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Covariation in leaf and root traits for native and non-native grasses along an altitudinal gradient in New Zealand

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Abstract Across 30 grassland sites in New Zealand that ranged from native alpine grasslands to low elevation improved pastures, there were consistent patterns of leaf and root traits and significant differences between native and non-native grasses. Plants of high altitude sites have low N concentrations in both their leaves and roots, have thick leaves and roots, yet no differences in tissue density or photosynthetic water use efficiency when compared to plants of low altitude sites. Both the leaves and roots of the low altitude plants were enriched in ^{15}N relative to the plants of higher altitude, indicating that the low-N set of traits is associated with a more closed N cycle at high altitude. A second independent set of correlations shows that plants of wetter habitats have lower photosynthetic water use efficiency (more negative $\delta^{13}\text{C}$) and lower leaf and root tissue density than the plants of drier sites. For both leaves and roots, plants of native species consistently had traits associated with lower resource availability: lower N concentrations, denser tissues, more negative $\delta^{15}\text{N}$, and more positive $\delta^{13}\text{C}$ than non-native species. If root %N is correlated with root longevity as has been shown in other systems, root longevity may be able to be predicted from simple measurements of leaf %N, though a hysteresis in the relationship between leaf and root N concentrations may make prediction of high longevity roots difficult.

Keywords $\delta^{15}\text{N}$ Nitrogen · Nitrogen · Tissue density · Water use efficiency

Introduction

Plant species' fine root traits are important in determining ecosystem species composition (Ryser 1996; Craine et al. 2001), ecosystem resource use (Caldwell et al. 1985; Hendricks et al. 1993; Jackson et al. 1996), and response

to global change factors such as temperature, atmospheric CO_2 , and N deposition (Jackson et al. 1998; Boone et al. 1998; Van Noordwijk et al. 1998). Yet, direct measurement of root traits, such as root longevity, can be exceedingly difficult to obtain. As such, our understanding of the patterns of root traits across species and environmental gradients is rudimentary and this is likely to constrain our understanding of species composition and ecosystem function.

The lack of directly measured data is exacerbated by the lack of techniques that can be used to quantify root traits indirectly (Norby and Jackson 2000). Any ability to predict key root traits from leaf traits across major environmental gradients could greatly increase our power to understand changes in species abundance while allowing better parameterization of models of ecosystem function. Within a site, there can be quite strong relationships among root and leaf traits (Aerts and Berendse 1989; Ryser 1996; Schlaepfer and Ryser 1996; Craine et al. 2001). For example, plants with high N concentrations in their leaves have high N concentrations in their roots. Though root longevity data is rare, as paralleled in leaves (Ryser 1996; Reich et al. 1997), both root N concentrations and root tissue density have been linked to the longevity of fine roots of different species within a site (Ryser 1996; Eissenstat et al. 2000; Nadelhoffer 2000; Craine et al. 2002; but see Burton et al. 2000). If there are strong links between leaf traits and either of these root traits, root longevity may be predicted simply by measuring the traits of leaves. Although relationships between leaf traits, root traits and root longevity may exist for a site, there are few data on the generality of these relationships, especially across major ecological gradients.

The availability of resources can influence root functional traits and their relationship with leaf traits. In leaves, as nutrient supply increases, leaf nutrient concentrations increase and tissue density decreases (Monk 1966; Berendse et al. 1994; Grime et al. 1997; Craine et al. 2001). Similarly, Yin and Perry (1991) and Hendricks et al. (2000) found that in forests, root N concentrations

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increased with increasing N availability. Measurements across novel gradients and among different ecosystem types are necessary to more robustly understand variation in root and leaf trait relationships. Even though both leaf and root N concentrations may increase with increasing nutrient supply, how any relationship between leaf and root traits changes across nutrient supply gradients is uncertain. For example, if root N concentration is constrained at higher fertility, leaf N concentrations can increase without any concomitant increase in root N concentration, potentially altering the complexity of the equations used to predict root N concentration, if not decreasing its predictability.

Across environmental gradients, it is important to know how the functional traits of individual species change as well as how the traits of species differ within a site. Average differences between sites could be due to species plasticity or differences in the relative abundance of species. Responses of individual species to changes in environmental conditions may not be the same as differences between sites that are due to changes in species abundance. For New Zealand grasslands, native species are more abundant at high elevation, low-productivity sites and non-native, mostly European species at low elevation, high-productivity sites. Whether (and if so, how) species native and not native to New Zealand differ in their functional traits has not been rigorously explored. As high fertility, grazed pastures are relatively recent in the evolutionary history of New Zealand (Lee et al. 2001) it is likely that native species are poorly adapted to being productive and/or competitive at higher nutrient supply. As has been attempted in other regions (Crawley et al. 1996, Baruch and Goldstein 1999, Smith and Knapp 2001), quantifying differences between native and non-native species in both their above- and belowground traits should also aid in understanding the maintenance of current species distributions as well as patterns of invasions of non-native species into native grasslands as well as native species into pastures.

