

Different sets of belowground traits predict the ability of plant species to suppress and tolerate their competitors

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Abstract

Background and aims Functional traits may underlie differences in niches, which promote plant species co-existence, but also differences in competitive ability, which drive competitive exclusion. Empirical evidence concerning the contribution of different traits to niche differentiation and the ability to suppress and tolerate competitors is very limited, particularly when considering belowground interactions.

Methods We grew 26 temperate grassland species along a density gradient of interspecific competitors to determine which belowground traits a) explain species' ability to suppress and tolerate neighbours and b) contribute to niche differentiation, such that species with dissimilar trait values experience reduced competition.

Results We found that having larger root systems with extensive horizontal spread and lower root tissue density enabled efficient suppression of neighbours but did not significantly contribute to the ability to tolerate competition. Species with deeper root systems, lower specific

root length and less branched roots were better at tolerating competition, but these traits did not significantly affect the ability to suppress neighbours. None of the measured traits contributed significantly to niche differentiation, either individually or in combination.

Conclusions This study provides little support for belowground traits contributing to species co-existence through niche differentiation. Instead, different sets of weakly correlated traits enable plants to either suppress or tolerate their competitors.

Keywords Belowground interactions · Species coexistence · Competitive ability · Functional traits · Niche differentiation · Phenotypic plasticity · Rooting depth · Stress tolerance

Introduction

Plant functional traits have been extensively used as a means to understand plant community assembly (Lavorel and Garnier 2002; de Bello et al. 2010; Gotzenberger et al. 2012). Differences in functional traits have been used as a proxy for stabilising niche differences, based on the principle of limiting similarity, which predicts stronger competition between species with similar trait values (MacArthur and Levins 1967; Chesson 2000). However, plant traits may also influence competitive ability and contribute to competitive exclusion of species with inferior trait values (Chesson 2000; Grime 2006; Mayfield and Levine 2010). Indeed, an increasing number of studies have found that commonly used functional traits are more

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strongly related to competitive hierarchies than to niches (Kunstler et al. 2012; Herben and Goldberg 2014; Kraft et al. 2015). This apparent contradiction highlights the current lack of a mechanistic understanding of the way in which easily measured traits relate to plant function and the way in which different traits contribute to competitive hierarchies versus niche differentiation between species (Kraft et al. 2015; Shipley et al. 2016).

The existence of competitive hierarchies among plant species is well documented. Plant size has been repeatedly identified as a trait that is central to competitive success (Goldberg 1996; Keddy et al. 2002). Several aboveground traits such as greater maximum height, larger leaf area, higher leaf nitrogen content and lower leaf dry matter content have also been shown to provide an advantage in interspecific competition (Goldberg and Landa 1991; Herben and Goldberg 2014; Bennett et al. 2016). Competitively superior species are also frequently characterised by high specific leaf area (Kunstler et al. 2012; Fort et al. 2014; Kraft et al. 2015). These traits are often used to describe the leaf economics spectrum and suggest that species with strongly acquisitive traits are good competitors, whereas species with more conservative traits are less competitive (Wright et al. 2004; Reich 2014). Despite the importance of roots for plant functioning and ecosystem processes (Bardgett et al. 2014) and competition for soil-based resources being equally or even more important than competition for light (Kiaer et al. 2013), most trait-based studies of plant community assembly have concentrated on aboveground traits.

Current knowledge of belowground traits and their contribution to species co-existence still consists of too few traits and species to draw robust generalisations. It has been shown that higher competitive ability is associated with greater root system size, either achieved through greater allocation of biomass to roots or through higher specific root length (Wang et al. 2010; Ravenek et al. 2016). Specific root length and a combination of specific root length and aboveground traits have also been shown to contribute to niche differentiation (Kraft et al. 2015; Bennett et al. 2016). Species differences in rooting depth have long been assumed to form a main axis of niche differentiation, promoting productivity in diverse plant communities (Parrish and Bazzaz 1976; Berendse 1982). However, recent empirical studies have found little support for this hypothesis and suggest that rooting depth may instead be more closely related to competitive ability (Mommer et al. 2010; Kraft et al. 2015; Hoekstra et al. 2015).

