

Integrated plant phenotypic responses to contrasting above- and below-ground resources: key roles of specific leaf area and root mass fraction

Grégoire T. Freschet¹, Elferra M. Swart² and Johannes H. C. Cornelissen²

¹Centre d'Ecologie Fonctionnelle et Evolutive, UMR 5175 (CNRS – Université de Montpellier – Université Paul-Valéry Montpellier – EPHE), 1919 route de Mende, Montpellier 34293, France; ²Systems Ecology, Department of Ecological Sciences, VU University, de Boelelaan 1085, Amsterdam 1081 HV, the Netherlands

Summary

Author for correspondence:

Grégoire T. Freschet

Tel: +33 4 67 61 33 40

Email: gregoire.freschet@cefe.cnrs.fr

Received: 29 October 2014

Accepted: 3 February 2015

New Phytologist (2015) **206**: 1247–1260

doi: 10.1111/nph.13352

Key words: biomass allocation, functional equilibrium hypothesis, leaf mass fraction (LMF), light and nutrient treatments, plant phenotypic plasticity, root mass fraction (RMF), specific leaf area (SLA), specific root length (SRL).

- Plants adapt phenotypically to different conditions of light and nutrient supply, supposedly in order to achieve colimitation of these resources. Their key variable of adjustment is the ratio of leaf area to root length, which relies on plant biomass allocation and organ morphology.
- We recorded phenotypic differences in leaf and root mass fractions (LMF, RMF), specific leaf area (SLA) and specific root length (SRL) of 12 herbaceous species grown in factorial combinations of high/low irradiance and fertilization treatments.
- Leaf area and root length ratios, and their components, were influenced by nonadditive effects between light and nutrient supply, and differences in the strength of plant responses were partly explained by Ellenberg's species values representing ecological optima. Changes in allocation were critical in plant responses to nutrient availability, as the RMF contribution to changes in root length was 2.5× that of the SRL. Contrastingly, morphological adjustments (SLA rather than LMF) made up the bulk of plant response to light availability.
- Our results suggest largely predictable differences in responses of species and groups of species to environmental change. Nevertheless, they stress the critical need to account for adjustments in below-ground mass allocation to understand the assembly and responses of communities in changing environments.

Introduction

Plant above- and below-ground structures have as their primary role the acquisition of resources for growth and defence. Although there is broadly coordinated interspecific variation of leaf and root biomass allocation (Enquist & Niklas, 2002) and morphology above- vs below-ground (Freschet *et al.*, 2010a; Laughlin *et al.*, 2010), these relationships can be largely modified by plant phenotypic adjustments to variable environmental conditions (Liu *et al.*, 2010; Poorter *et al.*, 2012b; Freschet *et al.*, 2013a). For instance, changes in the availability of any one resource, either above or below ground, generally trigger phenotypic changes in functional traits that determine the ability of plants to acquire, and thereby mitigate the constraints imposed by, the limiting resource (Chapin *et al.*, 1987; van Kleunen & Fischer, 2005; Valladares *et al.*, 2007). Above ground, decreasing light supply commonly drives an increase in total leaf area for a given unit of plant biomass (i.e. leaf area ratio (LAR); Reich *et al.*, 1998). This results from increases in the area of light capture per biomass invested in leaves (i.e. specific leaf area (SLA); Rijkers *et al.*, 2000; Evans & Poorter, 2001), and/or the proportion of total plant mass allocated to leaves (i.e. leaf mass fraction (LMF); Poorter *et al.*, 2012b). In a corresponding way, below

ground, increasing nutrient limitation generally drives increases in the length of root for a given unit of plant mass (i.e. root length ratio (RLR); Ryser & Lambers, 1995). This follows from increases in the total length of root achieved per unit root mass (specific root length (SRL); Hill *et al.*, 2006; Ostonen *et al.*, 2007) and/or the proportion of total plant mass allocated to roots (i.e. root mass fraction (RMF); Poorter *et al.*, 2012b).

A recent meta-analysis by Poorter *et al.* (2012b) suggests that, in response to variation in resource availability above ground, the major part of plant adjustments in leaf area is driven by plastic changes in SLA, rather than in LMF. However, no such analysis has been conducted below ground and it is still unclear whether plastic changes in root morphology also play a stronger role than changes in RMF in the total length of root deployed below ground. Estimating this would tell us how important these processes are in the overall response of plants to changes in environmental conditions. Large changes in RMF below ground would, for instance, stress the need to include biomass allocation changes in predictions of plant responses to environmental conditions (Poorter *et al.*, 2012b) and the subsequent effects of these changes on ecosystem properties (Freschet *et al.*, 2013b).

While plant responses to changes in any single resource have been the focus of many studies, it remains largely overlooked

how plants deal with concomitant variation in several environmental factors (Chapin, 1991; de Kroon *et al.*, 2005; Freschet *et al.*, 2013a). Indeed, acquisition of the main above-ground resource (i.e. carbon through photosynthesis) relies, for instance, on several other resources, especially light, water and nutrients (Maire *et al.*, 2012). As a result, the effect of light shortage on SLA can shift from positive to null when nitrogen (N) supply decreases (Meziane & Shipley, 1999). To understand the effects of environmental changes on plants, it is thus critical to better document the complex integration of plant responses to multiple environmental stresses (Chapin, 1991; de Kroon *et al.*, 2005). Estimating the nonadditive component of plant responses to changes in light and nutrient availability, for instance, is critical to our understanding of the assembly of plant communities and their responses to ongoing global changes.

Despite some degree of variation in plant resource requirements across species, basic needs regarding investments in primary metabolic machinery (e.g. chloroplasts, mitochondria, ribosomes) are common to all plant species (Elser *et al.*, 2010). Consequently, acquiring elemental resources such as carbon and nutrients in a way that is consistent with these basic requirements (i.e. achieving carbon–nutrient colimitation) can maximize plant benefits while minimizing resource acquisition costs (Maire *et al.*, 2013). Many aspects of plants' life strategy impact on their above- and below-ground resource acquisition capacities. This includes, for instance, species' potential nutrient uptake per unit root deployed (Maire *et al.*, 2009), plant size and architecture (Fourcaud *et al.*, 2008; Rees *et al.*, 2010), the type and intensity of mycorrhizal associations (e.g. Ryser, 2006; Osmont *et al.*, 2007), species' capacity to take up distinct forms of the same resource (e.g. N in nitrate, ammonium, amino acids; Ashton *et al.*, 2010), and species' ability to recycle nutrients from senescing tissues (above ground (Chapin & Moilanen, 1991) and below ground (Freschet *et al.*, 2010b)) and to optimize the absolute difference between carbon assimilation and respiration (Lambers *et al.*, 2002). Nevertheless, a particularly critical and widespread way to modulate resource acquisition and to achieve carbon–nutrient colimitation is for plants to modify their ratio of total surface of leaves for capturing light to total length of roots for exploring soil (Ryser & Eek, 2000; Reich, 2002). In a subalpine flora, this ratio indeed appeared critical enough that it showed only little variation among co-occurring species (Freschet *et al.*, 2014; see explanation in Fig. 1a). However, different species achieved similar total leaf area to total RLRs either by maintaining more biomass in leaves but with lower SLA and less biomass in roots but with higher SRL, or by maintaining more biomass in roots but with lower SRL and less biomass in leaves but with higher SLA (Fig. 1b). It is still unknown how the balance between these two trends varies across contrasting conditions of above- and below-ground resource supply for different species (Fig. 1c).

Whereas it is generally assumed that most plant species would show at least the same directional response to the same change in a single environmental variable, evidence is growing that distinct resource requirements between species (e.g. xerophilous vs hygrophilous species), distinct tolerance to stresses (e.g.

shade-tolerant vs shade-intolerant) or differences in species' preferences for distinct forms of nutrients (McKane *et al.*, 2002; Turner, 2008) can potentially lead to variable responses of species to the same environmental change (both in amplitude and in direction of the response in allocation and morphology; Valladares *et al.*, 2000; Poorter *et al.*, 2009, 2012b). Such differences, probably mediated by variation in plant physiology, size or growth form, may be frequent and greatly complicate predictions of plant responses to environmental changes (Thuiller *et al.*, 2008; Lavorel *et al.*, 2009). However, whereas phylogenetic conservatism of plant responses to environmental change is generally rare (Bennett & Cahill, 2013), it remains poorly understood whether clear predictable patterns exist across major taxa, such as between monocots and eudicots, or between plant functional types, such as shade-tolerants vs shade-intolerants, or nutrient-conservative vs nutrient-demanding species (e.g. Poorter *et al.*, 2009).

Here we first test the hypothesis that species morphology and biomass allocation respond to light and nutrient availability according to the common assumptions described earlier, such that species ratios of leaf area to root length increase from the most nutrient-limited to the most light-limited treatment. Second, we hypothesize that, symmetrically to what was previously described above ground for LAR, below-ground differences in RLR across resource regimes are predominantly driven by plastic adjustments in root morphology rather than by adjustments in root biomass allocation. Third, we test the hypothesis that contrasting amounts of above- and below-ground resources trigger nonadditive effects on plant leaf and root biomass allocation and morphology. Fourth, we hypothesize that if all species tend towards achieving carbon–nutrient colimitation, there will probably be little variation in species ratio of total leaf area to total root length within each treatment, owing to the counteracting effect of leaf and root morphology (i.e. SLA and SRL) on the leaf–root biomass allocation. Finally, we hypothesize that plant responses vary among species, owing to differences in, for example, species growth form as related to deep time evolutionary history (monocot vs eudicot) and plant environmental optima with regard to light and nutrients (Ellenberg's indicator values; Ellenberg, 1988; see Table 1). We test these hypotheses by recording the responses in root and leaf mass allocation and morphology of 12 temperate herbaceous species to each of four factorial combinations of high and low irradiance and fertilization treatments.