To determine the relationships between leaf traits and root traits across a fertility gradient both within and between species, we sampled monotypic patches of two to five grassland species at each of 30 sites in the South Island of New Zealand. Sites varied in altitude, precipitation, temperature, and productivity. Both native and improved grasslands were sampled and many sites contained both native and non-native species. For each individual species at each site, we measured N concentration, tissue density, thickness/diameter, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of both leaves and fine roots.

Our two main goals were to: (1) quantify relationships between leaf and root traits among plants across a broad environmental gradient (site altitude), and (2) compare native and non-native species in their traits across the altitudinal gradient. Later, we examine the ability of leaf N concentrations to predict estimated root longevity compared to root N concentrations and examine the limitations of this indirect approach.

Based on the results of previous research, we hypothesized that across sites, leaf and root N should be positively correlated as well as leaf and root tissue density. We also hypothesized that plants growing at high N availability should have both leaves and roots with high N concentrations and low tissue density. We use the $\delta^{15}\text{N}$ of plants as an index of N supply (Garten and Van Miegroet 1994; Hogberg 1997). More positive plant $\delta^{15}\text{N}$ is associated with elevated N cycling. Although differences in plant $\delta^{15}\text{N}$ can be due to differences in N source within an ecosystem (e.g. Nadelhoffer et al. 1996), enrichment of plant $\delta^{15}\text{N}$ is positively correlated with N mineralization rates (Garten and Van Miegroet 1994). In ecosystems with high mineralization rates, the proportion of N that is acquired from NH_4^+ (which is enriched in ^{15}N during nitrification) can be higher, as can be rates of denitrification and leaching. All three processes enrich the N pool that plants acquire, suggesting that higher $\delta^{15}\text{N}$ can be used as an indicator of N limitation. As such, we hypothesize that $\delta^{15}\text{N}$ enrichment should be positively associated with N concentrations both in leaves and roots and negatively with tissue density. Lastly, we expect that the non-native species found along the New Zealand altitudinal gradient should be more adapted to high fertility, having higher N concentrations and lower tissue density than native species, even when compared at a common site.

Materials and methods

Roots and leaves of 118 plants of 24 grass species were collected from 30 grassland sites in January and February of 2001. Sites were located on the southern half of the South Island of New Zealand and ranged in altitude from 45 m to 1,205 m above sea level (Table 1). Sites included native and improved grasslands (i.e. fertilized and/or planted with non-native species), alpine as well as subalpine grasslands, and span a wide range of precipitation (399–1,959 mm year⁻¹) and habitats (e.g. roadsides, floodplains, hillsides, and dunes).

At each site, at least one monospecific patch of each of the most abundant grasses was selected (multiple patches were bulked to give one sample). A 10- to 20-cm-diameter patch of the grass was excavated to a depth of 10–20 cm. Three portions of green leaf blades, generally 3–15 cm in length, were removed and their thickness and width determined with calipers. Thickness was measured halfway between the edge and middle of the leaf. These leaf segments were later dried and weighed to determine their tissue density (mass per unit volume). Samples of two to five species per site were collected.

Roots of each sample were washed clean of soil under running water and fine roots (<1 mm diameter) separated from other belowground material. A small subsample of fine roots (average mass 0.07 g) was removed, placed in a 10×15-cm clear plastic tray, and scanned at 1,200 dpi on an Epson Expression 1600 scanner. Images were analysed with WinRhizo (Régent Instruments, Quebec) to determine average diameter and total root volume. For both fine roots and green leaves, N concentrations, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were determined on a Europa Scientific Integra mass spectrometer at the University of California, Davis Stable Isotope Facility.

At each site, approximately six 2.5-cm-diameter, 10-cm-deep cores of soil were removed, dried, passed through a 2-mm sieve, and the pH and $\delta^{15}\text{N}$ of the soil determined. Environmental data on temperature and precipitation were derived from climate surface estimates (Leathwick et al. 1998).

