



Different plant trait scaling in dry versus wet Central European meadows

Francesco de Bello, Štěpán Janeček, Jan Lepš, Jiří Doležal, Jana Macková, Vojtěch Lanta & Jitka Klimešová

Keywords

Biodiversity; Carbon isotope; Environmental filtering; Grassland; Land use; Leaf dry matter content; Management; Specific leaf area

Nomenclature

Kubát et al. (2002)

Received 11 January 2011

Accepted 2 January 2012

Co-ordinating Editor: Hans Henrik Bruun

de Bello, F. (corresponding author, fradebello@ctfc.es), **Janeček, Š.** (janecek.stepan@centrum.cz), **Doležal, J.** (jiriddolezal@gmail.com), **Klimešová, J.** (Klimešova@butbn.cas.cz) & **Lanta, V.** (vojlan@utu.fi): Institute of Botany, Czech Academy of Sciences, Dukelská 135, CZ-379 82, Třeboň, Czech Republic

de Bello, F., Lepš, J. (suspa@prf.jcu.cz) & **Macková, J.** (jana.macek@yahoo.com): Department of Botany, Faculty of Sciences, University of South Bohemia, Na Zlaté Stoce 1, CZ-370 05, České Budějovice, Czech Republic

Lepš, J.: Institute of Entomology, Biology Centre, Czech Academy of Sciences, CZ-370 05, České Budějovice, Czech Republic

Lanta, V.: Section of Ecology, University of Turku, FIN-20014, Turku, Finland

Abstract

Question: Trait scaling relationships involve multiple trade-offs and allometric constraints between the traits of co-existing species. Alternative trait relationships, particularly between plant size and other traits, are expected in response to combinations of different biotic and abiotic filters. To what extent does the expected convergence in plant attributes to water shortage and disturbance produce different trait scaling relationships in dry vs wet meadows under various disturbance regimes?

Locations: One test meadow in South Bohemia and one in South Moravia (i.e. wet vs dry Central Europe meadows), Czech Republic.

Methods: Selected species were sampled in mown and abandoned plots within each site. Several plant traits were measured to provide information on plant strategies related to growth, resource acquisition and carbon–water economy.

Results: Trait differentiation among co-existing species was strong in both meadows, and generally stronger than differentiation caused by mowing and site effects. Different trait scaling relationships with plant height were observed across the meadows, particularly showing a more independent trait differentiation linked to water–carbon economy at the dry site. Mowing tended to reinforce the effect of water limitation on traits and on trait scaling. In contrast, mowing cessation tended to resemble the effect of humid conditions on trait relationships.

Conclusions: The results advocate incorporating trait scaling relationships between species into vegetation models and community assembly assessments, therefore accounting for processes of plant co-existence along combined spectra of light, water and disturbance regimes. These gradients affect alternative life-history strategies and possibly sustain different species co-existence patterns based on different trait scaling. The results particularly advocate a convergence in traits and trait scaling relationships in response to the combination of disturbance and water limitation.

Introduction

Trait differences between species are expected to facilitate the maintenance of diversity within a site, with alternative life-history strategies allowing species to exploit different niches (Stubbs & Wilson 2004). It is becoming increasingly evident that under different environments, such trait differences could be underlined by alternative trait scaling relationships between co-existing species (i.e. bivariate relationships between traits of species within a site; En-

quist et al. 1999; Westoby et al. 2002). Trait scaling relationships involve trade-offs and allometric constraints between multiple traits and particularly between plant size and other functional traits linked to resource allocation. Most likely this within-site trait scaling arises because the ecological opportunities for each species depend strongly on which other species are present, in other words, because the set of species at a site is a mixture of strategies (Westoby et al. 2002) where different available niches are occupied (Mason et al. 2011).

Various authors have proposed that plant size is one of the main attribute involved in these trait scaling relationships, thus creating a mixture of alternative size-related strategies at a site (see review of Falster & Westoby 2003). Recently, Falster & Westoby (2005) recognized alternative trait scaling relationships with plant height in a species-diverse tropical forest along gradients of light acquisition and succession. We hypothesize that such alternative trait scaling is also important in other species-rich systems, for instance across species-diverse temperate grasslands, but that trait scaling relationships should vary across grasslands under different environmental conditions. The combined effect of resource availability and frequent disturbance regime on plant height should lead to different trait-scaling consequences of plant traits, for example when both water limitation and disturbance cause a convergent selection of similar attributes (Grime 2001; Rusch et al. 2009). Plant size is involved in the response to both resource availability and disturbance, as it relates to both competitive ability and tolerance/avoidance of disturbance and drought (Milchunas et al. 1988; Osem et al. 2004). Competition for light is considered to increase with taller growth forms and larger leaves (Grime 2001), but taller plants, particularly herbaceous ones, may be more vulnerable to disturbance and drought (Díaz et al. 2001; Osem et al. 2004). In meadows, trait differences between wet and dry conditions should therefore increase when comparing disturbed sites to undisturbed sites, because of the expected convergence in plant attributes to both resource scarcity and disturbance (Grime 2001; Rusch et al. 2009). This convergence has been identified along moisture (Milchunas & Lauenroth 1993) and fertility gradients (Grime 2001; Osem et al. 2004), but the examination of these relationships is still incipient (Fine et al. 2006; Rusch et al. 2009) and sometimes contradictory (Grime 2006).

To obtain a better understanding of these patterns, we propose here that the exploration of trait scaling relationships should complement more traditional analyses focusing on the shift in trait values across different vegetation types. For instance, it has been long recognized that dry conditions should select for species with traits related to a more conservative resource strategy as compared to wet conditions (for comparable herbaceous species: lower canopy, denser tissues, more conserved nutrients and water at drier sites; Fonseca et al. 2000; Cornelissen et al. 2003; Díaz et al. 2004; Osem et al. 2004). Although this 'filtering' process should result in a shift in trait values from wet to dry conditions (e.g. lower average plant height in dry sites), high variability between co-existing species should possibly equate, if not override, trait difference between sites (Westoby et al. 2002; de Bello et al. 2009a, 2011). As such, differences between biomes should be observable in different trait scaling rather than in just a shift in trait

values. According to classical ecological theories (Milchunas et al. 1988) we could moreover expect different space occupancy patterns in dry vs wet vegetation in response to disturbance: more horizontal (rather than vertical) regrowth in the dry conditions, where competition for soil water is higher, and the opposite in the wet and undisturbed conditions, where competition for light is more intense. As biomass removal with disturbance increases light availability, competition for light should be reduced, especially at the wet site. At the same time, this higher light availability should cause an increase in water stress, especially in drier conditions, leading plants to have more water-saving strategies during photosynthesis (Flexas et al. 2008). The regrowth after disturbance should also increase soil nutrient depletion, with plants investing in new tissues with higher nutrient concentrations (Loiseau et al. 2005). It is, however, uncertain how such complex changes will affect the ensemble of trait scaling relationships in a given community.

