root longevity (r = −0.48, P = 0.0076, n = 30); (c) leaf N\text{mass}–leaf longevity (r = −0.77, P = 0.0012, n = 14); (d) fine root N\text{mass}–root longevity (r = −0.60, P < 0.001, n = 29); (e) specific leaf area (SLA)–leaf longevity (r = −0.61, P = 0.02, n = 14); (f) fine root SRL–root longevity (r = 0.03, P = 0.89, n = 30).
Table 2 Nonparametric rank correlation coefficients for leaf and fine root traits among grassland and savannah species

<table>
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<tr>
<th>Leaf–root trait</th>
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<th>Spearman’s ρ</th>
<th>P &gt;</th>
<th>P</th>
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<td>Leaf C : N–fine root C : N</td>
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<td>Leaf SLA–fine root SRL</td>
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<td>Leaf Rmass–fine root Rmass</td>
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<tr>
<td>Leaf Narea–fine root Rlength</td>
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<td>Leaf longevity–root longevity</td>
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<td>0.50</td>
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</tbody>
</table>

n is number of species; SLA, specific leaf area; SRL, specific root length.

Fig. 4 Specific respiration rates (Rmass, nmol g⁻¹ s⁻¹) of leaves (△, ▲) and fine roots (▽, ▼) in relation to nitrogen concentration (Nmass) for legumes (closed symbols) and the combined C₃ and C₄ grass and forb groups (open symbols). Type II regression lines are shown for pooled leaf and fine root data for legumes (r = 0.71, P = 0.01, n = 11) and non-legume species (r = 0.85, P < 0.001, n = 51).

Although our root samples reflect the architecture of the finest root orders of all species, potentially important variation in longevity within root systems (Wells & Eissenstat, 2001; Anderson et al., 2003) may be averaged across when using sequential soil coring to estimate root turnover, and longevity estimates may differ among methods (Matamala et al., 2003). Within a species, the finest roots and lowest root orders tend to have higher N concentrations, higher specific respiration rates, higher SRL and shorter lifespans than higher-order roots (Pregitzer et al., 1997, 1998; Eissenstat et al., 2000; Wells & Eissenstat, 2001; Anderson et al., 2003) and exhibit declining metabolic activity with age (Volder et al., 2005). These within species patterns largely parallel the broad interspecific patterns described in the present study.

Unlike the concordant rankings observed in leaf and root N, rates of respiration, and longevity among species, SLA and SRL were unrelated, representing a contrast between leaf and root trait syndromes among these grassland and savannah species. In addition, both SRL and SLA failed to differ among the a priori functional groups in agreement with Craine et al. (2001) and Diaz et al. (2004). Moreover, unlike leaves, SRL was unrelated to root longevity or N concentration. Among grasses, leaf and root tissue density (dry mass to volume ratio) is positively correlated with tissue longevity (Ryser, 1996); however, leaf thickness and not leaf density is related to Aarea in 14 grass species (Garnier et al., 1999) and among woody plants (Niinemets, 1999). Among species in the present study, SRL and mean fine root diameter were uncorrelated (r = -0.19, P = 0.25, n = 36, not shown), suggesting that differences in SRL among these savannah and grassland species may be related more to differences in tissue density than diameter (Wähl & Ryser, 2000; Craine et al., 2001).

Overall, our findings demonstrate a common leaf and root trait syndrome of N concentration, respiration rate and longevity among a diverse array of sympatric grassland taxa when grown in a common garden. These findings support the concept of key trait syndromes that arise from trade-offs in plant traits and function (Lambers & Poorter, 1992; Aerts & Chapin, 2000; Lavorel & Garnier, 2002; Westoby et al., 2002; Evener & Chapin, 2003; Díaz et al., 2004). In both leaves and roots, increasing tissue N concentrations were associated with increased rates of metabolic activity and declining tissue longevity, reflecting a tradeoff between rapid acquisition of resources and conservation of resources within protected tissues (Díaz et al., 2004). The strong leaf and root trait relationship with tissue N shown in the present study perhaps reflects the low N availability in this grassland ecosystem (Tilman, 1988; Craine et al., 2002). Low tissue nutrient concentrations and turnover rates are key determinants of high nutrient retention in nutrient-poor environments (Aerts & Chapin, 2000). Finding consistent trait differences among species is one objective in assembling comparative datasets of plant functional types across wide-ranging sites and timescales (Wilson et al., 1999; Garnier et al., 2001). Although mean values of many of the traits differed among the a priori functional groups in this study, the trait values were largely continuously distributed among the species. Thus, our findings demonstrate that trait differences among species may be represented in terms of their bivariate trait relationships that link structural traits to function and, in turn, provide insight into the mechanisms governing species effects on ecosystem processes.