Materials and Methods

Experimental design

The experiment consisted of 240 plants from 12 northwest-European herbaceous species (see Table 1 for a list of species and characteristics) grown in four factorial combinations of high and low irradiance crossed with high- and low-fertilization treatments with five replicate plants per treatment. Seeds were collected from several ecosystems in the Sheffield region, UK, including limestone grassland, heathland and grassland on acidic sandstone, woodlands and ruderal sites (details in Cornelissen *et al.*, 2011).

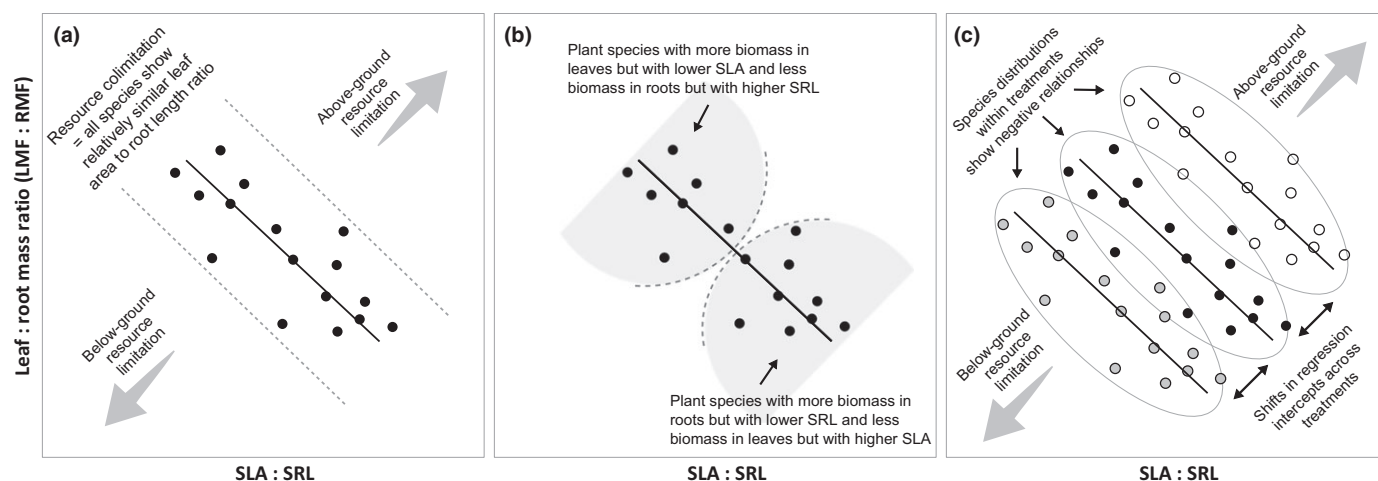


Fig. 1 Plant species are likely to grow and survive more successfully when they achieve resource colimitation above and below ground. Therefore, we expect that most plant species show relatively similar ratios of total leaf area capturing light to total root length exploring soil (LAR : RLR, leaf area ratio : root length ratio). As $LAR = LMF \times SLA$ and $RLR = RMF \times SRL$, this ratio varies according to two other ratios: the ratio of leaf to root biomass (LMF : RMF, leaf mass fraction : root mass fraction) and the ratio of leaf to root morphology (SLA : SRL, specific leaf area : specific root length). (a) A negative relationship between species LMF : RMF and SLA : SRL, showing a slope close to -1 , would indicate that all species show relatively similar LAR : RLR; and (b) the spread of species along this negative relationship reflects how different species can all achieve similar LAR : RLR, despite contrasting investments in leaf vs root biomass and SLA vs SRL. Finally, the intercept of this negative relationship (c) is expected to change across treatments, following changes in above- or below-ground resource availability, as plants phenotypically adjust leaf and root biomass and morphology.

Table 1 Species list and characteristics

	Plant clade	Ellenberg's indicator values		Average (and range) of trait values across treatments			
		Nutrient	Light	SLA ($\text{cm}^2 \text{g}^{-1}$)	SRL (m g^{-1})	LMF (g g^{-1})	RMF (g g^{-1})
<i>Anthoxanthum odoratum</i>	Monocot	3	7	263 (139–472)	217 (82–440)	0.53 (0.34–0.71)	0.31 (0.12–0.54)
<i>Arrhenatherum elatius</i>	Monocot	7	7	367 (199–626)	137 (25–361)	0.46 (0.23–0.78)	0.33 (0.05–0.63)
<i>Brachypodium pinnatum</i>	Monocot	3	7	303 (165–502)	269 (148–427)	0.52 (0.29–0.72)	0.33 (0.10–0.64)
<i>Chamerion angustifolium</i>	Eudicot	5	6	557 (287–987)	296 (149–444)	0.70 (0.57–0.85)	0.23 (0.09–0.43)
<i>Chenopodium album</i>	Eudicot	7	7	408 (192–715)	383 (183–543)	0.26 (0.13–0.41)	0.14 (0.06–0.31)
<i>Digitalis purpurea</i>	Eudicot	5	6	465 (151–871)	338 (91–550)	0.74 (0.44–0.90)	0.25 (0.10–0.45)
<i>Eriophorum vaginatum</i>	Monocot	1	8	86 (48–127)	178 (79–463)	0.51 (0.34–0.76)	0.17 (0.02–0.30)
<i>Galium aparine</i>	Eudicot	8	6	554 (364–861)	437 (49–789)	0.53 (0.37–0.69)	0.22 (0.03–0.41)
<i>Leontodon hispidus</i>	Eudicot	3	8	406 (259–621)	151 (18–260)	0.56 (0.18–0.91)	0.39 (0.09–0.82)
<i>Lotus corniculatus</i>	Eudicot	2	7	271 (135–425)	98 (41–149)	0.41 (0.17–0.63)	0.33 (0.09–0.64)
<i>Plantago lanceolata</i>	Eudicot	4	7	268 (145–461)	228 (101–434)	0.72 (0.29–0.93)	0.28 (0.05–0.62)
<i>Poa annua</i>	Monocot	7	7	422 (168–1034)	510 (200–1000)	0.55 (0.24–0.70)	0.22 (0.08–0.64)

Nomenclature follows Stace (1991). SLA, specific leaf area; SRL, specific root length; LMF, leaf mass fraction; RMF, root mass fraction.

Seeds of all 12 species were germinated on moist filter paper and then transferred to sand-filled trays. Seed batches were first germinated to determine the average time needed for each species to display well-developed leaves, and then a second time in order to obtain well-developed seedlings of all 12 species simultaneously. The experimental treatment started once 20 random seedlings of each species had been transferred to individual 275 ml pots filled with an even mix of sand and low-nutrient soil ($70 \text{ g l}^{-1} \text{ N}$; 60 g l^{-1} phosphorus (P)), and randomly dispatched into five experimental blocks.

Along the experiment, temperature fluctuated with day : night cycles within the boundaries of 10 and 30°C . Plants were watered

regularly, from once a day at the start of the experiment to twice a day at later stages. The high-fertilization treatment received, in addition, $10 \text{ g m}^{-2} \text{ N}$, 2.5 g m^{-2} potassium (K) and 1 g m^{-2} P by additions of a nutrient solution containing $0.45 \text{ g l}^{-1} \text{ N}$, $0.11 \text{ g l}^{-1} \text{ K}$ and $0.045 \text{ g l}^{-1} \text{ P}$ made from NH_4NO_3 and K_2HPO_4 in increasing amounts over time: from 5 to 35 ml over the course of the experiment. The low-nutrient treatment received no additional fertilization. In the growth chamber, light was supplied by 400 W metal halide lamps with a 12 : 12 h, light : dark cycle. Within each block, plant position was regularly rearranged to avoid side-effects. The high-light treatment had an average photon flux density of $c. 400 \mu\text{mol m}^{-2} \text{ s}^{-1}$, whereas the

low-light treatment (using black shading mesh) received an average of $c. 100 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Plant processing and trait measurements

Plant species were harvested (in June–July 2008) from 6 to 12 wk after the start of the experiment (in early May 2008) depending on their stage of development, that is, during the major growth before peak biomass and before heading. Signs of heading (reproductive phase) were only recorded for individuals of *Chenopodium album* and *Poa annua* in the high-light \times high-nutrient treatment. All individuals of the same species were harvested on the same day. Signs of pot-boundness were observed for most individuals of the high-light \times high-nutrient treatment, but this did not occur in other treatments. Nevertheless, as pot-boundness strongly limits total plant biomass but only slightly affects plant traits and the distribution of biomass between the different organs of plants, this slight bias should not affect our analyses of how organ mass fractions and traits vary across treatments (Poorter *et al.*, 2012a). Plant individuals were wrapped in moist filter paper and sealed in a polythene bag at 4°C for a maximum of 24 h before processing. For the low-light \times high-nutrient treatment, only two replicate individuals of the species *Eriophorum vaginatum* survived and this species \times treatment combination was excluded from further analysis. After harvest, each individual plant was cleaned with water and sorted into its leaf, stem, flower and root components. For grasses, only laminae were taken as leaves, and sheaths were considered part of the stems that they tightly embraced. For each individual, the total leaf area was measured using a Li-Cor 3100 leaf area meter (Li-Cor, Lincoln, NE, USA), and the total root length was estimated following Tennant's 1.5 cm line-intercept method (Tennant, 1975). All plant parts were subsequently dried at 60°C for 48 h and weighed. Total plant dry mass was calculated as the sum of all plant parts. SLA ($\text{cm}^2 \text{g}^{-1}$) and SRL (m g^{-1}) were estimated as the ratio of leaf area to leaf dry mass, and root length to root dry mass, respectively. These morphological traits represent the surface or length of organ formed per amount of biomass invested, that is, in the context of resource acquisition, the organ-level cost of above-ground and below-ground resource interception (notwithstanding respiration costs; Poorter *et al.*, 2009). In herbaceous species (without thick primary roots for resource storage, as in our study) where entire root systems are generally considered, SRL rather than specific root area is preferably measured because it better represents the volume of soil potentially explored by roots and is largely driven by roots of the thinnest root orders (Ryser, 2006), which are more strongly associated with the absorption function (Guo *et al.*, 2008). For each plant, the LMF and RMF were calculated as proportions of the total plant dry mass (g g^{-1}). Species-level trait values and ratios for each light \times fertilization treatment were estimated as averages of values and ratios of all five replicate individuals per treatment, in order to give equal weight to each plant individual independent of its size. Species LAR and RLR, that is, the leaf area and fine root length per total plant dry mass (in $\text{cm}^2 \text{g}^{-1}$ and m g^{-1} ,

respectively), were calculated as the product of species SLA and LMF, and of SRL and RMF, respectively.