Table 1 Site descriptions including latitude, longitude, elevation, mean annual temperature (*MAT*, °C), mean annual precipitation (*MAP*, mm), soil pH, soil %N (0–10 cm), and soil $\delta^{15}\text{N}$. *n/a* Not applicable

Site no.	Latitude	Longitude	Elevation	MAT	MAP	pH	Soil %N	Soil $\delta^{15}\text{N}$
1	45°28.541'	170°49.558'	45	10.3	524	5.11	0.53	6.37
2	45°53.424'	170°39.962'	129	10.0	727	6.98	0.00	n/a
3	45°53.617'	170°40.882'	218	9.6	777	5.92	0.60	4.51
4	45°04.344'	169°10.458'	231	10.1	399	5.46	0.05	4.75
5	45°12.068'	169°23.894'	247	9.7	402	6.3	0.18	2.90
6	45°38.366'	168°17.436'	287	9.9	930	5.56	0.57	3.87
7	44°42.875'	169°17.602'	361	9.9	519	6.12	0.18	3.73
8	45°10.154'	170°01.722'	393	8.8	782	6.62	0.45	4.88
9	45°32.091'	168°10.931'	439	9.1	805	6.27	0.36	-0.03
10	45°32.211'	168°11.109'	454	8.9	1,065	5.40	0.31	2.03
11	45°28.673'	168°06.024'	486	8.5	1,181	4.76	0.48	0.93
12	45°50.071'	170°27.099'	527	7.8	1,198	4.95	0.54	2.56
13	45°33.860'	168°10.897'	619	8.1	1,091	5.24	0.37	1.45
14	45°19.769'	168°12.382'	619	8.3	1,107	4.75	0.40	2.12
15	45°43.550'	167°21.440'	641	7.0	1,378	5.03	1.14	2.16
16	45°48.015'	170°29.707'	641	7.2	1,348	4.40	1.70	0.44
17	45°00.202'	169°59.343'	643	7.9	765	5.67	0.26	3.12
18	45°18.232'	168°15.315'	666	8.1	1,124	5.46	0.16	0.23
19	45°48.058'	170°29.752'	671	7.0	1,365	4.51	0.98	2.58
20	45°13.271'	168°19.936'	706	7.6	754	5.08	0.11	1.15
21	45°13.353'	168°20.131'	729	7.7	1,147	4.83	0.37	3.32
22	45°44.103'	167°24.731'	747	6.9	1,268	5.61	0.14	0.87
23	45°35.993'	169°54.486'	757	6.5	958	4.98	0.41	1.64
24	45°35.304'	169°54.197'	841	6.1	876	4.84	0.35	3.88
25	45°31.882'	169°56.480'	847	6.0	984	4.91	0.46	3.86
26	45°27.108'	168°44.789'	987	5.8	813	4.68	0.24	3.67
27	45°27.622'	168°45.286'	1,122	5.5	1,236	4.85	0.21	4.46
28	45°45.282'	167°23.352'	1,191	4.0	1,796	4.94	0.77	2.01
29	45°45.076'	167°23.120'	1,192	3.7	1,959	4.65	0.66	1.71
30	45°45.220'	167°23.286'	1,205	3.8	1,827	4.69	0.88	5.16

All statistical analyses were performed in JMP 4.0.1 (SAS Institute, Cary, N.C.). Pairwise relationships between selected traits were determined with a model II regression, which is required when there is no clearly defined independent variable and/or there exists measurement error for both variables (Sokal and Rohlf 1994, p 545). The model II regression is the equivalent of the first principal component or a reduced major axis for the variables. To determine the relationships among leaf and root traits, we calculated pairwise correlation coefficients for each pair of traits. The ten parameters (%N, tissue density, thickness/diameter, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ for both leaves and roots) were then included in a principal components analysis (PCA) to determine the patterns of relationships among traits. The first two axes were determined to be the most biologically significant (eigenvalue of axis 3=1.3, which only explains 13% of the total explained variation, 30% more variation than expected by chance). These two axes were then rotated with the Varimax procedure in order to strengthen the contrasts between axes.

To determine the relative importance of differences among species and sites in determining the two axes, we ran regression models that predicted the plants' scores on the PCA axes with species identity and site identity. To test for differences between native and non-native species in traits and composite axis scores, we compared them at a mean altitude with a regression that included site altitude, a categorical classification of species as native or non-native, and the interaction between the two. Five plants of the *Festuca* genus could not be reliably identified to species and were excluded from comparisons of native and non-native species.

Results

Among the 118 plants sampled across the 30 sites, the N concentration and tissue density of leaves were correlated

with those of fine roots. Plants with leaves that have high N concentrations also have roots with high N concentrations ($r=0.55$, $P<0.001$) (Fig. 1a). Leaf N concentrations were on average 78% greater than root N concentrations ($1.51\% \pm 0.67\%$ vs. $0.86\% \pm 0.27\%$). Leaf tissue density correlated positively with root tissue density, though the correlation was weaker than for leaf and root N concentrations ($r=0.29$, $P<0.001$) (Fig. 1b). Root %N and tissue density were not strongly correlated ($r=-0.19$, $P<0.04$) (Fig. 1c).