Such hypotheses indicate the need to understand (1) how the relationship between height and other traits varies under different environmental conditions, and (2) how these effects are modified by disturbance. Overall, we were interested in understanding whether trait differences between these conditions could be observed in alternative trait scaling relationships or in a shift in trait values. However, when considering meadows, comparisons between sites with different climate conditions are often difficult because of inherent differences in disturbance regimes (i.e. intensity, frequency and evolutionary history of disturbance), which are especially evident when considering livestock grazing effects (Pakeman 2004; Rusch et al. 2009). These limitations can be overcome by considering the effects of uniform and controlled disturbance regimes, such as traditional mowing. Mowing, in contrast to grazing, has the advantage of creating an equal disturbance across the vegetation, while avoiding any issues related to herbivore selectivity resulting from plant quality or secondary compounds. Interestingly, relatively few studies have assessed the effect of mowing on plant traits (Louault et al. 2005; Quetier et al. 2007; Kahmen & Poschold 2008; Römermann et al. 2009; Duru et al. 2010). To the best of our knowledge, none of these studies have used a comparable design under different environmental conditions. Here, within two selected grassland types (corresponding to wet vs dry meadows), similar sets of species were sampled for trait measurements in both mown and short-term abandoned conditions (two seasons of abandonment). Several plant traits were chosen for this study, i.e. specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content, leaf carbon isotope composition, stem height per plant biomass and below-ground organ water content (either root or rhizome), to provide information on plant

strategies related to growth, resource acquisition and to carbon–water economy. Our list of traits is by no means exhaustive, as it was limited by the design imperative to quantify traits across significant numbers of individuals in the field. We focused on traits considered to interact with both resource availability and disturbance regime and also with expected trait scaling relationships with plant size (Díaz et al. 2001; Westoby et al. 2002; Cornelissen et al. 2003; Pakeman et al. 2009).

Methods

Study sites and experimental design

The experiment was set up in two species-rich meadows (Table 1), Čertoryje ('dry') in South Moravia and Ohrazení ('wet') in South Bohemia, both in the Czech Republic. In these two meadows, largely differing in soil moisture conditions, long-term experiments dealing with the impact of management on community response have been carried out (Klimešová et al. 2010; Janeček et al. 2011; Mason et al. 2011). Together with differences in soil moisture, the 'dry' site is generally less limited in soil nutrients but has lower plant productivity (Table 1). After abandonment, similar *Molinia* species become dominant at the two sites: *Molinia arundinacea* in the dry meadow and *Molinia caerulea* in the wet meadow. These two dominant grasses are considered as two separate species in the regional flora (Kubát

et al. 2002), although historically they were considered as two subspecies.

Mowing is the traditional management applied to these ecosystems and occurs usually in June (Lanta et al. 2011). The experiment was set up in early Jun 2005 at both sites where mowing was either maintained or abandoned. Different blocks were marked at the beginning of the growing season (six blocks in Čertoryje and five in Ohrazení). Within each block, two 3 × 3-m plots were established and were either mown in mid-June (traditional management) or abandoned. In total there were 22 experimental plots. The mowing treatment was applied equally at both sites, i.e. at the same height of about 5 cm (as for the traditional mowing) in Jun 2005 and 2006. In the second half of Sep 2006, randomly sampled individuals belonging to a selected list of species (Fig. 1) were collected in each mown and each abandoned plot within each block. Sampling took place in September in order to allow the expression of vegetation re-growth after mowing. In this sense, plants had generally already terminated their vegetative growth, but mostly without senescence symptoms, in both mown and unmown conditions. We cannot, however, rule out that differences in trait values between mown and unmown plants were due to different compensatory effects during regrowth. We will discuss these potential effects when explaining the response of some traits to mowing. Overall, our main objective was to study whether differences between sites vary after a similar disturbance.

Individuals were marked randomly at the beginning of the growing season to avoid collecting only the largest individuals within each species. During the time of plant harvesting, soil samples at two different depths (0–15, 15–30 cm) were collected almost simultaneously in all plots within a site and the fresh weight determined. Soil water content was estimated after drying samples at 60 °C until constant weight. Meteorological sensors placed above the soil surface in some of the mown and unmown plots generally showed more extreme temperatures (i.e. higher diurnal ranges) and lower relative air humidity in mown conditions, probably as a consequence of lower biomass accumulation under this treatment (Doležal unpubl. data).

Trait measurements and plant strategies

The selected species (Fig. 1, all of them herbaceous) were the most common taxa at both locations and represent the main growth forms abundant in these communities (e.g. grasses, sedges, legumes, prostrate and erect forbs). In particular, trait measures were carried out on species representing most of the biomass in each plot (the species selected generally represent more than 80% of the total biomass in the vegetation; Pakeman & Quested 2007).

Table 1. Characteristics of the dry and wet meadow sites where the sampling was conducted.

	'Dry' meadow	'Wet' meadow
Locality	Čertoryje	Ohrazení
Geographical coordinates	48 °54' N, 17 °25' E	48 °57' N, 14 °36' E
Description and management	Dry meadow with scattered <i>Quercus</i> spp. trees, mown once a year in June	Wet meadow, mown once a year in June
Above-ground dry biomass	250 g·m ⁻²	320 g·m ⁻²
Cover of vascular plants	70%	80%
Mean annual precipitation	464 mm	700 mm
Mean annual temperature	9.4 °C	7–8 °C
Altitude	440 m a.s.l.	500 m a.s.l.
Soil	Deep calcium-rich soil	Acidic
Mean soil properties	pH: 5.99 Total N: 0.47% Total C: 5.45% P: 0.243 mg·g ⁻¹	pH: 4.5 Total N: 0.39% Total C: 4.94% P: 0.089 mg·g ⁻¹
Mean soil water content during sampling	0–15 cm: 17.2% 15–30 cm: 15.9%	0–15 cm: 39.6% 15–30 cm: 28.6%

Source of information: Climate Atlas of Czechia (2007) and Klimešová et al. (2010).