Data analysis

We used three-way type III ANOVAs to test the effects of species identity, light and nutrient supply, and their interactions on LAR and RLR as well as their respective components, SLA and LMF, and SRL and RMF. As we were interested in the interactive effects among species identity, light and nutrient supply on the proportional changes in SLA, SRL, LMF, RMF, LAR and RLR, rather than on their absolute changes, we performed all analyses on \log_2 -transformed data. Indeed, data transformation can interfere with the interpretation of interaction terms (Day & Quinn, 1989). Here, for instance, irrespective of whether a species SLA value is high or low, each phenotypic change doubling SLA value will be represented by the same proportional increase in \log_2 -transformed SLA. All \log_2 -transformed data satisfied the assumptions of parametric analyses.

Standardized major axis (SMA) regressions (SMATR package; see details in Warton *et al.*, 2006) were used to describe the variations, across light \times fertility treatments, of interspecific relationships in several leaf and root characteristics: LMF and RMF, LAR and RLR, and SLA and SRL. Further, in order to test for changes in these relationships across treatments, the homogeneity of SMA regression slopes of all treatments was tested via a permutation test. We used SMA regressions because, in contrast to simple linear regressions, they do not assume a unidirectional effect of one parameter over the other.

For each plant species separately, we calculated the relative contributions of plastic changes in LMF and SLA to variation in plant LAR, and of RMF and SRL to plant RLR, following the variance partitioning method of Rees *et al.* (2010). Specifically, we partitioned the variance in LAR and RLR of every plant species across all four treatments at once, and across all pairs of treatments separately. Nevertheless, this variance partitioning was only performed when substantial variation (i.e. $\geq 15\%$) in LAR or RLR was observed across treatments, so as to avoid meaningless results. Briefly, given that $\text{LAR} = \text{LMF} \times \text{SLA}$ and $\text{RLR} = \text{RMF} \times \text{SRL}$, these equations can be expressed as: $\text{lar} = \text{lmf} + \text{sla}$, and $\text{rlr} = \text{rmf} + \text{srl}$, where lowercase characters are used to indicate \log_e -transformed variables. Consequently, the variance decomposition of lar , for instance, can be expressed as: $\text{Var}(\text{lar}) = \text{Var}(\text{lmf}) + \text{Var}(\text{sla}) + 2 \times \text{Cov}(\text{lmf}, \text{sla})$, where Var is the variance and Cov is the covariance. Following this, the contribution of variation in lmf to the variation in lar can be written as: $\text{Cont}(\text{lmf}) = (\text{Var}(\text{lmf}) + \text{Cov}(\text{lmf}, \text{sla})) / \text{Var}(\text{lar})$. Additionally, we partitioned the variation in LAR and RLR of each species for changes in single environmental parameters.

We also used SMA regressions to test for potential counteracting effects of the SLA to SRL and LMF to RMF ratios on the ratio of LAR to RLR. For each light \times fertility treatment, SMA regression was performed between (\log_2 -transformed) ratios of LMF : RMF and SLA : SRL across all 12 species. Homogeneity among SMA regression slopes of all four treatments was tested via a permutation test and, assuming constant slopes, differences

Three-way type III ANOVAs were also used to test the effect of species phylogeny (monocot vs eudicot) on species response to different light and nutrient supplies (i.e. the interaction between phylogeny and either of light and nutrient supply). Finally, three-way type III ANCOVAs were used to test the effect of species' Ellenberg's indicator values for light and nutrient preferences on species responses to different light and nutrient supplies, respectively (i.e. the interaction between Ellenberg's values and either light or nutrient supply). Ellenberg's indicator values are observation-based numbers on a scale from 1 to 9 describing the environmental optimum of a given species in relation to a given environmental factor (Ellenberg, 1988). Ellenberg's values for British plants were taken from Hill *et al.* (1999).

Species ratios of leaf area to root length increase from the most nutrient-limited to the most light-limited treatment

Total plant leaf and root mass varied strongly between treatments (Fig. 2a). Within each treatment, species showed coordinated variation in leaf and root mass (Fig. 2a). Nevertheless, no significant relationships were observed between LMF and RMF within any of the four treatments ($P > 0.12$ in all cases; Fig. 2b), indicating that these relationships were driven by differences in total plant mass rather than by coordination between plant allocation in LMF vs RMFs. Total plant leaf area and root length also varied strongly between treatments (Fig. 2c). Within each treatment, species showed coordinated variation in leaf area and root length (Fig. 2c). However, in contrast to leaf mass:root mass relationships, most leaf area:root length relationships remained significant after correcting for plant mass (Fig. 2d), indicating that these relationships were only partly driven by differences in total plant mass.

Table 2 Effects of species identity, light and nutrient supply, and their interactions, on leaf and root characteristics as revealed by three-way type III ANOVAs

Leaf traits										Root traits																			
SLA					LMF					LAR					SRL					RMF					RLR				
Effect	df	+/-	F	P _≤	+/-	F	P _≤	+/-	F	P _≤	+/-	F	P _≤	+/-	F	P _≤	+/-	F	P _≤	+/-	F	P _≤							
Species	10		56.3	0.001		62.32	0.001		71.97	0.001		36.31	0.001		18.50	0.001		19.79	0.001										
Light	1	-	819.6	0.001	-	112.29	0.001	-	839.78	0.001	-	43.18	0.001	+	136.34	0.001	+	4.13	0.04										
Nutrient	1	+	72.5	0.001	+	144.24	0.001	+	236.98	0.001	-	54.30	0.001	-	480.45	0.001	-	524.42	0.001										
Species × light	10		6.3	0.001		2.22	0.02		4.31	0.001		1.38	0.19		2.72	0.004		1.16	0.32										
Species × nutrient	10		10.9	0.001		18.47	0.001		13.44	0.001		3.08	0.001		3.07	0.001		2.55	0.007										
Light × nutrient	1	-	20.8	0.001	o	0.00	0.98	-	11.08	0.001	o	2.71	0.10	+	15.01	0.001	o	1.53	0.22										
Species × light × nutrient	10		1.8	0.07		1.31	0.23		1.05	0.41		0.54	0.86		1.77	0.07		1.12	0.35										

+/-/-o indicate positive, negative and absent trends between effects and traits, respectively. SLA, specific leaf area; LAR, leaf area ratio; SUR, specific root length; RMF, root mass fraction; RLR, root length ratio.