Do a priori functional groups differ in trait scaling relationships?

The regression analysis of trait relationships permitted examination of functional group effects in terms of the slope (scaling relationship) and its elevation. The C₃ and C₄ grass species groups together differed from forbs and legumes in the elevation of the slopes of the leaf photosynthesis–N
relationships but not respiration. As expected (prediction 4) based on well-documented differences between photosynthetic pathways (Sage & Pearcy, 1987; Ehleringer & Monson, 1993), C_4 grasses exhibited higher instantaneous efficiencies of N use (PNU), water use (WUE) and carbon use (A : R) compared with the C_3 grasses, forbs and legumes. Somewhat unexpected was the increased PNU of the C_3 grasses relative to the forbs and legumes, the similarity of PNU of C_4 and C_3 grasses and the comparable slope of the leaf photosynthesis–N relationship for C_4 and C_3 grasses despite their different photosynthetic pathways. Thus, for C_3 and C_4 grass species together, the increased efficiencies were manifested in shifts in elevation but not slopes of the regression relationships that, in effect, resulted in greater A_max or A_area for a given N_max or N_area. In addition, the grasses and C_4 grasses in particular had the lowest leaf N concentrations compared with the other functional groups. These findings suggest that compared with forbs and legumes, the C_4 and C_3 grasses exhibit increased leaf-level resource-use efficiencies, which are reflected in a shift in elevation of the leaf-trait regression slopes.

In a greenhouse study of seedlings of many of the same species, C_4 grasses did have a different elevation of the photosynthesis–N relationship than the C_3 grasses, which followed the same relationship as all other C_4 plants (Reich et al., 2003). Moreover, the young seedlings of the C_3 grasses had similar tissue N_max (%) and lower photosynthetic rates than the C_4 grasses. Perhaps plant maturation under field conditions leads these species in different ontogenetic pathways, with the C_4 grasses exhibiting progressively lower N concentrations and concomitantly, having slightly lowered photosynthetic rates relative to the C_3 grasses. Among woody plant species, trait correlations may differ between juvenile and mature plants (Cornelissen et al., 2003).

The photosynthesis–N relationships for legumes were shifted upward along a common slope that included the forb species (supporting prediction 5). Consequently, legumes had higher leaf and fine root N concentrations, increased CO_2 exchange rates and reduced tissue longevity than forbs. These structural and functional traits in large part contributed to the more rapid N cycling in legumes in monoculture compared with the other functional groups among these grassland species (Craine et al., 2002). By contrast to the common photosynthesis–N relationship among legumes and forbs, both leaf and fine root respiration–tissue N relationships exhibited a lower elevation in legumes compared with the forb or grass species groups. The legumes had lower rates of respiration for a given tissue N concentration compared with the other functional groups. This was somewhat surprising, especially in roots, given the expectation that respiratory carbon costs are higher for roots that support N_2 fixation compared with nitrate assimilation. Maintenance respiration is thought to scale generally with the adenylate demand associated with turnover of protein, maintenance of ion gradients and nutrient assimilation (Amthor, 2000). The respiration–N relationship in legumes may, in effect, be altered through increased concentrations of organic nitrogen in roots and leaves, reflected in the substantially lower root C : N ratio in legumes compared with the other functional groups (24 vs ≥ 38). Production, storage and transport of organic forms of N in legumes would also presumably differ in underlying respiratory carbon costs on a per unit N basis compared with non-N_2-fixing forbs. For a given root N concentration, a comparatively lower net CO_2 efflux from roots of legumes may, in part, result from re-fixation of respired CO_2 in roots and root nodules (Atkins et al., 2001).