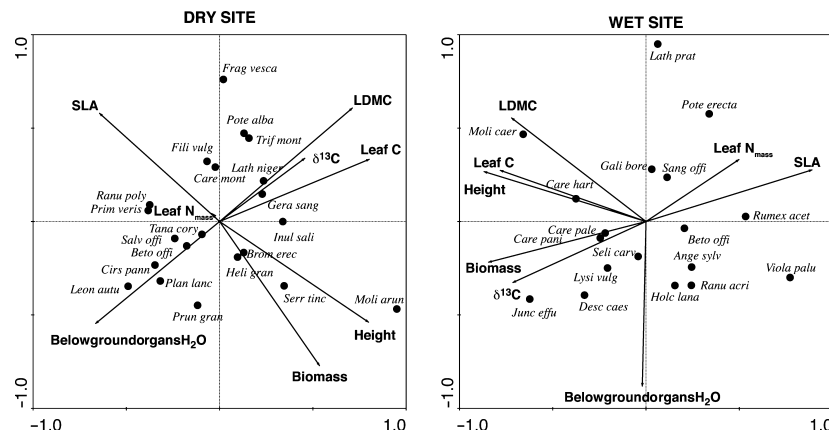


Fig. 1. Principal components analysis (PCA) of species traits for the two study sites [Čertoryje (dry) and Ohrazení (wet)]. The angle between arrows denotes trait correlations. Species (see below) are denoted by dots. The ratio of the first to the second PCA eigenvalue axis was higher at the wet site than at the dry site: $0.520/0.177 = 2.93$ for the wet site and $0.394/0.265 = 1.48$ for the dry site. Species at the dry site: *Betonica officinalis* L., *Bromus erectus* HUDS., *Carex montana* L., *Cirsium pannonicum* (L. fill) LINK, *Clematis recta* L., *Filipendula vulgaris* MOENCH, *Fragaria vesca* L., *Geranium sanguineum* L., *Helianthemum grandiflorum* (SCOP.) DC., *Inula salicina* L., *Lathyrus niger* (L.) BERNH., *Leontodon autumnalis* L., *Molinia arundinacea* SCHRANK, *Plantago lanceolata* L., *Potentilla alba* L., *Primula veris* L., *Prunella grandiflora* (L.) SCHOLLER, *Ranunculus polyanthemos* L., *Salvia officinalis* L., *Serratula tinctoria* L., *Tanacetum corymbosum* (L.) HULZ-BIP., *Trifolium montanum* L., Species at the wet site: *Angelica sylvestris* L., *Betonica officinalis* L., *Carex hartmanii* CAJANDER, *Carex pallens* L., *Carex panicea* L., *Deschampsia cespitosa* (L.) PB., *Galium boreale* L., *Holcus lanatus* L., *Juncus effusus* L., *Lathyrus pratensis* L., *Lysimachia vulgaris* L., *Molinia caerulea* (L.) MOENCH, *Potentilla erecta* (L.) RÄUSCHEL, *Ranunculus acris* L., *Rumex acetosa* L., *Sanguisorba officinalis* L., *Selinum carvifolia* (L.) L., *Viola palustris* L.

Various individuals per species were dug out in each plot (1–6 depending on their size, see below). Digging of individuals was intended to collect most of the underground organs of each individual, i.e. rhizomes and large roots. For clonal–rhizomatous species, e.g. for both *Molinia* spp., the distinction of physically independent individuals in the field is a complex task. Digging, in this case, was limited to a representative number of connected ramets per species (expected to reflect a unit of biomass influencing resource use and space occupancy). Senescent individuals were discarded for trait measurements.

The maximum reproductive height of each plant was measured in the field once each individual plant had been dug out. The whole fresh above- and below-ground biomass was also weighed (using a field Kern & Sohn GmbH CM500-GN1 balance, Balingen, Germany). After these measurements, a minimum of one leaf per individual was removed from the stem and weighed fresh in the field (more leaves were considered for species with smaller leaves). The number of individuals selected per species in a given plot was chosen in a way that produced a sufficient amount of leaves and below-ground material for weighing. As we were not primarily interested in determining the species variability within blocks, but rather across treatments, we used this approach as a method to characterize differences between species. Leaves were selected following the criteria defined in Cornelissen et al. (2003), where only non-shaded (at the time of sampling), undamaged,

fully expanded leaves were sampled. For the leafless *Juncus effusus* a part of a young photosynthetic stem (length >4 cm) was considered to be a leaf (Cornelissen et al. 2003). In the laboratory, the one-side projected leaf area for each sample was measured with a leaf area meter AM200 (ADC BioScientific Ltd., Hoddesdon, UK). The leaf dry weight and the dry biomass for each sampled plant were measured after the material had been dried to constant weight at 80 °C (min. 24 h).

These leaf measurements enabled calculation of two important traits: SLA, which is the ratio of leaf area to dry weight ($\text{mm}^2\cdot\text{mg}^{-1}$) and LDMC, which is the ratio of leaf dry mass divided by fresh mass (expressed here as a percentage, i.e. $\text{mg}\cdot 100\cdot\text{mg}^{-1}$). LDMC tends to scale with $1/\text{SLA}$, although the two traits may not always capture the same functions (Cornelissen et al. 2003). SLA and LDMC are two of several inter-correlated leaf traits, representing a fast–slow continuum in leaf economy across species (Díaz et al. 2004; Wright et al. 2004). Higher SLA is associated with shorter leaf life span, higher leaf nitrogen, shorter nutrient residence time and higher relative growth rate (Westoby et al. 2002). The relationship between LDMC and these traits is generally rather weak, while leaves with high LDMC tend to be relatively tough and more resistant to herbivory and decomposition (Cornelissen et al. 2003; Pálková & Lepš 2008). Species with high SLA and low LDMC are generally capable of rapid height growth (Cornelissen et al. 2003), but may as a result have increased mortality.

After drying, the leaves of different individuals of a given species and plot were ground to a powder using a mixer mill MM 200 (Retsch, Haan, Germany) to provide material for chemical analyses. Nitrogen (N), carbon (C) content and leaf carbon isotope composition ($\delta^{13}\text{C}$) were measured from these pooled samples, using standard techniques and a NC 2100 analyser (ThermoQuest Italia S.p.A., Milano, Italy). Across species, *leaf N content* (N_{mass} , $\text{mg}\cdot\text{g}^{-1}$) and the content of N per unit area (N_{area} , i.e. $N_{\text{mass}}/\text{SLA}$) tend to be closely correlated with maximum photosynthetic rate, (on a mass or area base, respectively; Cornelissen et al. 2003; Wright et al. 2004). Within species, N content tends to vary significantly also with N availability in the environment. The *leaf carbon content* ($\text{mg}\cdot\text{g}^{-1}$; 'Leaf C') reflects the amount of C per leaf dry matter (Cornelissen et al. 2003). The *carbon isotope composition* ($\delta^{13}\text{C}$) of leaf tissues, which measures the ratio of ^{13}C to ^{12}C (‰), is an integrated, long-term measure of the ratio between internal and ambient CO_2 concentrations (c_i/c_a), which affects discrimination against the carbon isotope ^{13}C during CO_2 fixation by Rubisco (Farquhar et al. 1989). Although a number of factors might influence the variation of $\delta^{13}\text{C}$, it can be used to provide first insights into water–carbon relations in plants (with less negative $\delta^{13}\text{C}$ values largely suggesting higher stomata closure within a species and, across taxa at a given location, species with higher intrinsic water use efficiency; Seibt et al. 2008; de Bello et al. 2009b).