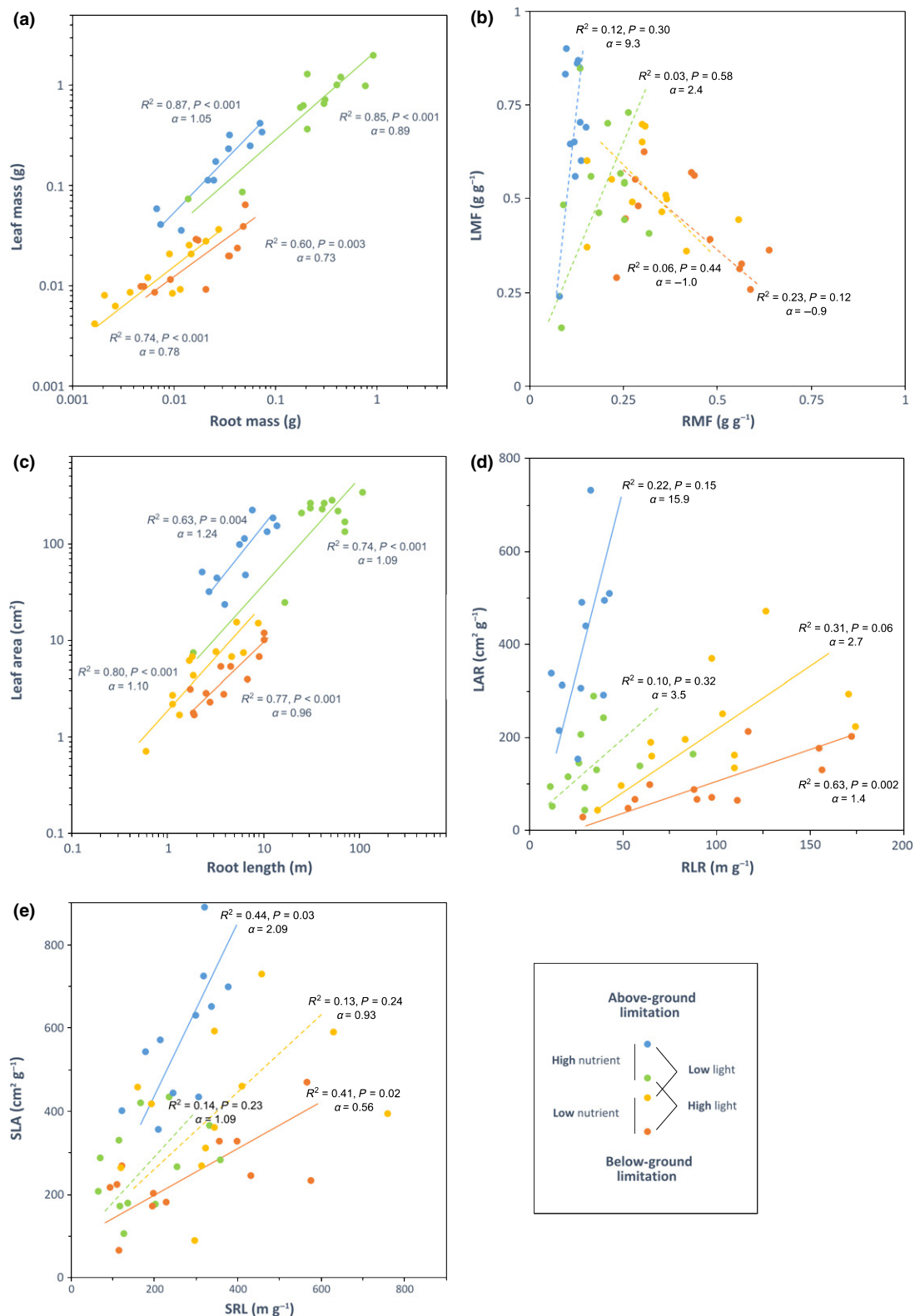


Fig. 2 Standardized major axis (SMA) regressions between leaf mass and root mass (a), leaf and root mass fractions (LMF and RMF, respectively) (b), leaf area and root length (c), leaf area ratio and root length ratio (LAR and RLR, respectively) (d), and specific leaf area and specific root length (SLA and SRL, respectively) (e) across the 12 herbaceous species within each factorial light \times nutrient supply treatment. Significant relationships (solid lines) and nonsignificant relationships (dashed lines, $P > 0.05$) are shown; α -values are the regression slopes.

Table 3 Relative contribution of the variation in leaf and root mass fractions (LMF and RMF, respectively) vs specific leaf area and specific root length (SLA and SRL, respectively) across treatments to the total variation in leaf area ratio (LAR) and root length ratio (RLR) of each plant species

Leaf area ratio (LAR = LMF × SLA)	Variation in light supply				Variation in nutrient supply				Variation in light and nutrient supply	
	Low nutrient		High nutrient		Low light		High light		SLA	LMF
	SLA	LMF	SLA	LMF	SLA	LMF	SLA	LMF		
Monocots (average for)	0.56	0.44	0.66	0.34	0.58	0.42	–	–	0.64	0.36
<i>Anthoxanthum odoratum</i>	0.64	0.36	0.79	0.21	0.61	0.39			0.67	0.33
<i>Arrhenatherum elatius</i>	0.61	0.39	0.58	0.42	0.44	0.56	0.49	0.51	0.57	0.43
<i>Brachypodium pinnatum</i>	0.50	0.50	0.66	0.34	0.51	0.49			0.50	0.50
<i>Eriophorum vaginatum</i>	0.50	0.50							0.64	0.36
<i>Poa annua</i>			0.63	0.37	0.78	0.22			0.80	0.20
Eudicots (average for)	0.72	0.28	0.78	0.22	0.45	0.55	–	–	0.67	0.33
<i>Chamerion angustifolium</i>	0.93	0.07	0.81	0.19	0.62	0.38			0.79	0.21
<i>Chenopodium album</i>	0.72	0.28	0.66	0.34					0.58	0.42
<i>Digitalis purpurea</i>	0.80	0.20	0.99	0.01					0.93	0.07
<i>Galium aparine</i>			0.88	0.12					0.75	0.25
<i>Leontodon hispidus</i>	0.72	0.28	0.56	0.44	0.25	0.75	0.31	0.69	0.47	0.53
<i>Lotus corniculatus</i>	0.38	0.62	0.78	0.22	0.49	0.51			0.47	0.53
<i>Plantago lanceolata</i>	0.77	0.23	0.77	0.23					0.71	0.29
Root length ratio (RLR = RMF × SRL)	SRL	RMF	SRL	RMF	SRL	RMF	SRL	RMF	SRL	RMF
Monocots (average for)	–	–	–	–	0.22	0.78	0.23	0.77	0.30	0.70
<i>Anthoxanthum odoratum</i>					0.22	0.78	0.44	0.56	0.41	0.59
<i>Arrhenatherum elatius</i>					0.06	0.94	0.45	0.55	0.21	0.79
<i>Brachypodium pinnatum</i>					0.05	0.95	–0.45	1.45	–0.15	1.15
<i>Eriophorum vaginatum</i>	2.10	–1.10					–0.12	1.12	0.51	0.49
<i>Poa annua</i>			–0.20	1.20	0.54	0.46	0.84	0.16	0.50	0.50
Eudicots (average for)	–	–	–	–	0.16	0.84	0.37	0.63	0.25	0.75
<i>Chamerion angustifolium</i>					0.06	0.94	0.66	0.34	0.32	0.68
<i>Chenopodium album</i>	0.10	0.90			0.23	0.77	0.15	0.85	0.18	0.82
<i>Digitalis purpurea</i>					0.33	0.67	0.37	0.63	0.36	0.64
<i>Galium aparine</i>					0.50	0.50	0.61	0.39	0.56	0.44
<i>Leontodon hispidus</i>					–0.26	1.26	0.06	0.94	–0.09	1.09
<i>Lotus corniculatus</i>					–0.01	1.01	0.25	0.75	0.16	0.84
<i>Plantago lanceolata</i>			–0.78	1.78	0.31	0.69	0.50	0.50	0.30	0.70

Changes in LAR and RLR were partitioned into their organ biomass and morphology components only when substantial variations ($\geq 15\%$) in LAR and RLR, respectively, were observed so as to avoid meaningless results. Note that negative contributions of any given trait indicate that this trait contributes in the opposite direction to that of LAR or RLR; and because the sum of both trait contributions always amounts to 100%, the other trait appears to contribute $> 100\%$.

SLA : SRL (Fig. 2e) relationships ($P < 0.001$ in all five cases), indicating strong and predictable changes in these ratios across treatments.

Predominance of morphological changes above ground vs change in biomass allocation below ground

Above ground, the LAR of all species responded significantly to changes in light supply, and 38–99% of the plastic variation in LAR was the result of changes in SLA, depending on the species considered (Table 3). Averaged across all species, SLA contributed 72% of the variation in LAR, compared with 28% for LMF, a 2.5-fold larger contribution (Fig. 3). By contrast, in response to changes in nutrient supply, only seven of the 12 species showed significant changes in LAR, and the contribution of SLA to changes in LAR varied from 25 to

78% among these seven species, with an average of only 50% across species.

Below ground, in most species, RLR did not respond significantly to changes in light supply (Table 3). However, in all species, RLR responded significantly to changes in nutrient supply, and from 0 to 84% of the plastic variation in RLR was the result of changes in SRL depending on the species (Table 3). Averaged across all species, SRL contributed only 29% of the variation in RLR, as compared with 71% for LMF, a 2.5-fold smaller contribution (Fig. 3).

Additive and nonadditive effects of light and fertilization on plant biomass allocation and morphology

A significant interaction was found between nutrient and light on LAR, as mediated by significant interaction between nutrient and

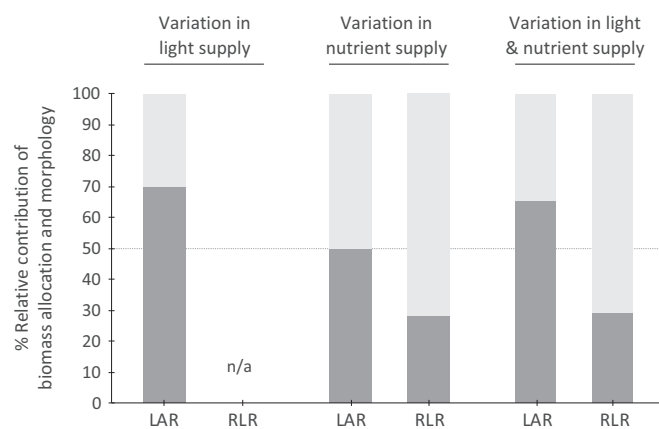


Fig. 3 Relative contribution of leaf and root biomass allocation (leaf mass fraction (LMF) or root mass fraction (RMF), light grey bars) and morphology (specific leaf area (SLA) or specific root length (SRL), dark grey bars) variables to the total variation in leaf area ratio (LAR) and root length ratio (RLR), averaged across all species (Table 4). n/a, not applicable, as most species displayed no variation in RLR in response to variation in light supply.

light on SLA but not on LMF (Table 2). Specifically, SLA and LAR increased more in response to low light supply at high vs. low nutrient supply (Fig. 4). A significant interaction was also found between nutrient and light supplies on RMF (Table 2; Fig. 4).