As expected since both leaves and roots are derived from the same resource supply, the $\delta^{15}\text{N}$ of leaves and roots were also correlated ($r=0.49$, $P<0.001$), as well as the $\delta^{13}\text{C}$ of leaves and roots ($r=0.75$, $P<0.001$). On average, the $\delta^{15}\text{N}$ of leaves was 1.0% less than roots (-2.4 ± 0.3 vs. -1.4 ± 0.2). The slope of the orthogonal (model II regression) relationship of leaf $\delta^{15}\text{N}$ vs. root $\delta^{15}\text{N}$ was 1.43 (95% confidence interval: 1.22–1.69), indicating an increasing difference in $\delta^{15}\text{N}$ between leaves and roots as root $\delta^{15}\text{N}$ increases. Leaf $\delta^{13}\text{C}$ was slightly higher than root $\delta^{13}\text{C}$ (-27.7 ± 0.1 vs. -28.2 ± 0.1), with the relationship between leaf and root $\delta^{13}\text{C}$ not significantly different than 1 (1.34; 95% confidence interval 0.95–1.90).

With so many leaf and root functional traits and potential indirect correlations among them, broader patterns of functional traits are best analysed through multivariate analysis. In a PCA of leaf and root N concentrations, tissue density, thickness, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$, there were two independent sets of correlations in the data

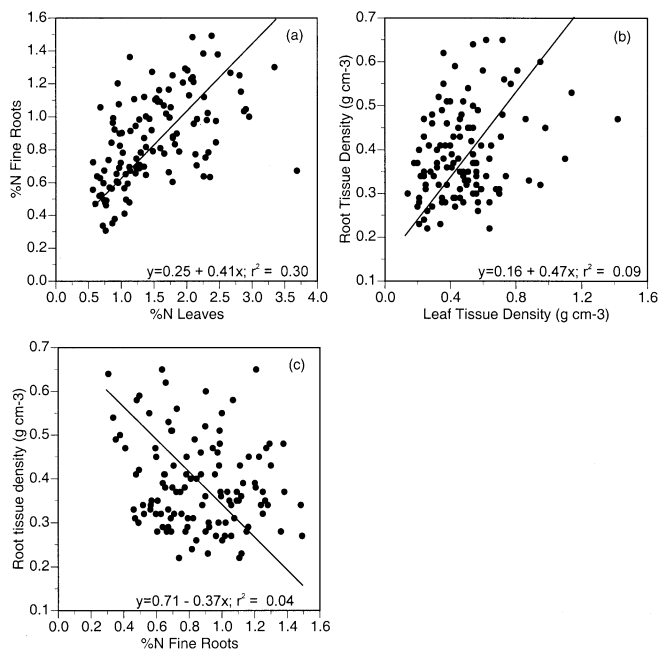


Fig. 1 Relationships between leaves and roots of 118 grass samples from the South Island of New Zealand for N concentrations (a) and tissue density (b), and the relationships between root tissue density and root N concentration (c). Regression lines are derived from model II regressions (see text for details)

Table 2. Eigenvectors for the first two principal components analysis axes. *Data in italics* indicate variable correlated with axis at $P < 0.01$. Also included is the percentage of the total variation explained by all the principal components analysis (PCA) axes that is associated with each axis

Variable	Axis 1	Axis 2
$\delta^{15}\text{N}$ leaves	<i>0.91</i>	0.02
$\delta^{15}\text{N}$ roots	<i>0.79</i>	-0.08
$\delta^{13}\text{C}$ leaves	-0.11	<i>0.69</i>
$\delta^{13}\text{C}$ roots	-0.10	<i>0.74</i>
%N leaves	<i>0.76</i>	-0.24
%N roots	<i>0.70</i>	-0.32
Leaf thickness	-0.67	-0.02
Root diameter	-0.47	0.02
Leaf density	-0.15	<i>0.45</i>
Root density	0.14	<i>0.75</i>
% Variation explained	32%	20%

set. The first set of correlations (Table 2), axis 1, is associated with differences in plant $\delta^{15}\text{N}$, %N, leaf thickness, and root diameter. Axis 1 accounted for 32% of the explained variation, 3.2 times more than expected by chance. Plants from habitats with high N supply (as inferred from the more positive leaf and root $\delta^{15}\text{N}$) have high N concentrations in both their leaves and roots and have leaves and roots that are thin, yet there are no differences in tissue density or $\delta^{13}\text{C}$.