Moreover, we still lack mechanistic understanding of the pathways by which traits affect competitive outcome (Gruntman et al. 2014; Aschehoug et al. 2016; Shipley et al. 2016). Goldberg (1996) proposed that plants can persist and reproduce in the face of competition by exhibiting a strong competitive effect – i.e. the ability to suppress the growth of their competitors – and/or by possessing a good competitive response, i.e. the ability to minimise the negative impact of sharing resources with neighbours. While some studies have reported positive correlations between competitive effect and competitive response (Novoplansky and Goldberg 2001; Wang et al. 2010), others have not detected significant correlations (Cahill et al. 2005; Baron et al. 2015). Strong competitive effects have been linked to plant size and resource pre-emption but traits related to competitive response remain to be elucidated (Cahill et al. 2005; Wang et al. 2010). As root competition is more size-symmetric than shoot competition and involves multiple resources (Schwinning and Weiner 1998; Cahill and Casper 2000), competitive success in belowground interactions may be less reliant on size and more dependent on diverse traits conferring competitive tolerance (Keddy et al. 1998; Belter and Cahill 2015).

The aim of this study was to determine which belowground traits a) enhance species' ability to suppress and tolerate neighbours (i.e. competitive effect and response, respectively) or b) allow niche differentiation (demonstrated by reduced competitive suppression between species with dissimilar traits). Competitive outcome and relationships between functional traits and competitive ability may be strongly influenced by the choice of the competitor species (Wang et al. 2010; Baron et al. 2015). In natural conditions, community species composition and spatial structure determine the identities of interacting individuals, and the frequency of such interactions is a factor regulating local adaptation in competing species (Turkington 1989; Grondahl and Ehlers 2008; Lankau 2012; Abakumova et al. 2016). To assess interspecific interactions in a realistic setting, we therefore conducted an experiment in which we selected 26 focal species characteristic of temperate grasslands and grew them in interspecific competition with a species they encounter as a common neighbour in their home community (Semchenko et al. 2013). As competitive outcomes may also be strongly dependent on the density of neighbours (Goldberg and Landa 1991; McPhee and Aarssen 2001), we grew plants at a range of neighbour densities. This also allowed us to assess trait plasticity in

response to neighbour density and the contribution of trait plasticity to species' competitive ability. Correlations between seven belowground traits and the dependence of competitive outcome on focal and neighbour trait values, and trait distances, were examined. As recent studies have found significant links between root morphology and mycorrhizal colonisation (Maherali 2014; Cortois et al. 2016), we assessed whether measured root traits were correlated with a species-level index of mycorrhizal association based on previously published data (Gerz et al. 2016). We hypothesised that a) competition is least intense between species differing in multiple traits, and b) the ability to suppress neighbours is driven by size-related traits while tolerance of competition is related to trait plasticity, root morphology and associated mycorrhizal status, and root spatial distribution.

Materials and methods

Experimental design

Seeds of all species used in the experiment were collected from seven temperate grassland communities differing in species richness, productivity and management history in Estonia (Table S1; Semchenko et al. 2013). All seeds were air-dried and stored at 4 °C until the experiment began. Twenty-six plant species (17 forbs, 5 graminoids and 4 legumes) were selected as focal species.