For each species, the height per biomass ratio was calculated. The biomass cost per length of stem reflects a fundamental facet of a species' growth strategy. Yet despite significant variation among species (Poorter et al. 2003), this trait has received relatively little attention to date (here it was expressed as height per biomass ratio, i.e. ' h/b_{mass} '). Successional early species are hypothesized to economize on stem biomass (Falster & Westoby 2005), thereby facilitating rapid growth. Similarly, plants higher in the canopy generally show stronger vertical growth to maintain their leaves in the canopy, i.e. with a high height per biomass ratio. Here, for rhizomatous grasses, this index was intended to reflect a general vertical growing strategy, i.e. without taking into account the whole above-ground biomass of each individual, which is often impossible to distinguish and collect. In this case, we collected only a representative amount of tillers of rhizomatous species connected to the excavated rhizome. In this way, we tried to represent a tussock unit, being aware that this may underestimate, especially for larger species, the real individual biomass.

For the below-ground organs, we calculated the percentage water content for each individual sampled (i.e. 1 minus the ratio of dry weight to fresh weight). The *below-ground water content* should reflect (similar to LDMC) different strategies of water conservation and allocation

during growth. The relevance of this trait remains poorly assessed to date, probably due to the demanding work connected with below-ground organs; nonetheless this trait might hold promise for understanding water use patterns when combined with other above-ground traits, such as LDMC and $\delta^{13}\text{C}$. Below-ground organs were of different types across and within species. Here we distinguished between rhizomatous species and non-rhizomatous species. For the first, the below-ground organs largely corresponded to most of the plant base where the rhizomes are connected (the water content of these organs was called ' BaseH_2O '). For non-rhizomatous species, main and secondary roots were considered (the water content of these organs was called ' RootH_2O '). In some cases, the rhizomatous species also had a considerable amount of roots in addition to the rhizome. In this case, we calculated both BaseH_2O and RootH_2O (within each species, with the water content that was not markedly different from other organ types). Depending on the analysis, we considered these different organs either separately or together.

Data analysis

For each species, an average trait value for all individuals collected in each plot was calculated and considered as one observation. Using nested ANOVA, we tested the effects of site (dry vs wet), mowing treatment (mown vs unmown) and species identity on trait values. This was done, for each trait separately, considering site and mowing regime as fixed factors, species identity as a random factor nested within site, and also block (of the experimental design) as a random factor nested within the site. SPSS 10.0 for Macintosh (SPSS Inc., Chicago, IL, USA) was used for this purpose. In this analysis, the effect of site largely corresponds to differences in species composition at the two sites, while the effect of mowing underlay the extent of species trait response to mowing regime. In a second analysis, trait values were tested for each site separately. There was only one species common to both sites, i.e. *Betonica officinalis* (Fig. 1).

The ANOVA was further extended to assess the degree of functional differentiation between species at the two sites. Trait variance within a site, in fact, corresponds to the measure of functional diversity between co-existing species (de Bello et al. 2009a, 2011). For each trait, we computed the variance of trait values (with equation 5 in de Bello et al. 2009a) for each block within a site. The average of trait variance (across all blocks of a site) was compared across sites. The significance of differences between sites was obtained by comparing the observed ratio of trait variance to randomizations (i.e. the ratio of the observed mean trait variance of the two sites was compared to the ratio expected by chance through randomly reallocating block

trait variance across sites, with 999 randomizations per trait).

To assess changes in the trait scaling relationships between species, bivariate trait relationships were compared both between sites and in response to mowing within each site. For comparisons between sites, a mean trait value for each species and trait was calculated (pooling together all trait values from both mown and unmown plots). For comparisons between mowing regimes within a site, a separate mean trait was calculated for each species in mown and unmown conditions. Bivariate trait relationships between species were analysed by fitting standardized major axes regressions (SMA). Line fitting, within individual sets with 95% slope confidence intervals, was calculated with the SMATR package for the R software (R Development Core Team, Vienna, Austria). SMA estimates of the line fitted between two variables (i.e. the main axis along which two variables are correlated) are superior to ordinary linear regression estimates for our purposes, because residual variance is minimized in both X and Y dimensions, rather than the Y dimension alone (Falster & Westoby 2005). To compare the observed bivariate trait relationships between groups of species (i.e. dry vs wet site and mown vs unmown), function tests for statistical differences between the slopes and the intercepts of the SMA relationships were examined. First we tested for significant heterogeneity between slopes of the two sets by estimating a common slope and permuting residuals from the common slope (following Warton et al. 2006). Given a common slope (i.e. test for heterogeneity not significant), we tested for elevation differences between sets (Falster & Westoby 2005).

Together with the SMA analyses, we used principal components analysis (PCA) to obtain a visual approximation of the ensemble of trait correlations within each site. The angle between arrows in a PCA (after centring and standardization of traits) gives a general approximation of the correlation between traits, which was later explored in more detail when performing specific SMA analyses. A separate PCA was calculated for each site, where each species was characterized for each trait by its mean trait value per site (pooling together all trait values from both mown and unmown plots, as for the SMA analyses mentioned above). From the PCA, we removed those traits that, by definition, are expected to be strongly correlated with other traits. We removed traits such as leaf N_{area} , leaf C:N and h/mass. Including these traits and below-ground organ content in the analyses or not produced largely comparable results. The main results were also comparable when assessing PCAs in dry vs wet sites under comparable mowing regimes (using trait values only from mown or unmown conditions) probably because there was a restricted effect of mowing regime on traits (see Results).

Hence, we only show the results of general PCA, disregarding the effect of mowing regime. Using CANOCO 4.5 (ter Braak & Šmilauer 2002) centring and standardization of traits was applied to account for different trait units.

Results

Soil water content was higher at the wet site (on average 34% vs 17% at the dry site; site effect was always significant, i.e. $P < 0.05$, except in the triple interaction site \times mowing \times soil depth; results of the full ANOVA analysis are shown in Appendix S1). It decreased with soil depth and with mowing, but the decrease with mowing was more pronounced at the dry site (mowing \times site interaction $P = 0.042$). The decrease was around 7% at the wet site and 12% at the dry site compared to unmown plots.

Trait values and variability within sites

The analysis of trait values revealed that species in the wet meadow tended to be taller, with lower biomass per stem height (although site differences were influenced by mowing), higher below-ground water content and more negative $\delta^{13}C$ (Fig. 2, Appendix S2).

The effects of mowing were generally weak. When significant, the effect of mowing was more marked at the dry site and for certain species (see interactions mowing \times site and mowing \times species in Appendix S2 'Both sites'). Moreover (Fig. 2, Appendix S2), the decrease in height and biomass connected with mowing was followed, particularly at the dry site, by an increase in leaf N_{mass} (with consequent lower leaf C:N) and below-ground organ water content. The $\delta^{13}C$ tended to be less negative when the meadow was mown ($P = 0.083$), particularly at the dry site (Fig. 2, Appendix S2). SLA, LDMC and height per biomass changed idiosyncratically in response to mowing. Overall, given the fact that the vegetation was mown to the same height at both sites, taller vegetation in the mown treatments of the wet site indicated higher vertical regrowth compared to the dry site ($P < 0.05$ in the nested ANOVA with only mown blocks).