To determine which environmental factor is most closely linked to axis 1, we averaged axis 1 scores for all plants at a site and analysed the partial correlations among axis 1, altitude, precipitation, mean annual temperature,

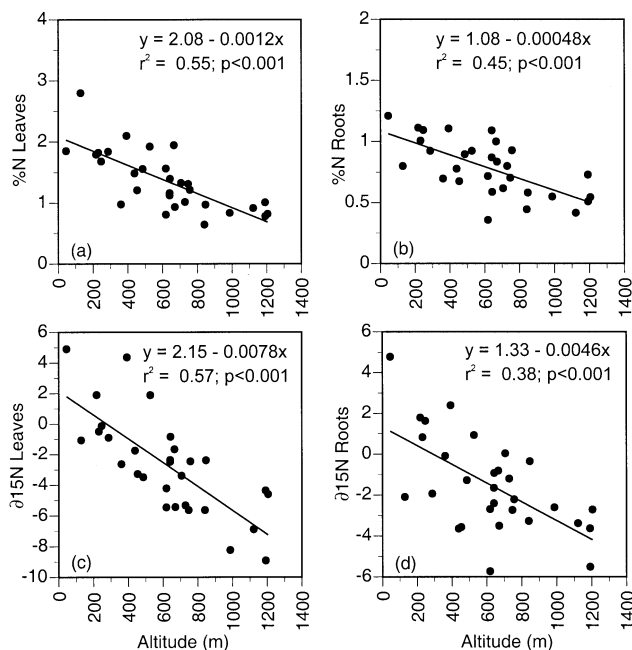


Fig. 2 Relationship between altitude and N concentrations and $\delta^{15}\text{N}$ of leaves (a, c) and roots (b, d). Data for all plants were averaged for each site before regressing against altitude

and soil pH. Although all factors are significantly correlated ($P < 0.001$) (high altitude sites have lower temperature, higher precipitation, and lower pH), axis 1 was more directly correlated with altitude ($r = -0.63$) than mean annual temperature ($r = -0.28$), precipitation ($r = -0.16$), or soil pH ($r = 0.17$). As altitude increases, the four main components of axis 1 (leaf and root %N and $\delta^{15}\text{N}$) all decrease (Fig. 2a–d). From sea level to 1,200 m, leaf N concentrations decline from 2.1% to 0.6% and root N concentrations from 1.1% to 0.5%. Leaf and root $\delta^{15}\text{N}$ decrease by 9.4‰ and 5.5‰, respectively, over the same altitudinal range. The high importance of leaf and root $\delta^{15}\text{N}$ in axis 1 could not be explained by differences in soil $\delta^{15}\text{N}$ (Table 1). There was no correlation between altitude or site-averaged axis 1 scores of the plants and soil $\delta^{15}\text{N}$ ($r = -0.17$, $P = 0.37$; $r = 0.29$, $P = 0.12$).

The high-N set of traits is associated with differences within species across sites as well as differences among species. Both site identity and species identity explained significant amounts of variation in axis 1 scores ($r^2 = 0.91$; site, $SS = 13.0$, $F = 3.6$, $P < 0.001$; species, $SS = 36.2$, $F = 8.3$, $P < 0.001$). For the seven most sampled species, all but one had decreasing axis 1 scores (lower N concentrations, thicker leaves and roots, and a more negative $\delta^{15}\text{N}$) with increasing altitude. At a common altitude, the four non-native species (*Agrostis capillaris*, *Anthoxanthum odoratum*, *Dactylis glomerata* and *Holcus lanatus*; $n = 13, 17, 7, 12$) had consistently higher scores on axis 1 than the two native species (*Chionochloa rubra*, *Festuca novae-zealandii*, and *Poa cita*; $n = 7, 10, 11$) (Fig. 3).

Across the entire data set, non-native species were consistently lower in their scores on axis 1 than non-

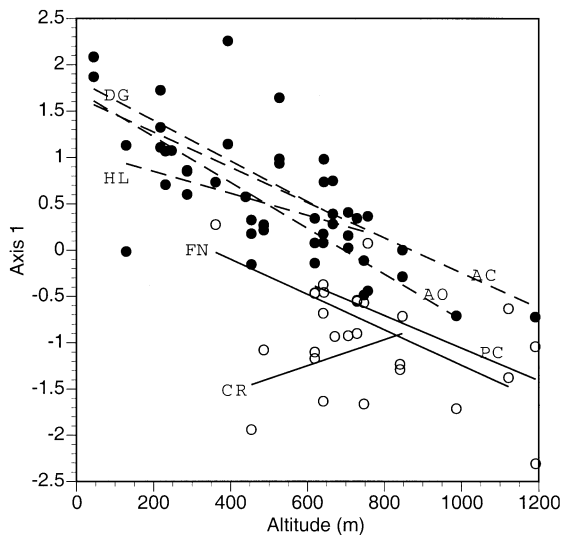


Fig. 3 Relationship between altitude and axis 1 for the four most collected non-native species [●, dashed lines: *Agrostis capillaris* (Ac), *Anthoxanthum odoratum* (Ao), *Dactylis glomerata* (Dg) *Holcus lanatus* (Hl)] and the three most collected native species [○, solid lines: *Chionochloa rubra* (Cr), *Festuca novae-zealandii* (Fn), and *Poa cita* (Pc)]