Counteracting effect of leaf and root morphology on the leaf–root biomass allocation

Significant negative relationships with slopes close to -1 were found among all 12 species between their ratio of SLA : SRL and their leaf mass : root mass ratio, for the low-nutrient \times high-light treatment and for the low-nutrient \times low-light treatment (Fig. 5a). This relationship was negative but nonsignificant for the high-nutrient \times high-light treatment, and absent for the high-nutrient \times low-light treatment (Fig. 5a). Nevertheless, when excluding the two species that showed signs of heading, the relationship of the high-nutrient \times high-light treatment became stronger and significant ($P=0.03$; $R^2=0.46$; $\alpha=0.81$). No significant difference was observed between regression slopes of all four treatments ($P=0.52$), but significant differences in elevation (i.e. line intercept) were found between each and every pair of regression lines (all $P<0.001$). Consistent with this increase in line intercept from the most nutrient-limited treatment to the most light-limited treatment (Fig. 5a), we observed, for each of the 12 species taken separately, a positive slope of SMA regression across all four phenotypes (Fig. 5b).

Variable responses of plant biomass allocation and morphology to light and fertilization among species

The effect of light treatment was significantly different among species for all leaf traits (SLA, LMF, LAR), but for root traits it

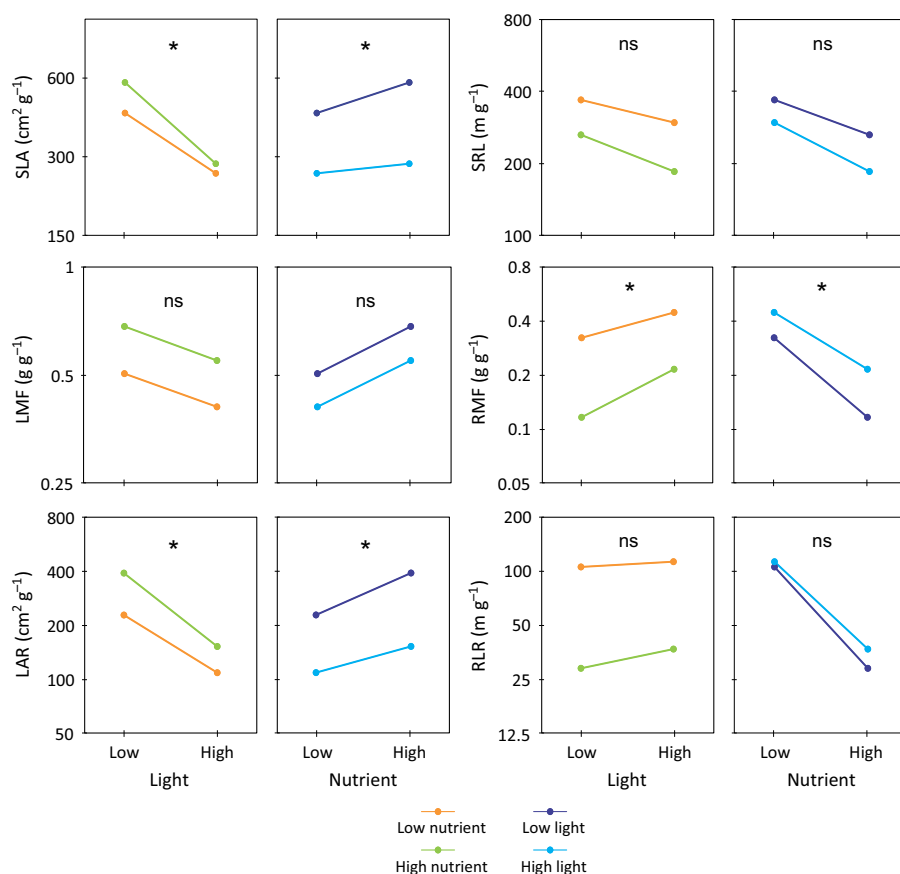


Fig. 4 Average species responses of leaf and root biomass allocation and morphology to contrasting light and nutrient treatments. The significance (*, $P<0.05$; ns, $P>0.05$) of interactions between light and nutrient treatments, as taken from Table 2, is displayed in each panel. SLA, specific leaf area; LMF, leaf mass fraction; LAR, leaf area ratio; SRL, specific root length; RMF, root mass fraction; RLR, root length ratio.

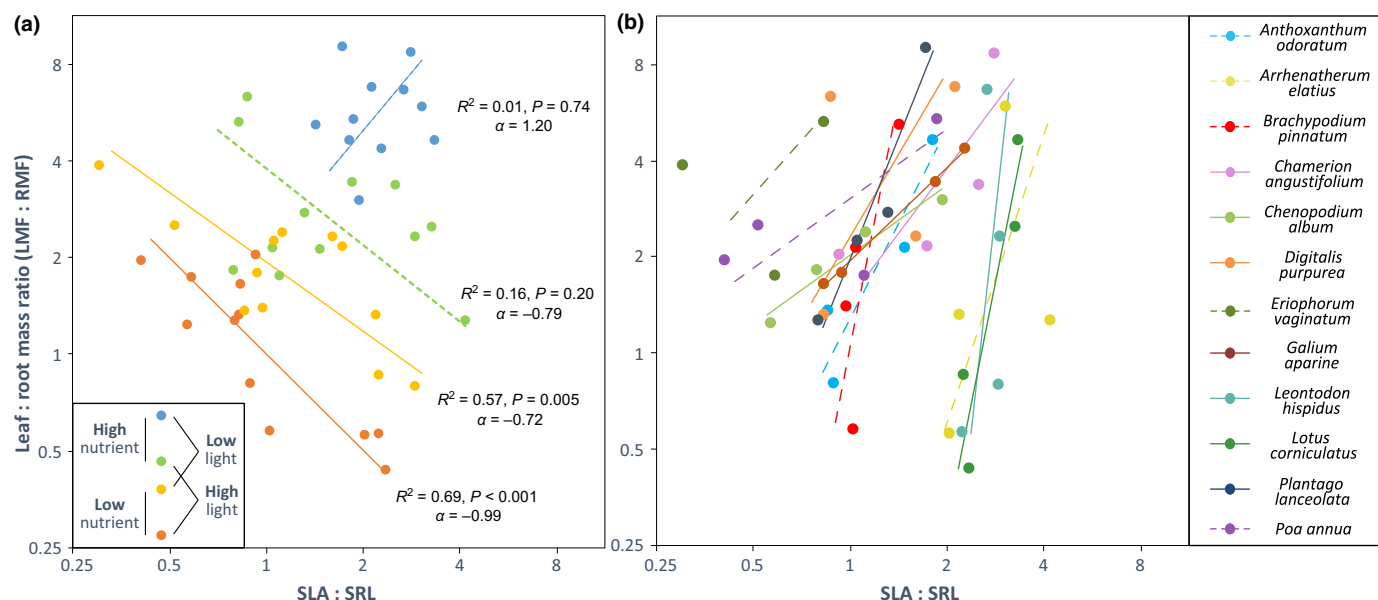


Fig. 5 Standardized major axis (SMA) regressions between the specific leaf area : specific root length (SLA : SRL) ratio and the leaf : root mass ratio, (a) across the 12 herbaceous species within each factorial light × nutrient supply treatment, and (b) across all four phenotypes of each individual species. Both ratios are expressed on a log₂-scale. In (a), the two low-light treatments show significant relations (solid lines), whereas the two high-nutrient treatments show nonsignificant relations (dashed lines, $P > 0.05$); α -values are the regression slopes. In (b), monocots are represented by dashed regression lines and eudicots by solid ones.

was only significantly different for RMF (Table 2). In all four cases, these differences stemmed from contrasting strength of a few species responses rather than differences in the direction of species responses (Fig. 6). Regarding the nutrient treatment, significantly different responses were observed among species for all leaf and root traits (Table 2), owing to both contrasting strength of species responses and differences in the direction of a few species responses (Fig. 6). Owing to the initiation of heading phase in *C. album* and *P. annua* species, which is likely to have diverted some biomass from leaves and roots into reproductive organs, the significant differences between species observed for LMF and RMF should be interpreted with caution. The interactive effect of light and nutrient treatment on plant above- and below-ground traits did not differ significantly among species for any of the traits (Table 2).

Plant responses to different intensities of light supply were not influenced by whether species belonged to the eudicot or monocot clade, or by species' Ellenberg's value for light preference (Table 4, all $P > 0.05$). However, we note that because species' Ellenberg's value for light varied between 6 and 8 only (Table 1), these differences were possibly too low to detect a potential interaction between species' environmental optima for light and our light supply treatment. By contrast, the responses of monocot species to different amounts of nutrient supply were much stronger than those of eudicot species for both SLA and LAR (Table 4, $P < 0.05$ in both cases). Changes in LMF, LAR, SRL and RMF in response to changing nutrient supply were also significantly influenced by species' Ellenberg's value for nutrients (Table 4, all $P < 0.05$). Specifically, as nutrient supply increased, LMF and LAR increased significantly more for species with higher Ellenberg's nutrient value; and SRL and RMF decreased more and

less, respectively, with increasing species' Ellenberg's nutrient value.

Discussion

Consistent role of phenotypic plasticity in biomass allocation and leaf and root morphology in plant responses to environmental changes

Plant phenotypic increases in leaf : root mass ratio across conditions ranging from below-ground to above-ground resource limitation are consistent with the functional equilibrium hypothesis, which postulates that as a plant gets limited by one resource, it will invest more biomass in the organ responsible for acquiring that resource (Brouwer, 1963; Poorter *et al.*, 2012b). Although less well documented so far (but see precursor work by Ryser & Eek, 2000), the concomitant increase in the ratio of SLA to SRL also reflects phenotypic differences consistent with increasing the resource acquisition efficiency of that organ responsible for acquiring the limiting resource (Evans & Poorter, 2001; Ostonen *et al.*, 2007). These results therefore support our first hypothesis of strong adaptive phenotypic differences in leaf area to RLR, as mediated by differences in both plant biomass allocation and leaf and root morphology, along a continuum of treatments from high below-ground resource limitation to high above-ground resource limitation. In other words, allometric relationships between leaves and roots depend strongly on environmental conditions.