Trait differentiation among co-existing species was strong in both meadows and generally stronger than differentiation caused by mowing and site effects (see species identity effects, significant for all traits at $P < 0.05$, and the corresponding F -value in the nested ANOVA, Appendix S2). In this sense, the variance of each trait within sites revealed the importance of the between-species differentiation in each meadow (i.e. functional diversity). Following randomization procedures (see Methods for details), traits more related to plant size, i.e. height and biomass and water, i.e. $\delta^{13}C$ and below-ground water content, reached

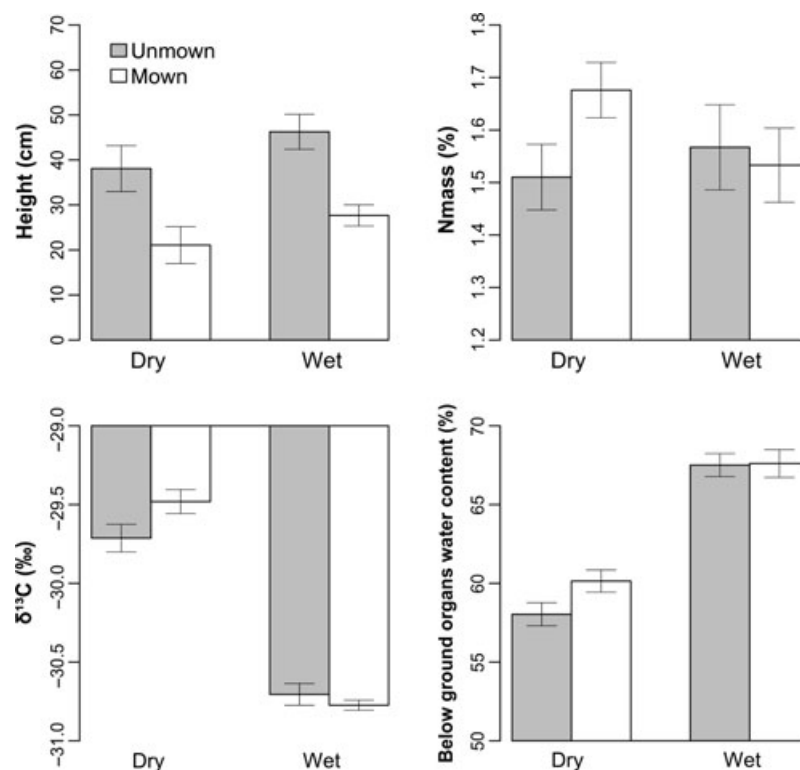


Fig. 2. Site and mowing effects on the mean value of the selected trait values (after Appendix S2). Error bars indicate ± 1 SE. Panels refer to results for plant height ('Height'), leaf nitrogen content ('N_{mass}'), leaf carbon isotope composition ($\delta^{13}\text{C}$), and below-ground organ water content.

a significantly higher variance at the dry site ($P = 0.029$ for height, $P = 0.037$ for biomass and $P = 0.011$ for $\delta^{13}\text{C}$), indicating higher functional diversity for these traits when water was more limiting. For some other traits related to nutrient acquisition, such as SLA and leaf N (both based on mass and area), trait variance, and therefore functional diversity, was higher at the wet site ($P = 0.012$ for SLA and $P = 0.008$ and $P = 0.014$ for leaf N based on mass and area, respectively).

Trait scaling

Overall, the trait differentiation between species revealed different trait scaling patterns at the dry vs wet site. The PCA diagrams (Fig. 1) indicate at both sites there was a trade-off between tall species with low SLA and shorter species with high SLA (with the angle between traits arrows giving an approximation of the correlation between traits). However, while many traits co-varied with height at the wet site, most of the traits were decoupled from height at the dry site, particularly several traits related to carbon–water economy, i.e. $\delta^{13}\text{C}$ and LDMC. Also, the first PCA axis explained more variability at the wet site (the eigenvalues for the first PCA axis were 0.520 and 0.394 for the wet and dry site, respectively; the second axis values were 0.197 and 0.265, respectively).

The bivariate trait relationships, using SMA, confirmed and expanded the general patterns revealed in the PCA analyses. Most of the selected traits varied with plant height, especially at the wet site, with site conditions and mowing regime altering the bivariate relationships between individual traits and height in a number of ways (either shown as change in slope or elevation in the SMAs; Appendix S3, Fig. 3). In particular, the results showed increased species stature per biomass at the wet site in comparison with the dry site, and a co-variation between height and LDMC, C:a, and $\delta^{13}\text{C}$ at the wet site (Fig. 3, not shown for C:N). The increased species stature per biomass at the wet site was also observable after removal of grasses from the analyses (see different symbols in Fig. 3). The effect of mowing on trait scaling relationships with height tended to resemble the effect of dry conditions (and vice versa wet and unmown conditions). For example, at both sites mowing produced higher biomass per plant height (i.e. higher SMA intercept: Fig. 4, Appendix S3). Lower plant height per biomass was also observed at the wet site as compared to the dry one (Fig. 3). Similarly, the relationship between height vs leaf N_{area} and root water content was stronger at the dry site when mown, while the relationship between height vs LDMC and leaf C with height was stronger at the wet site when unmown (Appendix S3, partly, Fig. 4).

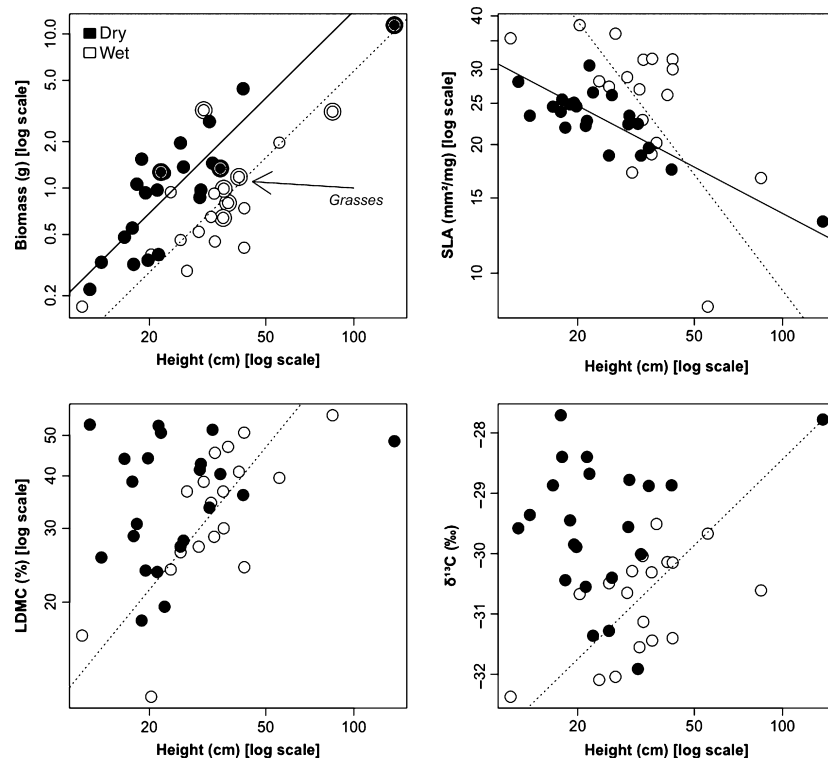


Fig. 3. Selection of relevant bivariate relationships between different traits and height, in dry vs wet meadows (full results shown in Appendix S3). Significant ($P < 0.05$) lines are shown (full line for the dry site, dotted line for the wet site). Panels refer to the results for sampled plant biomass ('Biomass'), specific leaf area ('SLA'), leaf dry matter content ('LDMC'), and leaf carbon isotope composition (' $\delta^{13}\text{C}$ '). For the height to biomass relationship, different circles indicate graminoids sampled as representative tussock units.