The following spring, seeds were germinated on moist sand in a greenhouse. Upon germination, seedlings were transplanted into pots filled with a mixture of commercial potting compost (pH 6.5, water-soluble N 100 mg/l, P 80 mg/l, K 400 mg/l), sand and limestone powder. Substrate was prepared for species collected from different study sites by mixing commercially available soil, sand and lime powder so as to match the N content and pH of natural soil at the site of collection as closely as possible (Semchenko et al. 2013). Soil from the site of seed collection was also added to the substrate mixture to provide plants with natural soil biota. Groups of species from different habitats were grown in pots of different volume to reduce growth constraint by pot walls in species from productive habitats characterised by larger plant size, which would be inevitable if the same pot volume was used for all species. Pots with volume of 3.5 l and a diameter of 17.1 cm were used for plants from Sites 2 and 7; 5 l pots with a diameter of

20.4 cm were used for plants from Sites 1, 3 and 4; 7.5 l pots with a diameter of 23.4 cm were used for plants from Sites 5 and 6. Pot volume and initial soil N content were kept constant across all neighbour density treatments for species groups from the same habitat. A focal plant was planted at the centre of each pot. Each focal species was subjected to a density gradient of heterospecific neighbours belonging to a single species: no neighbours, 1, 2, 3, 4, 6 or 8 neighbouring individuals. Neighbouring plants were established equidistantly around the focal plant, in a circle with a radius equal to 2/3 of the pot radius. For each focal species, the heterospecific neighbour chosen was a species commonly encountered by the focal species in its native community, as identified in a previous field survey (Table S1; Semchenko et al. 2013). Each focal species × neighbour density (0–8 neighbours) combination was replicated twice (i.e. 14 pots per focal species). Two additional replicates of every focal and neighbour species were grown as single plants to enable growth potential in the absence of competition to be estimated. The total number of pots established for the experiment was 436, but measurements were limited to 410 pots by the end of the experiment because of seedling mortality. Full details of the experimental design are given in Semchenko et al. 2013.

Pots were placed randomly in a single block in a common garden. The positions of pots were re-randomised twice during the experiment. During the first 2 weeks of the experiment, seedlings that failed to survive after transplantation were replaced. Plants were exposed to ambient precipitation, and received additional watering during spells of dry weather. Plants were harvested after 11–14 weeks of growth as follows. Aboveground biomass was removed and dried at 70 °C for 48 h. Intact soil from each pot was frozen at –18 °C and then sliced horizontally at depths of 5 cm and 10 cm below the soil surface. The defrosted surface of soil slices at 5 cm and 10 cm depths was scanned (Epson perfection V700 PHOTO, Long Beach, CA, USA) and the number of root intersections visible on the scanned images was recorded. For eight species from Site 2, all root tips visible within the central area of the scanned image, corresponding to 2/3 of the pot radius, were recorded (data used in Semchenko et al. 2013). For the remaining species, root tips visible within 19 circular sampling plots (each with an area of 1 cm²) distributed in a hexagonal pattern across the central area of the scanned image were recorded (Fig. S1).

Horizontal spread of root systems

The horizontal spread of the root systems of each focal and neighbouring species in the absence of neighbours was calculated as the number of root tips located at the periphery of the pot at 5 cm soil depth (reaching 2/3 of the pot radius) divided by the total number of root tips recorded at 5 cm soil depth (see Fig. S1 for graphical representation).

Vertical distribution of root systems

For 18 focal species, differences in root colour allowed the root tips of focal and neighbouring species to be counted separately, enabling assessment of the spatial distribution of roots of focal and neighbouring plants along the neighbour density gradient (0–8 neighbours). The vertical distribution of focal species roots was calculated as the number of root tips at 5 cm soil depth divided by the total number of focal root tips recorded at 5 cm and 10 cm soil depths. The same calculations were performed to describe the vertical distribution of the roots of neighbour species.

Root mass and morphology

After scanning soil slices, the roots of focal and neighbouring plants were carefully washed out from the top 5 cm soil layer and separated. Roots of 22 focal species were successfully separated and available for morphological analysis. For nine focal species, roots were only washed out of a fraction of the replicate pots because of the difficulty of disentangling and retrieving intact roots (Table S1). Two representative root axes with associated higher order laterals were selected from each focal plant and its neighbours for morphological analysis. Roots were scanned (Epson perfection V700 PHOTO, Long Beach, CA, USA) and root length, volume, number of root tips and mean root diameter were calculated using the program WinRhizo 2008a (Regent Instruments Inc., Quebec, Canada). Scanned roots, the rest of the roots and aboveground biomass were dried at 70 °C for 48 h and weighed separately. Specific root length (SRL) was calculated as the ratio of root length to dry mass of the scanned root sample. Root tissue density (dry root mass/root volume) and branching frequency (number of root tips/root length) were also calculated. The extent to which each studied species associates with mycorrhizal fungi (mycorrhization index) was estimated