Conclusions

Concordance in above- and below-ground traits was evident in similar rankings in leaf and root tissue N, respiration rate and longevity among this set of sympatric grassland species. Moreover, the relationships between tissue N and respiration rate exhibited a common regression relationship for leaves and fine roots. Although trait values were continuously distributed among species, a priori functional group differences were manifested in differing elevations of regression slopes of certain gas exchange and structural trait relationships. In this regard, C_3 and C_4 grasses had lower leaf N concentrations but higher photosynthesis rates across a similar range of leaf N compared with forbs and legumes. Similarly, legumes had lower respiration rates for a given tissue N concentration compared with grasses and forbs. Functional shifts in ecophysiological traits reflect key trade-offs in tissue structure and function that resulted in greater tissue level resource-use efficiency among C_4 and C_3 grasses compared with forbs and legumes. Tissue-level traits are associated with competitive abilities and species effects on ecosystem-scale processes in grasslands (Tilman & Wedin, 1991; Craine et al., 2001) and other ecosystems (Grime et al., 1997; Aerts & Chapin, 2000; Lavorel & Garnier, 2002; Westoby et al., 2002; Evine & Chapin, 2003; Diaz et al., 2004). Understanding leaf and root function in the context of integrated above- and below-ground trait syndromes will aid in predicting plant and ecosystem response to global change factors (Reich et al., 2001b).

Acknowledgements

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References


## Appendix 1

Mean leaf traits (± 1 SE) of North American grassland savannah species

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Aarea SE</th>
<th>Amass SE</th>
<th>gS SE</th>
<th>WUE SE</th>
<th>PNUE SE</th>
<th>SLA SE</th>
<th>Nmass SE</th>
<th>C : N SE</th>
<th>Narea SE</th>
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<td>661</td>
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<td>0.71</td>
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<td>23</td>
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<td>99</td>
<td>3.42</td>
<td>0.35</td>
<td>11.8</td>
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<td>4.9</td>
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- n: number of measurements
- Aarea: leaf area
- Amass: leaf mass
- gS: specific leaf area
- WUE: water use efficiency
- PNUE: photosynthetic nitrogen use efficiency
- SLA: specific leaf area
- Nmass: leaf nitrogen mass
- C : N: carbon:nitrogen ratio
- Narea: leaf nitrogen area
### Appendix 1 continued

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The number of samples (n) indicated for each species applies to all photosynthesis and respiration traits, except where indicated (*n* = 2). No data are indicated as dashes. Units for traits are: A<sub>area</sub>, area-based photosynthesis, µmol m<sup>–2</sup> s<sup>–1</sup>; A<sub>mass</sub>, mass-based photosynthesis, nmol g<sup>–1</sup> s<sup>–1</sup>; gs, stomatal conductance, mmol m<sup>–2</sup> s<sup>–1</sup>; WUE, water-use efficiency, mmol CO<sub>2</sub> mol<sup>–1</sup> H<sub>2</sub>O; PNUE, photosynthetic N-use efficiency, µmol CO<sub>2</sub> g<sup>–1</sup> N s<sup>–1</sup>; SLA, specific leaf area, cm<sup>2</sup> g<sup>–1</sup>; N<sub>mass</sub> %; C : N, carbon–nitrogen ratio; N<sub>area</sub> g m<sup>–2</sup>; R<sub>area</sub>, area-based respiration, µmol m<sup>–2</sup> s<sup>–1</sup>; R<sub>mass</sub>, mass-based respiration, nmol g<sup>–1</sup> s<sup>–1</sup>; leaf longevity, d. Note that leaf N-values are shown separately for both photosynthesis and respiration samples. Leaf longevity data are from Craine et al. (1999).
## Appendix 2

Mean root traits (± 1 SE) of North American grassland savannah species

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<td>0.03</td>
<td>0.22</td>
<td>0.10</td>
<td>0.21</td>
<td>0.07</td>
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<td>89.8</td>
<td>7.4</td>
<td>4</td>
<td>740</td>
<td>584</td>
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<tr>
<td><em>Poa pratensis</em></td>
<td>3</td>
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<td>1.1</td>
<td>1.00</td>
<td>0.15</td>
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<td>0.97</td>
<td>0.59</td>
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<td>57</td>
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<td>2.8</td>
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<tr>
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<td>4</td>
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<td>0.89</td>
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<td>1173</td>
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</tbody>
</table>

The number of individual plots (n) applies to all traits of each species, except where indicated (*n = 1*). No data are indicated by dashes. Units for traits are: $R_{mass}$, mass-based respiration, nmol g$^{-1}$ s$^{-1}$; $N_{mass}$, %; $R_{length}$, length-based respiration, nmol m$^{-1}$ s$^{-1}$; $N_{length}$, mg m$^{-1}$; SRL, specific root length, m g$^{-1}$; C : N, carbon–nitrogen ratio; longevity, d.