native species (Fig. 4). Comparing native and non-native species within sites via the paired *t*-test, non-native species have a more high-N strategy [mean difference (native non-native) = -0.79 ± 0.11 , $P < 0.001$]. In a regression model including altitude, plant classification as native or non-native, and the interaction between the two factors, both altitude and native/non-native classification were significant (SS=26.5, $F=79.0$, $P < 0.0001$; SS=15.2, $F=45.5$, $P < 0.0001$), without any significant interaction between the two (SS=0.1, $F=0.4$, $P=0.52$). Comparing at the mean altitude, plants of native species had lower %N, more negative $\delta^{15}\text{N}$, and thicker roots and leaves than non-native species (Table 3).

The second set of correlations in the principal component analysis (axis 2) explained less variation than axis 1 (20% of the explained variation), but still twice as much variation as expected by chance (Table 2). Axis 2 separates wet habitat plants that have lower photosynthetic water use efficiency (more negative $\delta^{13}\text{C}$) and lower tissue density from those drier site plants that have higher water use efficiency and higher tissue density (Table 2). Site identity and species identity explained similar amounts of variation in axis 2 scores (SS=38.9, $F=4.1$, $P < 0.001$; SS=33.7, $F=4.3$, $P < 0.001$). Averaging the axis 2 scores for each site, there was no relationship between the average axis 2 score and precipitation, altitude, or soil pH ($P=0.95$, $P=0.67$, $P=0.25$, respectively). Instead, sites that had plants with lower photosynthetic water use efficiency (more negative $\delta^{13}\text{C}$ Table 2) tended to be wetter sites, such as edges of bogs or adjacent to creeks (site descriptions not shown). Drier upland sites, saddles, and roadsides tended to have plants with higher water use efficiency and higher tissue density.

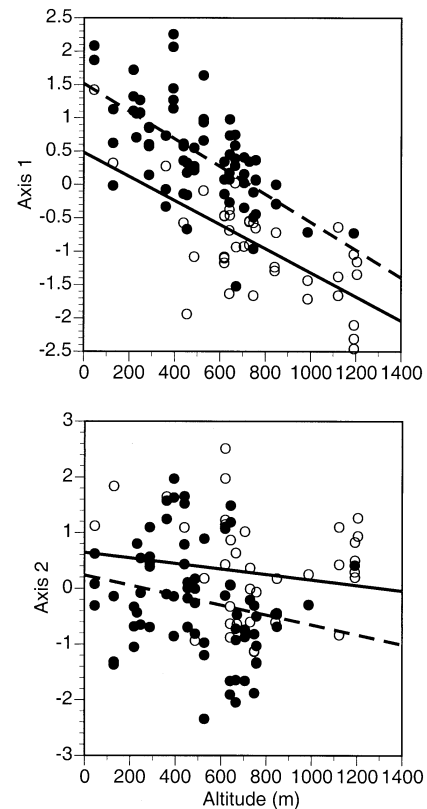


Fig. 4 Relationship between altitude and axis 1 and 2 scores for all sampled native species (○, solid lines) and non-native species (●, dashed lines). Five *Festuca* spp. could not be identified to genus and are not included

The differences in $\delta^{13}\text{C}$ and correlated variables were not just due to differences among sites – there were differences in these traits among species independent of sites. In a multiple regression model of axis 2 scores for plants that included both species and site identities, both site and species identity explained similar amounts of variation (SS=43, $F=4.7$, $P < 0.0001$; SS=35, $F=4.3$, $P < 0.0001$) and species differed independent of site.

Although we could not standardize for site wetness as we could for altitude, average axis 2 scores for species indicate that “wetter” species include *H. lanatus* (-1.00 ± 0.25), *Anthoxanthum odoratum* (-0.26 ± 0.19), and *C. rubra* (-0.40 ± 0.20), while “drier” species include *P. colensoi* (0.83 ± 0.42), and *D. glomerata* (0.25 ± 0.31). Across the entire data set, native species were significantly more positive on axis 2 than non-native species (Fig. 4), though differences were less strong than differences on axis 1 [mean difference (native non-native) = 0.57 ± 0.21 , $P < 0.05$]. This is associated with native species such as *C. rubra* having negative axis 2 scores (being “wet” species). For both leaves and roots, plants of native species on average had higher photosynthetic water use efficiency (a less negative $\delta^{13}\text{C}$) ($+0.72$, $P=0.01$; $+0.39$, $P=0.07$ for leaves and roots, respectively) and higher tissue density ($+29\%$, $P < 0.001$; $+10\%$ $P=0.04$) (Table 3).