as the proportion of published studies reporting the presence of mycorrhizal colonisation in the species, as described in Gerz et al. (2016) and using data from Harley and Harley (1987), Wang and Qiu (2006), Akhmetzhanova et al. (2012) and Hempel et al. (2013).

Statistical analysis

Growth response to competition

Growth response to competition (GRC) was calculated as the slope of the regression between ln-transformed aboveground focal species biomass and neighbour density. The slope (negative in the case of competition) expresses the impact of neighbour density on focal plant size. The more negative the value, the stronger the competitive response of the focal species and the competitive effect of the neighbour species. GRC was also calculated using root biomass, which was available for 22 species. Using root biomass instead of aboveground biomass to calculate GRC produced very similar results in all subsequent analyses. We therefore present results of models with GRC calculated using aboveground biomass, which was available for all 26 species.

Plasticity in response to neighbour density, and competitive ability

To examine how root vertical distribution and morphology was modified in focal species in response to neighbour density, individual linear models were constructed for each focal species using five root traits (root vertical distribution, SRL, root diameter, root tissue density and root branching frequency) as response variables, and neighbour density as an explanatory variable. *Directional plasticity* was quantified as the slope of the linear relationship between neighbour density and ln-transformed root traits (except for vertical distribution, which was square root transformed). It can be negative or positive, reflecting a linear increase or decrease in trait values with neighbour density. Additionally, the *magnitude of plasticity* was quantified as the absolute value of directional plasticity (i.e. absolute value of the slope).

To examine the role of root trait plasticity in determining competitive ability, linear models were constructed, with growth response to competition (GRC) as the response variable and the plasticity of each trait (directional or magnitude) as explanatory variables.

Interspecific relationships between trait means and competitive ability

Seven belowground traits for each focal and neighbour species were calculated as follows: a) mean horizontal spread and ln-transformed root mass of plants grown in the absence of competition, and b) mean root vertical distribution and ln-transformed SRL, root diameter, root tissue density and root branching frequency across all neighbour densities. Root horizontal spread and root biomass were calculated for plants grown in the absence of competition to reflect maximum growth potential under the experimental conditions. Also, horizontal spread could not be measured in pots with competitors as its estimation requires a plant to be located in the centre of the pot (which was not the case for neighbours in competition treatments), while root mass in pots with competitors was strongly correlated with shoot mass and GRC, which was the response variable in subsequent data analyses. The mean values for all other traits were calculated across all available pots (i.e. up to 14 replicates per focal species, Table S1).

To examine the effect of focal and neighbour species traits on growth response to competition (GRC), linear regression models were constructed using GRC as a response variable and either A) the mean trait value of the focal species and the mean trait value of the neighbour species or B) the mean Euclidean distance between the focal and neighbour species trait values as explanatory variables. Model A tests for the significance of focal and neighbour species root traits in explaining competitive suppression of focal species. In addition, pot volume and soil N content were added to models as covariates to determine whether these significantly affected any of the relationships between GRC and root traits. Variance inflation factors were calculated to assess possible collinearity between multiple predictors in the models (Zuur et al. 2010). Model B tests whether differences in root traits result in niche differentiation, with less severe competition occurring when there are greater distances between the focal and neighbour species trait values (i.e. a positive coefficient in Model B). Models were constructed for each of the seven root traits. Study site was not included as a random effect in the final models as it did not improve the fit of the models

significantly, and produced nearly identical fixed effect estimates.