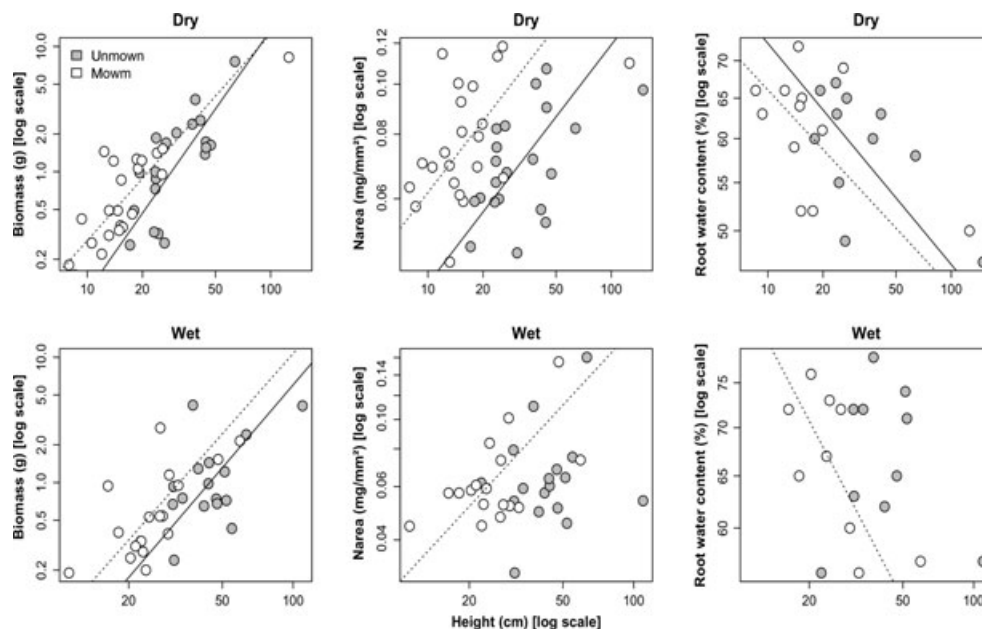


Fig. 4. Selection of bivariate relationships between different traits and height in the dry vs wet meadows in mown (empty circles) vs unmown (grey circles) conditions. Significant ($P < 0.05$) lines are displayed (full line for the unmown conditions and dotted line for the mown conditions).

Discussion

This study shows that changes in vegetation trait structure under combined effects of resource availability and disturbance regime can be highlighted through different trait scaling relationships between co-existing species. It shows that the effects on trait scaling were generally stronger (or at least the same) than the shift in trait average values *per se*. We revealed different trait scaling relationships, especially with respect to vertical vegetation structures in the dry vs wet meadows and mown vs unmown conditions. In total, these results support the view that differences between vegetation types are observable in alternative trait differentiation patterns and not necessarily only in changes in the average trait values across sites (Westoby et al. 2002).

It has been hypothesized that trait scaling relationships arise via strategic combinations of particular trait values (Westoby et al. 2002; Cornelissen et al. 2003). Traits connected directly to processes of dry mass investment within shoots (e.g. SLA, LDMC, biomass per height) are examples of such trade-offs (Huston & Smith 1987; Grime 2001), especially between species investing in rapid growth and resource acquisition (e.g. higher SLA, lower LDMC, low biomass per height) compared with more conservative species (with opposite sets of traits; Díaz et al. 2004; Wright et al. 2004; Falster & Westoby 2005). We could have assumed significant differences in slope between dry and wet sites in response to the convergence of two combined environmental filters (see Fonseca et al. 2000). This could indeed have been a consequence of the fact that the meadows had been abandoned only recently. Alternatively, the convergence in plant attributes to disturbance and water limitation could, more generally, result in additive effects in trait scaling relationships. In all cases, our study demonstrated the importance of trait scaling in relation to plant size under the combined effects of competition for light and water limitation. In particular, the trait combinations observed between species (Figs. 1–4) demonstrate a variety of viable life-history strategies co-existing together in the vegetation, with and without disturbance. The great variability in the traits of co-existing species was as strong, if not stronger, than the expected trait differences between species across sites and mowing regimes. These greater within-site than between-site variability patterns have been observed previously (e.g. Westoby et al. 2002; de Bello et al. 2009a), although they are often neglected when modelling vegetation structure. Yet few models predict the co-existence of a mix of height strategies as a conspicuous feature of vegetation (see review of Falster & Westoby 2003). The results of the present study suggest that incorporating trait scaling consequences for such a mix of height strategies in vegetation models could help

account for the processes of co-existence along both spectra of light income and disturbance regime. In this way, a single trait (like plant height) should not be used alone to predict vegetation dynamics, because it is not possible to distinguish between alternative strategies that allow species co-existence (Westoby et al. 2002; Poorter et al. 2003) and because the adaptive value of plant stature to disturbance varies with respect to its association with other traits (Klimešová et al. 2008; Lanta et al. 2011).

Site condition effects

The results concerning site effect revealed distinct axes of trait differentiation in the dry vs wet meadows. We hypothesize that the extent of trait differentiation, and its trait scaling changes, vary particularly in relation to the different space occupancy patterns in dry vs wet meadows (Milchunas et al. 1988) and how these are related to the most limiting resources at a site. We expect that at the wet site there were more constraints on plant height because of stronger competition for light, whereas at the dry site there were more constraints related to water acquisition. Overall, at the wet site we observed decreasing height differences between species but increasing importance of trait scaling between height and other traits. In contrast, at the dry site we observed increasing importance of alternative water use strategies, which were more decoupled from plant size. Stronger trait scaling changes at the wet site between various leaf traits (LDMC, C:N and $\delta^{13}\text{C}$) and height could suggest, for example, more complex differentiation between species in the acquisition and processing of resources with respect to the vertical structure of the vegetation (Appendix S3, Figs. 1, 3). These results indicate that when competition for light is the more important constraint on plant height, more pronounced trait scaling relationships between height and other traits can be expected. On the contrary, the higher differentiation in $\delta^{13}\text{C}$ at the dry site, with different traits linked to water processing ($\delta^{13}\text{C}$, LDMC below-ground water content) being rather independent of height (Figs. 1, 3), highlights alternative water use strategies when water is a more limiting factor. Although more research is required to confirm our hypothesis at larger scales, these results provide a first indication that the relevance of different trait scaling relationships could be shaped by the most limiting resource in a given vegetation type.