Additionally, the contrast between root morphology response to light supply (higher SRL at low light) and leaf morphology response to nutrient supply (lower SLA at low nutrient supply)

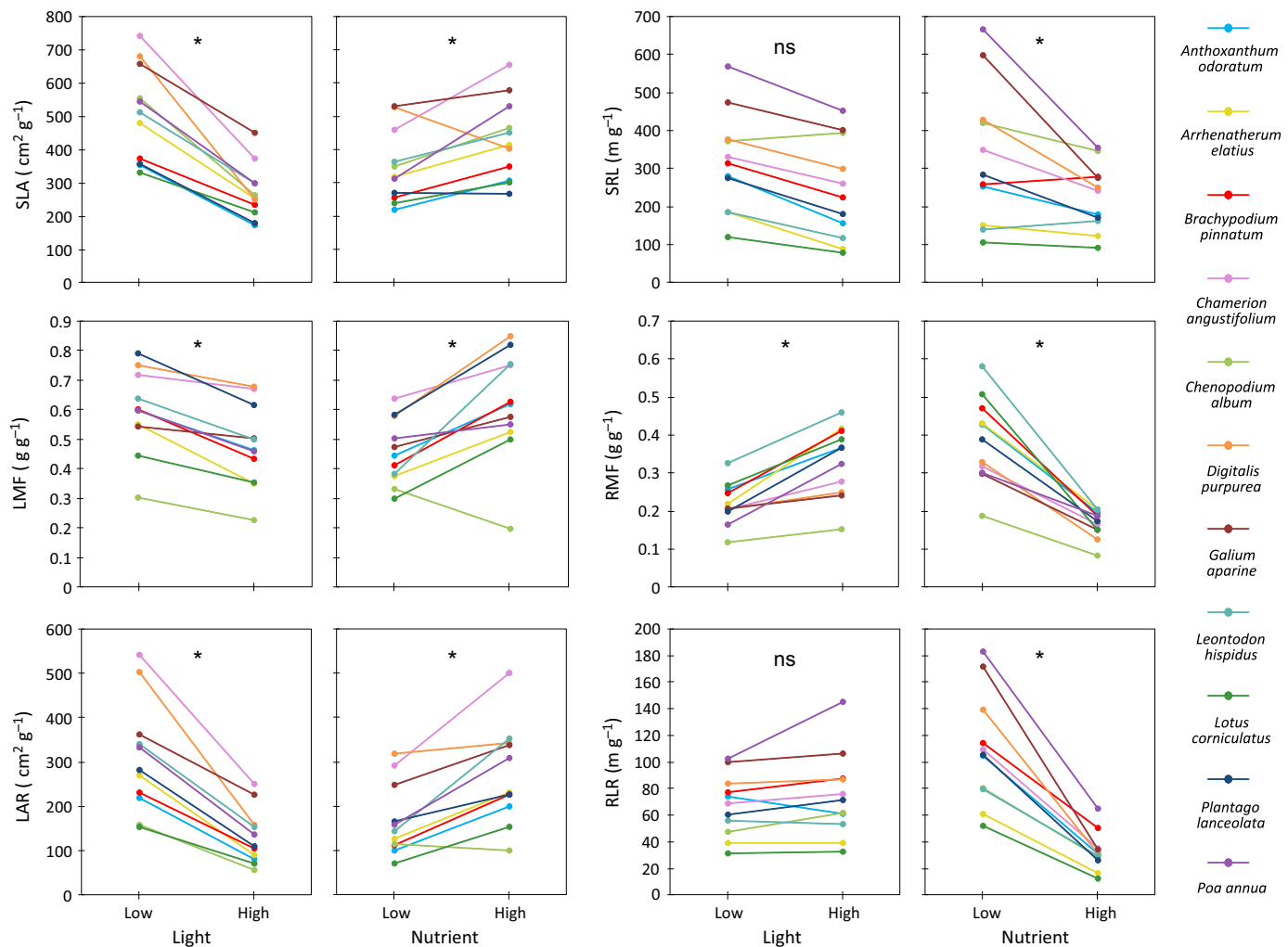


Fig. 6 Species-specific responses of leaf and root biomass allocation and morphology to contrasting light and nutrient treatments. The significance (*, $P < 0.05$; ns, $P > 0.05$) of interactions between species identity and resource supply treatments, as taken from Table 2, is displayed in each panel. SLA, specific leaf area; LMF, leaf mass fraction; LAR, leaf area ratio; SRL, specific root length; RMF, root mass fraction; RLR, root length ratio.

Table 4 Effects of species phylogeny and Ellenberg's indicator values on species leaf and root responses to contrasting light and nutrient supply, as revealed by their interactions in type III ANOVAs (monocot–eudicot) and ANCOVAs (with Ellenberg's indicator values as covariates)

Effect	df	Leaf traits						Root traits					
		SLA		LMF		LAR		SRL		RMF		RLR	
		F	P≤	F	P≤	F	P≤	F	P≤	F	P≤	F	P≤
Nutrient supply × monocot–eudicot	1	7.350	0.007	0.069	0.793	3.941	0.048	0.083	0.773	2.653	0.105	2.284	0.132
Nutrient supply × Ellenberg's nutrient indicator	1	0.154	0.695	17.13	<0.0001	7.929	0.005	5.74	0.017	7.3	0.007	0.52	0.471
Light supply × monocot–eudicot	1	0.345	0.558	1.342	0.248	0.284	0.595	0.460	0.498	3.842	0.051	0.401	0.527
Light supply × Ellenberg's light indicator	1	1.598	0.208	1.915	0.168	0.053	0.817	1.127	0.290	2.235	0.136	0.004	0.949

SLA, specific leaf area; LMF, leaf mass fraction; LAR, leaf area ratio; SRL, specific root length; RMF, root mass fraction; RLR, root length ratio.

has interesting implications for plant ecology. The higher SRL at low light represents a more efficient use of carbon in the short term as this resource becomes scarcer, and counterbalances the lower relative investment in root biomass. As a consequence, shading did not reduce plant relative investment in root length

(i.e. RLR) while considerably increasing that in leaf area, suggesting that at low light, plants manage to secure both their above- and below-ground resources in the short term (Ryser & Eek, 2000). By contrast, the decrease in SLA with decreasing nutrient supply probably stems from the absence of benefits for plants to

maintain constant SLA when the concentration of N per unit leaf mass decreases (i.e. when the amount of photosynthetic machinery per leaf area decreases; Niinemets & Tenhunen, 1997; Meziane & Shipley, 1999). Nutrient shortage therefore substantially reduces plant relative investment in leaf area while increasing that in root length, suggesting that nutrient shortage can have critical consequences for plant above-ground resource acquisition capacities.

Below ground, root mass allocation responds more than root morphology

In line with the meta-analysis by Poorter *et al.* (2012b), variation in SLA contributed 2.5 times more to plastic differences in LAR than variation in LMF, across contrasting conditions of light supply. This was lower than the nearly sevenfold higher contribution of SLA than LMF estimated by Poorter *et al.* (2012b), potentially because our study did not cover the full dose–response curve of plant response to light treatment. Nevertheless, this result confirms the widespread idea that changes in biomass allocation are relatively marginal for plant response to environmental conditions, at least above ground. Besides, this is also in line with the much stronger contribution of SLA than LMF to differences in LAR found among species of the same plant community (e.g. Freschet *et al.*, 2014, who observed a ninefold higher contribution of SLA compared with LMF in a subalpine community).

In contrast to our third hypothesis, the opposite trend was observed below ground. In response to different nutrient supply, phenotypic adjustments in root biomass allocation were 2.5 times more important than in SRL to modulate plant relative length of roots exploring soil. This result is consistent with the 2.5-fold higher contribution of RMF than SRL to differences in RLR observed by Freschet *et al.* (2014) among species of a subalpine plant community. These results suggest, contrary to common expectations (Ryser, 2006), that plasticity in root functional traits, at least in terms of morphology, may not carry as much importance for plant resource acquisition as plasticity in leaf functional traits above ground (see also Boot & Mensink, 1990; Wahl *et al.*, 2001). Nonetheless, root traits involved in below-ground resource acquisition other than SRL were not investigated here and may show a higher degree of plasticity than SRL, for example, root hair length and density (Boot & Mensink, 1990) or intensity of mycorrhizal association. This highlights both the need for a better understanding of the role of root functional traits for plant economics, and the idea that plant biomass allocation patterns should be a central aspect of plant responses to changing environmental conditions, and, more particularly, to changes in soil fertility.

Light and fertilization interact to determine leaf and root biomass allocation and morphology

Consistent with results by Meziane & Shipley (1999), we observed an interaction between light and nutrient supply on SLA (and consequently plant relative investment in leaf area) that translated into a proportionally higher increase in SLA in

response to low light when nutrient availability was high. Such an interaction between light and nutrient supply was also observed on plant relative investment in root biomass, and provides further support for the functional equilibrium hypothesis. As light supply decreases, and more biomass is allocated to leaves, plant relative investment in root biomass decreases less at low than at high nutrient supply, suggesting that plant response to light shortage is lower in conditions of nutrient shortage, through both morphological and biomass allocation constraints. Together these results support our first hypothesis and build on growing evidence (e.g. Sultan *et al.*, 1998; Maire *et al.*, 2012) that plant phenotypic response to changes in the availability of any one resource is often intimately linked to the availability in other resources.