To assess whether closely related species had more similar root traits and GRC (i.e. to test for phylogenetic signal in the data; Revell 2010), the same models (A-B) were constructed using generalised least squares with a correlation structure that accounts for phylogenetic dependencies between species (function `gls` in package `nlme` and `corPagel` in package `ape`, λ estimated from the data). The models with and without phylogenetic correlation structure were compared using likelihood ratio tests (Revell 2010; Münkemüller et al. 2012). A phylogeny containing all the study species was obtained from Durka and Michalski (2012).

To examine possible niche differentiation in multi-dimensional trait space, we calculated Euclidean distance between the trait values of focal and neighbour species in one- to seven-dimensional trait space (i.e. producing a total of 127 combinations of the seven measured root traits). We then fitted linear models with growth response to competition (GRC) as a response variable and the distance between root trait values as a single explanatory variable. The analysis was performed on 14 focal species for which all of the seven root traits were measured. A positive relationship would indicate niche differentiation, with weaker competitive growth suppression in species pairs with larger trait distances, whereas a negative relationship would indicate weaker competitive suppression in species pairs with more similar trait values. The best-fitting models were identified using the Akaike Information Criterion (AIC). In addition, we calculated the relative response to hetero- and conspecific competition for each focal species as $GRC_{\text{hetero}} - GRC_{\text{con}}$, where GRC is the slope of the regression between ln-transformed aboveground biomass of the focal species and heterospecific or conspecific neighbour density, respectively (available from Semchenko et al. 2013). Positive values indicate that plants are more suppressed by conspecific than heterospecific competition, which is a theoretical condition for species co-existence. A significant positive relationship between the relative strength of heterospecific competition ($GRC_{\text{hetero}} - GRC_{\text{con}}$) and trait distances would indicate greater potential for co-existence among species with dissimilar trait values.

Data analyses were performed using R 3.3.2 (R Development Core Team 2016).

Results

Response to density of neighbours

Neighbour density significantly affected all measured root traits, but species differed widely in the direction and magnitude of their response and in the combination of traits that were significantly modified (Fig. S2). The relationship between any selected root trait and neighbour density was positive for some species but negative for others. Many species exhibited wide variation in root traits that could not be explained by neighbour density. No significant relationship was found between growth response to competition and the direction or magnitude of plasticity in any of the measured root traits (Table 1).

Relationships between root traits and competitive ability

Size-related root traits

The growth of focal plants was more suppressed when neighbouring species were characterised by larger root mass and/or wider root system horizontal spread (based on trait values measured on neighbouring species grown in the absence of competition; Fig. 1c-d; Table 2). However, the root mass and horizontal spread of focal species did not affect their ability to withstand competition from neighbours (Fig. 1a-b; Table 2). Therefore, the size of the root system was an important determinant of competitive effect but not of competitive response. There was a significant phylogenetic signal for the

relationship between growth response to competition and root horizontal spread (Table 2).

Root vertical distribution and morphology

Growth response to competition was explained by the traits of the focal plant but not those of the neighbouring species when considering root vertical distribution, SRL and root branching frequency (Table 2). Species with deeper root systems (Fig. 2a), lower SRL (Fig. 2c) and less branched roots (Fig. 2i) were better at tolerating interspecific competition, but these traits did not significantly affect the ability to suppress neighbours (Fig. 2b-j). Root tissue density had a significant effect on the competitive effect of neighbours, but not on the competitive response of focal species: neighbour species with lower root tissue density had a more suppressive effect on the growth of focal plants, but tissue density of focal plants did not affect ability to withstand competition (Table 2, Fig. 2 g-h). There was no significant relationship between growth response to competition and root diameter in either focal or neighbour species (Table 2; Fig. 2 e-f).

The inclusion of pot volume and soil N content as covariates in the models did not significantly improve model fits, except for root horizontal and vertical distribution, where model fit was improved (likelihood ratio test, $P = 0.045$ and $P = 0.047$, respectively) but the relationship between focal trait values and competitive response remained very similar (Table S2, Figs. S3–4). No phylogenetic signal was detected for the relationships between root morphological traits and growth response to competition (Table 2).