Mowing effects across sites

The mechanisms governing the response of diversity to disturbance under different environmental conditions remain elusive. As mentioned in the Introduction, we can expect that regrowth after disturbance is produced predominantly

on the vertical axis in wetter conditions and more along the horizontal axis in drier conditions (Milchunas et al. 1988). The lower investment in biomass per height in the wet meadow (Fig. 3) and in the unmown condition at both sites (Fig. 4), together with more productive (Table 1) taller vegetation and higher regrowth after disturbance at the wet site, reflect these predictions. In particular, if we assume that taller and more vertical-growing species have a competitive advantage for light (Falster & Westoby 2003; Jumpponen et al. 2005), the higher height per biomass ratios in the wet meadow and unmown conditions suggest that plants make a higher investment per unit of biomass to grow vertically and reach the canopy as a response to competition for light.

Our results also provide evidence of the rather similar effects of mowing and dry conditions (and vice versa, unmown and wet conditions) on similar traits and similar trait scaling relationships. Such effects were accompanied by a reinforced effect of mowing in decreasing soil water content at the dry site. According to classical studies of the effects of grazing on vegetation structure, the capacity for regrowth after disturbance largely results from a modification in resource availability (Milchunas et al. 1988). It also leads to a compensatory increase in photosynthetic capacity of the remaining or new leaves. In this sense, the already less negative $\delta^{13}\text{C}$ values at the dry site (suggesting stronger water saving strategies; Jumpponen et al. 2005; de Bello et al. 2009b) tended to be further increased by mowing. This suggests that the lower soil water content, accompanied by a less dense canopy with increased light irradiation and higher water vapour deficit, could lead to plants growing with more closer stomata and with increased water saving strategies. We cannot, however, rule out the small influence of changes in C signature due to C reallocation from storage organs, as new shoots are generally built with a higher contents of heavier C isotopes; this effect, however, should be minimized by collecting only mature leaves (Seibt et al. 2008). This hypothesis is also supported by more recent gas exchange measurements, showing that plants under a mown treatment had a higher photosynthetic rate at the same level of stomatal conductance, which means higher water use efficiency under similar environmental conditions (Macková unpubl. results). The increased below-ground water content under mown conditions at the dry site (Fig. 2) further supports this hypothesis, suggesting that water is used more efficiently when scarcer. The higher leaf N_{mass} under mowing at the dry site (Fig. 2) also supports the idea of decreased light competition with increased mobilization of N at the site where soil N was less limiting. Higher leaf N_{mass} and N_{area} are associated with a higher carboxylation capacity, which, in combination with higher mesophyll conductance, allows for maintaining a higher photosyn-

thesis rate even under low stomatal conductance (Flexas et al. 2008).

Conclusions

Overall, our results stress the fact that the expected convergence in plant attributes conferring tolerance to both resource scarcity and disturbance (Milchunas et al. 1988; Grime 2001; Osem et al. 2004; Rusch et al. 2009) should also be considered in the context of convergent trait scaling relations. Similar selection in different trait scaling relationships (under dry and mown conditions, on the one hand, unmown and wet conditions, on the other) indicate that changes in water and light availability under mowing could imply similar shifts in trait combinations. While the idea that disturbance reinforces water stress in a vegetation structure is generally accepted (Osem et al. 2004; Pakeman 2004), our results indicate that such patterns further involve rearrangements in community structure along different axes of trait combinations. Although based on only two environmentally contrasted locations, our study is an example of the potential comparisons that can be performed involving different trait scaling relationships. It also sets several new hypotheses about combined effects of biotic and abiotic filters on species co-existence that should be tested more widely. The main implication of these results is that in order to attain a general model of vegetation dynamics in response to environmental conditions, we should encourage comparative studies in different trait scaling relationships, especially in relation to the vertical structure of different vegetation types.

Acknowledgements

Several collaborators made this study possible thanks to their dedicated work in the field and in the laboratory: Eliška Padyšáková, Eliška Patáčová, Jan Altman, Jana Kantorová, Alena Bartušková, Vít Latzel and Tomáš Pícek. We also thank Robin Pakeman and three anonymous reviewers for many useful suggestions, and Jan W. Jongepier for language correction. This study was supported by projects GACR 206/09/1471, GACR 526/09/0963, MSMT 6007665801, GACR 526/07/0808 and CNRS APIC RT PICs 4876.

References

- de Bello, F., Thuiller, W., Lepš, J., Choler, P., Clement, J.C., Macek, P., Sebastia, M.T. & Lavorel, S. 2009a. Partitioning of functional diversity reveals the scale and extent of trait convergence and divergence. *Journal of Vegetation Science* 20: 475–486.