Co-occurring species all manage to balance resource acquisition capacities above ground and below ground, but in different ways

In support of our fourth hypothesis, at the level of individual treatments, there was relatively little variation among species in their ratio of total leaf area to total root length. Generally, the spectrum of potential responses of species ranged from maintaining more biomass in leaves but with lower SLA and less biomass in roots but with higher SRL, to maintaining more biomass in roots but with lower SRL and less biomass in leaves but with higher SLA. This was particularly true for the two nutrient-poor treatments, where substantial species differences in leaf and root morphology (i.e. SLA : SRL ratio) counteracted large species differences in leaf–root biomass allocation, as described for plants in the field by Freschet *et al.* (2014). Regarding the higher interspecific variation in the LAR : RLR of plants from the high-light \times high-nutrient treatment, it appears likely that the early start of heading in some species increased interspecific differences in biomass allocation. Nevertheless, for both treatments of high nutrient supply, one simple mechanism can explain some of the higher variation in leaf area to RLR (i.e. when light becomes increasingly limiting relative to nutrients). As discussed earlier, most species keep SRL relatively constant while strongly increasing their SLA when light becomes the most limiting factor (see also Ryser & Eek, 2000); this tends to reduce variation in SLA : SRL ratios among species that are more limited by light supply. As such, differences in SLA : SRL cannot counterbalance differences in leaf : root mass ratios, as observed in conditions of nutrient limitation. Overall, our results confirm that most species, when faced with the same conditions of resource availability, will respond in broadly similar ways with respect to the ratio of leaf area to root length deployed.

Largely similar responses among species, although with predictable group differences

Despite the increasing awareness that coexisting species can display contrasting responses in above-ground traits to the same environmental change, both within natural plant communities (Albert *et al.*, 2010; Lü *et al.*, 2012; Kichenin *et al.*, 2013;

Kumordzi *et al.*, 2014) and in controlled conditions without interspecific interactions (Sultan *et al.*, 1998; Hill *et al.*, 2006; Grassein *et al.*, 2010), the drivers of species-specific responses remain unclear. Here we show that contrasting species display partly homogeneous and partly heterogeneous responses to the same differences in environmental conditions, both above and below ground. On the one hand, for most traits considered above and below ground, the 12 herbaceous species generally showed a similar direction of trait response to contrasting amounts of nutrient and light supply. On the other hand, differences were observed in the type and intensity of plant responses. For instance above ground, all species showed increasing relative investment in leaf area with decreasing light supply, but the intensity of the response varied slightly among species. Moreover, while variation in SLA was consistently the main driver responsible for the response of LAR, some species showed exclusive changes in SLA while others showed comparable contributions of changes in SLA and LMF. Similarly below ground, while all species showed increasing relative investment in root length with decreasing nutrient supply, there was variation among species both in the intensity of the response and in the relative contribution of SRL vs RMF to this response.

Such interspecific differences in trait responses can stem from the different means and ranges of trait values potentially achieved by different species (Valladares *et al.*, 2007). For instance, species that display trait values at the edge of their adaptive plasticity would be limited in their response to changing environmental conditions (see also Albert *et al.*, 2010). Further, necessary coordination between leaf and root properties can be an obstacle to plant adaptive plasticity above and below ground (Osone *et al.*, 2008). Besides this, it is also likely that species differences in plant size and architecture (Campbell *et al.*, 1991), capacity to take up distinct forms of the same resource (e.g. nitrate, ammonium, amino acids; Ashton *et al.*, 2010) or ability to recycle nutrients from senescing tissues (Chapin & Moilanen, 1991) would also play a role in these different species responses. Additionally, our results provide further evidence that there is some degree of phylogenetic conservatism in species response to environmental factors (Niinemets & Valladares, 2006; Bennett & Cahill, 2013), at least at the level of major taxa such as monocots and eudicots. Exploring such ecological similarity within clades could, to some extent, help to better predict individual species responses to environmental changes. In the present study, the stronger response of monocot than eudicot species in SLA and LAR to differences in nutrient supply may be linked to the larger root biomass fraction of monocots relative to eudicots. By consistently maintaining higher biomass below ground, monocots would be less able to vary biomass above ground and would compensate by larger changes in SLA above ground. This finding suggests that ecological similarities via phylogenetic conservatism may also be adequately characterized by continuous measures of plant functional traits (here, average biomass fraction invested in roots).

Following a similar line of thinking, several authors have hypothesized that resource-exploitative species could be more dominant in favourable environments than resource-conservative species, thanks to their ability to express more phenotypic

plasticity and thereby better exploit the varying supply of light and nutrients (Crick & Grime, 1987; Bazzaz, 1996; Lavorel *et al.*, 2009). Here, we extend the work of Poorter *et al.* (2009) on SLA, and that of Grassein *et al.* (2010), by demonstrating that, indeed, some heterogeneity in species' above- and below-ground responses to different environmental conditions could be attributed to contrasting plant environmental optima. However, while our results on SRL support the higher phenotypic plasticity of species from fertile habitats (as observed for several leaf traits by Grassein *et al.*, 2010) across contrasting conditions of nutrient supply, these species showed by contrast lower plasticity in biomass allocation to leaves and roots. Generally, these results suggest that plants from infertile habitats, used to long-term resource deprivation, would more strongly rely on changes in biomass allocation, as compared with plants from fertile habitats, which would more strongly adapt their morphological trait values in response to short-term resource deprivation. Indeed, within infertile habitats, species generally face long periods of nutrient shortage, and species that display trait values typical of faster resource acquisition (e.g. high SLA and SRL; Laughlin *et al.*, 2010) would show high rates of nutrient loss that cannot be fully compensated by increased nutrient acquisition (Aerts, 1995). In the same way, large plastic changes that would increase short-term nutrient acquisition rate but cause nutrient loss in the long term would probably prove adaptive only for species from rather fertile habitats.

Conclusions

Few authors have studied the phenotypic response of plants in terms of biomass allocation and organ morphology simultaneously, and even less so at the level of the whole plant (Ryser, 2006; Poorter *et al.*, 2012b). In that respect, our study demonstrates, for the first time, that both types of phenotypic adaptations can be equally important, although under different circumstances above and below ground. Contrary to our expectations, we revealed the overarching importance of change in biomass allocation below ground in response to differences in nutrient supply. This aspect of plant response is often overlooked by ecophysiologists and community ecologists, even though it has important implications for our understanding of the assembly and responses of communities in changing environments. Besides, large changes in root biomass allocation should also have considerable consequences for ecosystem functioning. Properties of ecosystems dominated by root biomass production probably differ strongly from those of ecosystems dominated by shoot biomass production, for instance, in terms of soil organic matter build-up and nutrient recycling (Freschet *et al.*, 2013b). While we rejoice in the considerable attention that physiology and functions of root systems are currently attracting among plant community ecologists and ecosystem ecologists, our results clearly emphasize that plant biomass allocation patterns should be better integrated into these fields of research. Allometric relationships between leaves and roots in mass and length/surface strongly depend on environmental conditions, and LMFs and RMFs vary critically between species.

Finally, our results bring further support to the idea that non-additive effects occur between environmental parameters such as light and nutrient supply, which should complicate predictions of plant responses to environmental changes (Thuiller *et al.*, 2008). Different responses were also observed among species, even to single environmental factors, although these concerned mostly the intensity rather than the direction of plant responses. Some of these differences could, nonetheless, be explained by plants' environmental optimum, with, for instance, plants typically from fertile habitats responding more strongly to nutrient availability in terms of root morphology but less so in terms of biomass allocation to roots. These and previous results (e.g. Hill *et al.*, 2006) suggest that advancing our understanding of the functional characteristics that influence the type and magnitude of plant species responses to environmental parameters could strongly improve our predictions regarding the responses of individual plants and plant communities to environmental change.

Acknowledgements

We are grateful to Richard van Logtestijn for laboratory assistance. Ken Thompson kindly provided the seeds. We are most grateful to the two anonymous reviewers for their many, highly constructive comments on this manuscript.