Table 3 Least squares means (\pm SE) for native and non-native plants. Regression model for each factor included a categorical classification of native or non-native and site altitude. As such, category means are adjusted to the mean altitude and *P*-value represents whether native and non-native plants differed significantly for a given trait

	Native	Non-native	<i>P</i>
Leaf %N	1.06 \pm 0.01	1.63 \pm 0.01	<0.0001
Root %N	0.71 \pm 0.03	0.84 \pm 0.02	<0.01
Leaf tissue density (g cm ⁻³)	0.58 \pm 0.03	0.41 \pm 0.02	<0.0001
Root tissue density (g cm ⁻³)	0.41 \pm 0.02	0.37 \pm 0.01	<0.05
Leaf $\delta^{15}\text{N}$	-4.03 \pm 0.40	-1.41 \pm 0.30	<0.0001
Root $\delta^{15}\text{N}$	-2.04 \pm 0.35	0.99 \pm 0.26	0.03
Leaf $\delta^{13}\text{C}$	-27.3 \pm 0.2	-28.0 \pm 0.2	<0.01
Root $\delta^{13}\text{C}$	-28.0 \pm 0.2	-28.4 \pm 0.1	0.06
Leaf thickness (cm)	0.22 \pm 0.01	0.13 \pm 0.01	<0.0001
Root diameter (mm)	0.16 \pm 0.01	0.14 \pm 0.00	0.06
Axis 1	-0.56 \pm 0.10	0.30 \pm 0.07	<0.0001
Axis 2	0.39 \pm 0.16	-0.27 \pm 0.12	<0.01

Discussion

Leaf and root trait relationships along N supply and moisture gradients

As opposed to other studies (e.g. Craine et al. 2001), tissue density and N concentrations were not tightly linked. Although leaf and root N concentrations were correlated across both the N supply and moisture gradients, tissue density responded differently to the two gradients. Across all plants sampled, leaf N concentrations explained 30% of the variation in root %N and N concentrations decreased with both increasing altitude and decreasing site moisture. Yet, leaf tissue density only explained 9% of root tissue density overall, most likely due to there being no consistent (or weakly opposite) changes in root or leaf tissue density across the altitudinal gradient, although they were tightly linked across the moisture gradient. That N concentrations and tissue density are responding differently to different gradients led to a poor correlation between root tissue density and root %N.

High altitude sites and the native species that are more likely to grow there, such as *Chionochloa* species, *P. colensoi*, and *F. novae-zealandii*, have lower N concentrations in their leaves and roots than plants of low altitude sites. There are multiple reasons that this pattern can occur. High altitude grasslands are less likely to be fertilized, grazed, or planted with non-native grasses and legumes than lower altitude pastures. Also, the cold temperatures can inhibit mineralization leading to greater N limitation and lower N concentrations in plants at high altitude (e.g. Klingensmith and Van Cleve 1993). To our knowledge the N concentrations of green leaves observed in the native species of this study (often <0.75% N) and of fine roots (often <0.5% N) are amongst the lowest recorded for C₃ grasses anywhere. Understanding the trait relationships of the native grasses and grasslands of New Zealand in a global context will require more comparative work.

High altitude plants are less enriched in ¹⁵N, which likely reflects lower N supply rates at high altitude. Garten and Van Miegroet (1994) saw the opposite pattern in forests along an elevational transect in southeastern USA, where trees at high elevation were more enriched in

¹⁵N. Yet, in that elevational sequence, high altitude sites received higher levels of N deposition and had higher mineralization rates. As such, our results are consistent with those of Garten and Van Miegroet: sites where N mineralization should be higher are more enriched in ¹⁵N. The relationships in New Zealand between plant $\delta^{15}\text{N}$ and site altitude are not due to altitude per se, but instead factors that are correlated with altitude.

In New Zealand high altitude grasses have roots of higher average diameter [and lower specific root length (SRL)] than plants at low altitude. In contrast, Körner and Renhardt (1987) analysed the SRL of herbaceous dicots at low and high altitude and found higher SRL at higher elevations. It is unknown if differences between the two studies can be explained by differences in functional responses between monocots and dicots or if other factors are necessary. In New Zealand, high altitude grasses are more likely to be bunch grasses, which acquire resources from a larger ground area, as evidenced by the larger inter-tussock distance at high altitude than low (J. Craine, personal observation). This requires larger root networks and higher order roots that lead to a greater average diameter. Alternatively, the high average root diameter of high altitude plants could represent a greater dependence on mycorrhizae or the need for greater thrust potential (Fitter 1996), although there is evidence that high altitude herbaceous species may have lower mycorrhizal infection rates than those at low altitude (Haselwandter and Read 1980).