- de Bello, F., Buchmann, N., Casals, P., Lepš, J. & Sebastia, M.T. 2009b. Relating plant species and functional diversity to community delta C-13 in NE Spain pastures. *Agriculture Ecosystems and Environment* 131: 303–307.
- de Bello, F., Lavorel, S., Albert, C.H., Thuiller, W., Grigulis, K., Doležal, J., Janeček, Š. & Lepš, J. 2011. Quantifying the relevance of intraspecific trait variability for functional diversity. *Methods in Ecology and Evolution* 2: 163–174.
- ter Braak, C.J.F. & Šmilauer, P. 2002. *CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5)*. Microcomputer Power, Ithaca, NY, USA.
- Cornelissen, J.H.C., Cerabolini, B., Castro-Diez, P., Villar-Salvador, P., Montserrat-Marti, G., Puyravaud, J.P., Maestro, M., Werger, M.J.A. & Aerts, R. 2003. Functional traits of woody plants: correspondence of species rankings between field adults and laboratory-grown seedlings? *Journal of Vegetation Science* 14: 311–322.
- Díaz, S., Noy-Meir, I. & Cabido, M. 2001. Can grazing response of herbaceous plants be predicted from simple vegetative traits? *Journal of Applied Ecology* 38: 497–508.
- Díaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J.H.C., Jalili, A., Montserrat-Marti, G., Grime, J.P., Zarrinkamar, F., Asri, Y., Band, S.R., Basconcelo, S., Castro-Diez, P., Funes, G., Hamzehee, B., Khoshnevi, M., Perez-Harguindeguy, N., Perez-Rontome, M.C., Shirvany, F.A., Vendramini, F., Yazdani, S., Abbas-Azimi, R., Bogaard, A., Boustani, S., Charles, M., Dehghan, M., de Torres-Espuny, L., Falczuk, V., Guerrero-Campo, J., Hynd, A., Jones, G., Kowsary, E., Kazemi-Saeed, F., Maestro-Martinez, M., Romo-Diez, A., Shaw, S., Siavash, B., Villar-Salvador, P. & Zak, M.R. 2004. The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science* 15: 295–304.
- Duru, M., Ansquer, P., Jouany, C., Theau, J.P. & Cruz, P. 2010. Comparison of methods for assessing the impact of different disturbances and nutrient conditions upon functional characters of grassland communities. *Annals of Botany* 106: 823–831.
- Enquist, B.J., West, G.B., Charnov, E.L. & Brown, J.H. 1999. Allometric scaling of production and life-history variation in vascular plants. *Nature* 401: 907–911.
- Falster, D.S. & Westoby, M. 2003. Plant height and evolutionary games. *Trends in Ecology and Evolution* 18: 337–343.
- Falster, D.S. & Westoby, M. 2005. Alternative height strategies among 45 dicot rain forest species from tropical Queensland, Australia. *Journal of Ecology* 93: 521–535.
- Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 503–537.
- Fine, P.V.A., Miller, Z.J., Mesones, I., Irazuzta, S., Appel, H.M., Stevens, M.H.H., Saaksjarvi, I., Schultz, L.C. & Coley, P.D. 2006. The growth–defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* 87: S150–S162.
- Flexas, J., Ribas-Carbó, M., Díaz-Espejo, A., Dalmés, J. & Medrano, H. 2008. Mesophyll conductance to CO₂: current knowledge and future prospects. *Plant, Cell and Environment* 31: 602–621.
- Fonseca, C.R., McC Overton, J., Collins, B. & Westoby, M. 2000. Shift in trait-combinations along rainfall and phosphorus gradients. *Functional Ecology* 88: 964–977.
- Grime, J.P. 2001. *Plant strategies, vegetation processes and ecosystem properties*, 3rd edn. Wiley, Chichester, UK.
- Grime, J.P. 2006. Trait convergence and trait divergence in herbaceous plant communities: mechanisms and consequences. *Journal of Vegetation Science* 17: 255–260.
- Huston, M. & Smith, T. 1987. Plant succession – life history and competition. *The American Naturalist* 130: 168–198.
- Janeček, S., Lanta, V., Klimešová, J. & Doležal, J. 2011. Effect of abandonment and plant classification on carbohydrate reserves of meadow plants. *Plant Biology* 13: 243–251.
- Jumpponen, A., Mulder, C.P.H., Huss-Danell, K. & Hogberg, P. 2005. Winners and losers in herbaceous plant communities: insights from foliar carbon isotope composition in monocultures and mixtures. *Journal of Ecology* 93: 1136–1147.
- Kahmen, S. & Poschlod, P. 2008. Effects of grassland management on plant functional trait composition. *Agriculture, Ecosystems and Environment* 128: 137–145.
- Klimešová, J., Latzel, V., de Bello, F. & van Groenendael, J.M. 2008. Plant functional traits in studies of vegetation changes in response to grazing and mowing: towards a use of more specific traits. *Preslia* 80: 245–253.
- Klimešová, J., Janeček, S., Bartušková, A., Lanta, V. & Doležal, J. 2010. How is regeneration of plants after mowing affected by shoot size in two species-rich meadows with different water supply? *Folia Geobotanica* 45: 225–238.
- Kubát, K., Hrouda, L., Chrtěk, J., Kaplan, Z., Kirschner, J. & Štěpánek, J. 2002. *Klíč ke květeně České republiky*. Akademia, Prague, CZ.
- Lanta, V., Klimešová, J., Martincová, K., Janeček, S., Doležal, J., Rosenthal, J., Lepš, J. & Klimeš, L. 2011. A test of the explanatory power of plant functional traits on the individual and population levels. *Perspectives in Plant Ecology, Evolution and Systematics* 13: 189–199.
- Loiseau, P., Louault, F., Le Roux, X. & Bardy, M. 2005. Does extensification of rich grasslands alter the C and N cycles, directly or via species composition? *Basic and Applied Ecology* 6: 275–287.
- Louault, F., Pillar, V.D., Aufrere, J., Garnier, E. & Soussana, J.F. 2005. Plant traits and functional types in response to reduced disturbance in a semi-natural grassland. *Journal of Vegetation Science* 16: 151–160.
- Mason, N.W.H., de Bello, F., Doležal, J. & Lepš, J. 2011. Niche overlap reveals the effect of competition, disturbance and contrasting assembly processes in experimental grassland communities. *Journal of Ecology* 3: 788–796.
- Milchunas, D.G. & Lauenroth, W.K. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs* 63: 327–366.

- Milchunas, D.G., Sala, O.E. & Lauenroth, W.K. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. *The American Naturalist* 132: 87–106.
- Osem, Y., Perevolotsky, A. & Kigel, J. 2004. Site productivity and plant size explain the response of annual species to grazing exclusion in a Mediterranean semi-arid rangeland. *Journal of Ecology* 92: 297–309.
- Pakeman, R.J. 2004. Consistency of plant species and trait responses to grazing along a productivity gradient: a multi-site analysis. *Journal of Ecology* 92: 893–905.
- Pakeman, R.J. & Queded, H.M. 2007. Sampling plant functional traits: what proportion of the species need to be measured? *Applied Vegetation Science* 10: 91–96.
- Pakeman, R.J., Lepš, J., Kleyer, M., Lavorel, S., Garnier, E. & Consortium, V. 2009. Relative climatic, edaphic and management controls of plant functional trait signatures. *Journal of Vegetation Science* 20: 148–159.
- Pálková, K. & Lepš, J. 2008. Positive relationship between plant palatability and litter decomposition in meadow plants. *Community Ecology* 9: 17–27.
- Poorter, L., Bongers, F., Sterck, F.J. & Woll, H. 2003. Architecture of 53 rain forest tree species differing in adult stature and shade tolerance. *Ecology* 84: 602–608.
- Quetier, F., Lavorel, S., Thuiller, W. & Davies, I. 2007. Plant trait-based modelling assessment of ecosystem service sensitivity to land-use change. *Ecological Applications* 17: 2377–2386.
- Römermann, C., Bernhardt-Römermann, M., Kleyer, M. & Poschlod, P. 2009. Substitutes for grazing in semi-natural grasslands – do mowing or mulching represent valuable alternatives to maintain vegetation structure? *Journal of Vegetation Science* 20: 1086–1098.
- Rusch, G.M., Skarpe, C. & Halley, D.J. 2009. Plant traits link hypotheses about resource-use and response to herbivory. *Basic and Applied Ecology* 10: 466–474.
- Seibt, U., Rajabi, A., Griffiths, H. & Berry, J.A. 2008. Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia* 155: 441–454.
- Stubbs, W.J. & Wilson, J.B. 2004. Evidence for limiting similarity in a sand dune community. *Journal of Ecology* 92: 557–567.
- Warton, D.I., Wright, I.J., Falster, D.S. & Westoby, M. 2006. Bivariate line-fitting methods for allometry. *Biological Reviews* 81: 259–291.
- Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33: 125–159.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. & Villar, R. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.

Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Variation of soil water content at the time of sampling.

Appendix S2. Results of nested ANOVA on species traits.

Appendix S3. Comparison of bivariate SMA relationships of height with other traits.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.