References

- Aerts R. 1995. The advantages of being evergreen. *Trends in Ecology & Evolution* 10: 402–407.
- Albert CH, Thuiller W, Yoccoz NG, Soudant A, Boucher F, Saccone P, Lavorel S. 2010. Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology* 98: 604–613.
- Ashton IW, Miller AE, Bowman WD, Suding KN. 2010. Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* 91: 3252–3260.
- Bazzaz FA. 1996. *Plants in changing environments*. Cambridge, UK: Cambridge University Press.
- Bennett JA, Cahill JF Jr. 2013. Conservatism of responses to environmental change is rare under natural conditions in a native grassland. *Perspectives in Plant Ecology, Evolution and Systematics* 15: 328–337.
- Boot RGA, Mensink M. 1990. Size and morphology of root systems of perennial grasses from contrasting habitats as affected by nitrogen supply. *Plant and Soil* 129: 291–299.
- Brouwer R. 1963. Some aspects of the equilibrium between overground and underground plant parts. *Jaarboek van het Instituut Voor Biologisch en Scheikundig Onderzoek aan Landbouwgewassen* 1963: 31–39.
- Campbell BD, Grime JP, Mackey JML. 1991. A trade-off between scale and precision in resource foraging. *Oecologia* 87: 532–538.
- Chapin FS III. 1991. Integrated responses of plants to stress. *BioScience* 41: 29–36.
- Chapin FS III, Bloom AJ, Field CB, Waring RH. 1987. Plant responses to multiple environmental factors. *BioScience* 37: 49–57.
- Chapin FS III, Moilanen L. 1991. Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. *Ecology* 72: 709–715.
- Cornelissen JHC, Sibma F, Van Logtestijn RSP, Broekman RA, Thompson K. 2011. Leaf pH as a plant trait: species-driven rather than soil-driven variation. *Functional Ecology* 25: 449–455.
- Crick JC, Grime JP. 1987. Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasted ecology. *New Phytologist* 107: 403–414.
- Day RW, Quinn GP. 1989. Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs* 59: 433–463.
- Ellenberg H. 1988. *Vegetation ecology of Central Europe*. Cambridge, UK: Cambridge University Press.
- Elser JJ, Fagan WF, Kerkhoff AJ, Swenson NG, Enquist BJ. 2010. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist* 186: 593–608.
- Enquist BJ, Niklas KJ. 2002. Global allocation rules for patterns of biomass partitioning in seed plants. *Science* 295: 1517–1520.
- Evans JR, Poorter H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell & Environment* 24: 755–767.
- Fourcaud T, Zhang X, Stokes A, Lambers H, Körner C. 2008. Plant growth modelling and applications: the increasing importance of plant architecture in growth models. *Annals of Botany* 101: 1053–1063.
- Freschet GT, Bellingham PJ, Lyver POB, Bonner KI, Wardle DA. 2013a. Plasticity in above- and belowground resource acquisition traits in response to single and multiple environmental factors in three tree species. *Ecology and Evolution* 3: 1065–1078.
- Freschet GT, Cornelissen JHC, van Logtestijn RSP, Aerts R. 2010a. Evidence of the 'plant economics spectrum' in a subarctic flora. *Journal of Ecology* 98: 362–373.
- Freschet GT, Cornelissen JHC, van Logtestijn RSP, Aerts R. 2010b. Substantial nutrient resorption from leaves, stems and roots in a sub-arctic flora: what is the link with other resource economics traits? *New Phytologist* 186: 879–889.
- Freschet GT, Cornwell WK, Wardle DA, Elumeeva TG, Liu W, Jackson BG, Onipchenko VG, Soudzilovskaia NA, Tao J, Cornelissen JHC. 2013b. Linking litter decomposition of above and belowground organs to plant–soil feedbacks worldwide. *Journal of Ecology* 101: 943–952.
- Freschet GT, Kichenin E, Wardle DA. 2014. Explaining within-community variation in plant biomass allocation: a balance between organ biomass and morphology above versus belowground? *Journal of Vegetation Science*. doi:10.1111/jvs.12259.
- Grassein F, Till-Bottraud I, Lavorel S. 2010. Plant resource-use strategies: the importance of phenotypic plasticity in response to a productivity gradient for two subalpine species. *Annals of Botany* 106: 637–645.
- Guo D, Xia M, Wei X, Chang W, Liu Y, Wang Z. 2008. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytologist* 180: 673–683.
- Hill J, Simpson R, Moore A, Chapman D. 2006. Morphology and response of roots of pasture species to phosphorus and nitrogen nutrition. *Plant and Soil* 286: 7–19.
- Hill MO, Mountford JO, Roy DB, Bunce RG. 1999. *Ellenberg's indicator values for British plants. ECOFACT volume 2 technical annex*. Huntingdon, UK: Institute of Terrestrial Ecology.
- Kichenin E, Wardle DA, Peltzer DA, Morse CW, Freschet GT. 2013. Contrasting effects of plant inter- and intra-specific variation on community-level trait measures along an environmental gradient. *Functional Ecology* 27: 1254–1261.
- van Kleunen M, Fischer M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* 166: 49–60.
- de Kroon H, Huber H, Stuefer JF, Van Groenendaal JM. 2005. A modular concept of phenotypic plasticity in plants. *New Phytologist* 166: 73–82.
- Kumordzi BB, Wardle DA, Freschet GT. 2014. Plant assemblages do not respond homogeneously to local variation in environmental conditions: functional responses differ with species identity and abundance. *Journal of Vegetation Science* 26: 32–45.
- Lambers H, Atkin OK, Millenaar FF. 2002. Respiratory patterns in roots in relation to their functioning. In: Waisel Y, Eshel A, Kafafi U, eds. *Plant roots, the hidden half*. New York, NY, USA: Marcel Dekker, 521–552.
- Laughlin DC, Leppert JJ, Moore MM, Sieg CH. 2010. A multi-trait test of the leaf-height-seed plant strategy scheme with 133 species from a pine forest flora. *Functional Ecology* 24: 493–501.
- Lavorel S, Gachet S, Sahl A, Colace M-P, Gaucherand S, Burylo M, Bonet R. 2009. A plant functional traits data base for the Alps – understanding functional effects of changed grassland management. In: Spehn E, Körner C, eds. *Data mining for global trends in mountain biodiversity*. Boca Raton, FL, USA: CRC Press & Taylor and Francis, 106–123.

- Liu G, Freschet GT, Pan X, Cornelissen JHC, Li Y, Dong M. 2010. Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. *New Phytologist* **188**: 543–553.
- Lü X-T, Freschet GT, Flynn DFB, Han X-G. 2012. Plasticity in leaf and stem nutrient resorption proficiency potentially reinforces plant–soil feedbacks and micro-scale heterogeneity in a semi-arid grassland. *Journal of Ecology* **100**: 144–150.
- Maire V, Gross N, da Silveira Pontes L, Picon-Cochard C, Soussana J-F. 2009. Trade-off between root nitrogen acquisition and shoot nitrogen utilization across 13 co-occurring pasture grass species. *Functional Ecology* **23**: 668–679.
- Maire V, Gross N, Hill D, Martin R, Wirth C, Wright IJ, Soussana J-F. 2013. Disentangling coordination among functional traits using an individual-centred model: impact on plant performance at intra- and inter-specific levels. *PLoS ONE* **8**: e77372.
- Maire V, Martre P, Kattge J, Gastal F, Esser G, Fontaine S, Soussana J-F. 2012. The coordination of leaf photosynthesis links C and N fluxes in C₃ plant species. *PLoS ONE* **7**: e38345.
- McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwiatkowski BL, Laundre JA *et al.* 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* **415**: 68–71.
- Meziane D, Shipley B. 1999. Interacting determinants of specific leaf area in 22 herbaceous species: effects of irradiance and nutrient availability. *Plant, Cell & Environment* **22**: 447–459.
- Niinemets Ü, Tenhunen JD. 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant, Cell & Environment* **20**: 845–866.
- Niinemets Ü, Valladares F. 2006. Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. *Ecological Monographs* **76**: 521–547.
- Osmont KS, Sibout R, Hardtke CS. 2007. Hidden branches: developments in root system architecture. *Annual Review of Plant Biology* **58**: 93–113.
- Osone Y, Ishida A, Tatenos M. 2008. Correlation between relative growth rate and specific leaf area requires associations of specific leaf area with nitrogen absorption rate of roots. *New Phytologist* **179**: 417–427.
- Ostons I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Löhms K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A *et al.* 2007. Specific root length as an indicator of environmental change. *Plant Biosystems* **141**: 426–442.
- Poorter H, Bühler J, van Dusschoten D, Climent J, Postma JA. 2012a. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology* **39**: 839–850.
- Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**: 565–588.
- Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. 2012b. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* **193**: 30–50.
- Rees M, Osborne CP, Woodward FI, Hulme SP, Turnbull LA, Taylor SH. 2010. Partitioning the components of relative growth rate: how important is plant size variation? *The American Naturalist* **176**: E152–E161.
- Reich PB. 2002. Root–shoot relations: optimality in acclimation and adaptation or the ‘Emperor’s New Clothes’. In: Waisel Y, Eshel A, Kafafi U, eds. *Plant roots: The hidden half*. New York, NY, USA: Marcel Dekker, 205–220.
- Reich PB, Tjoelker MG, Walters MB, Vanderklein DW, Buschena C. 1998. Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. *Functional Ecology* **12**: 327–338.
- Rijkers T, Pons TL, Bongers F. 2000. The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. *Functional Ecology* **14**: 77–86.
- Ryser P. 2006. The mysterious root length. *Plant and Soil* **286**: 1–6.
- Ryser P, Eek L. 2000. Consequences of phenotypic plasticity vs. interspecific differences in leaf and root traits for acquisition of aboveground and belowground resources. *American Journal of Botany* **87**: 402–411.
- Ryser P, Lambers H. 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. *Plant and Soil* **170**: 251–265.
- Stace CA. 1991. *New flora of the British Isles*. Cambridge, UK: Cambridge University Press.
- Sultan SE, Wilczek AM, Bell DL, Hand G. 1998. Physiological response to complex environments in annual *Polygonum* species of contrasting ecological breadth. *Oecologia* **115**: 564–578.
- Tennant D. 1975. A test of a modified line intersect method of estimating root length. *Journal of Ecology* **63**: 995–1001.
- Thuiller W, Albert C, Araújo MB, Berry PM, Cabeza M, Guisan A, Hickler T, Midgley GF, Paterson J, Schurr FM *et al.* 2008. Predicting global change impacts on plant species’ distributions: future challenges. *Perspectives in Plant Ecology, Evolution and Systematics* **9**: 137–152.
- Turner BL. 2008. Resource partitioning for soil phosphorus: a hypothesis. *Journal of Ecology* **96**: 698–702.
- Valladares F, Gianoli E, Gómez JM. 2007. Ecological limits to plant phenotypic plasticity. *New Phytologist* **176**: 749–763.
- Valladares F, Wright JS, Lasso E, Kitajima K, Pearcy RW. 2000. Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology* **81**: 1925–1936.
- Wahl S, Ryser P, Edwards PJ. 2001. Phenotypic plasticity of grass root anatomy in response to light intensity and nutrient supply. *Annals of Botany* **88**: 1071–1078.
- Warton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. *Biological Reviews* **81**: 259–291.