Wet sites and species associated with “wetter” locations have lower water use efficiency than drier sites. As indicated by the PCA analyses, the trait relationships along this gradient of sites and species is not entirely independent of N. In general, more work is necessary to better understand the nature of nutrient limitation and water limitation (Hooper and Johnson 1999). Williams et al. (1978) found little variation in macroelement concentrations of grasses along topographically controlled drainage gradient in New Zealand. Similarly, we found only weakly consistent variation in N concentrations in leaves and roots. The only trait strongly associated with water use efficiency was tissue density. The low leaf and root tissue density of “wetter” plants could be due to the need for aeration and the formation of aerenchyma in roots (Visser et al. 2000; Eissenstat et al. 2000) and/or

that dry site grasses have less water with which to expand leaves.

Differences between native and non-native species

In general, native New Zealand grasses have traits that are associated with low resource availability. Although there were both native species associated with “wet” habitats (*C. rubra*) and non-native species associated with “dry” habitats (*D. glomerata*), on average plants of native species had higher scores on axis 2, higher water use efficiency, and higher tissue density than non-native species. A much stronger pattern was that native species had a more low-N strategy than non-native species. Non-native species that have been introduced are the ones that perform well in high fertility sites, which are a recent addition to New Zealand (Lee et al. 2001). Grasslands prior to Maori settlement were mostly in low productivity sites where trees were restricted by extreme conditions (boggy areas or alpine areas). There was no history of mammalian grazers or fire in New Zealand to maintain grasslands. Just as New Zealand has few endemic annual plants, associated with the rarity in space and time of conditions that are conducive for the growth of annuals, there must have been few stable high fertility grasslands and little opportunity for the evolution of high fertility grasses. Grasses that Europeans brought to New Zealand were largely selected for their ability to grow in pastures that were either fertilized and/or grown in association with non-native N-fixing plants such as clovers. Over the past 100 years, areas where fertilization or clover growth has ceased have often reverted back to native grasses and other low-N plants like bracken fern.

Smith and Knapp (2001) found little consistent differences among invasive non-native, non-invasive non-native, and native species in a North American tallgrass ecosystem. The only pattern found was that specific leaf area (SLA) (of which tissue density is a component along with thickness) was consistently higher for the non-native species. A similar pattern was found in Hawaii (Baruch and Goldstein 1999), where non-native species had higher SLA and N concentrations than non-native species. Although many factors such as disturbances and climate affect the pattern of plant invasions, it is possible that much of the patterns of plant invasion into historically nutrient-poor areas is associated with recent increases in nutrient availability. For example, Tilman (1987) observed that when nutrient-poor, diverse tallgrass communities were chronically fertilized with N, non-native, European grass species eventually dominated. These species have root and leaf traits such as lower tissue density (Craine et al. 2001), which in other studies at Cedar Creek have been linked to high N concentrations and appear to represent a general suite of traits for growth at high N supply (Grime et al. 1997; Craine et al. 2002). Although not an explanation for all patterns of non-native plant species establishment, the inherent ability for non-native species to grow and compete well at high nutrient

supply is likely to be a consistent factor determining their invasion success considering the global nature of eutrophication of ecosystems (Vitousek et al. 1997).

Estimating root longevity from leaf traits

If the relationships between leaf traits and root traits seen across the gradients measured here are general relationships, we could have the potential to predict a wide range of important root-associated parameters with simple measurements on leaves. For example, if there are general relationships between root longevity and root %N (Craine et al. 2002), leaf N could predict root longevity and help estimate belowground NPP. Consistent deviations in relationships between leaf and root traits would lead to consistent errors in estimating fine root longevity. For example, for the New Zealand plants sampled here, at low leaf %N there is a hysteresis in the relationship between leaf and root %N. There is little consistent change in leaf %N as root %N declines from 0.6% to 0.2% (Fig. 1). If root longevity would continue to increase over this range as root %N declined, then estimates of root longevity based on leaf %N will underestimate the longevity of potentially long-lived roots. As such, it is very important that attempts to estimate fine root longevity from leaf traits thoroughly examine the relationships between leaf and root traits.

Conclusions

There was consistent covariation in root and leaf traits along the two environmental gradients, yet the pattern of trait relationships differed between the N supply and site moisture gradients. This should be a note of caution regarding the generality of trait relationships, which need to be tested across multiple gradients. The finding that leaf traits could be used to predict root traits, though with caveats, is encouraging and further research that develops this potential is warranted. Standardizing for site differences, native species consistently had root and leaf traits that were associated with low N and water availability. This most likely reflects the longer evolutionary history of the non-native, generally European grass species with high fertility grasslands. As such patterns of plant species distribution and native and non-native invasions into established grasslands should benefit from considering resource availability.

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