Table 1 Summary of models assessing the relationship between competitive ability and the direction and magnitude of trait plasticity in response to the density of interspecific neighbours. Growth response to competition was used as the response variable in all models and each model contained a plasticity index in one of the root traits as an explanatory variable. Model coefficients, standard errors and statistical significance (NS - $P > 0.1$; \$ -

$P < 0.1$; *** - $P < 0.001$) are presented. Phyl. signal denotes likelihood ratio tests comparing models with and without phylogenetic correlation structure. Phylogenetic structure was included in the models if significant signal was detected. N – number of species for which there was sufficient data to estimate plasticity in response to neighbour density

Trait	Directional plasticity	Phyl. signal	Magnitude of plasticity	Phyl. signal	N
Vertical distribution	-1.33(0.96) NS	NS	-2.36(1.43) NS	NS	18
SRL (cm/mg)	-0.63(0.58) NS	NS	-1.60(0.80) \$	NS	13
Diameter (mm)	1.59(1.57) NS	NS	-4.24(2.45) NS	NS	13
Tissue density (g/cm ³)	0.48(0.58) NS	***	0.23(1.18) NS	***	13
Branching frequency(1/cm)	-1.25(0.80) NS	NS	-0.09(1.28) NS	NS	13

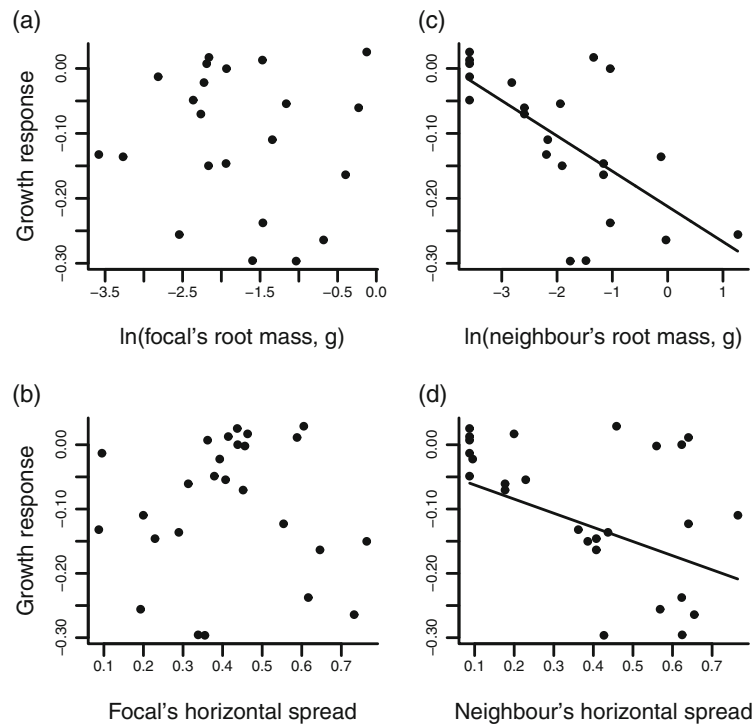


Fig. 1 Relationships between the growth response of focal species to competition and **a**) root mass of focal species; **b**) root horizontal spread of focal species; **c**) root mass of neighbour species; and **d**) root horizontal spread of neighbour species. The y-axis shows the slope of the relationship between focal plant mass and neighbour density, with more negative values indicating stronger growth suppression by neighbours. The y-axis reflects competitive response in the left-

hand column and competitive effect in the right-hand column of the panels. Horizontal spread was measured as the proportion of total root tips located at the periphery of the pots at 5 cm soil depth. All root traits were measured on plants grown in the absence of competition to estimate their growth potential. Solid lines show regression lines of relationships that were significant at $P < 0.05$. See Fig. S3 for partial regression plots

Niche differentiation based on root traits

None of the measured root traits contributed significantly to niche differentiation (i.e. there were no significant positive relationships between growth response to

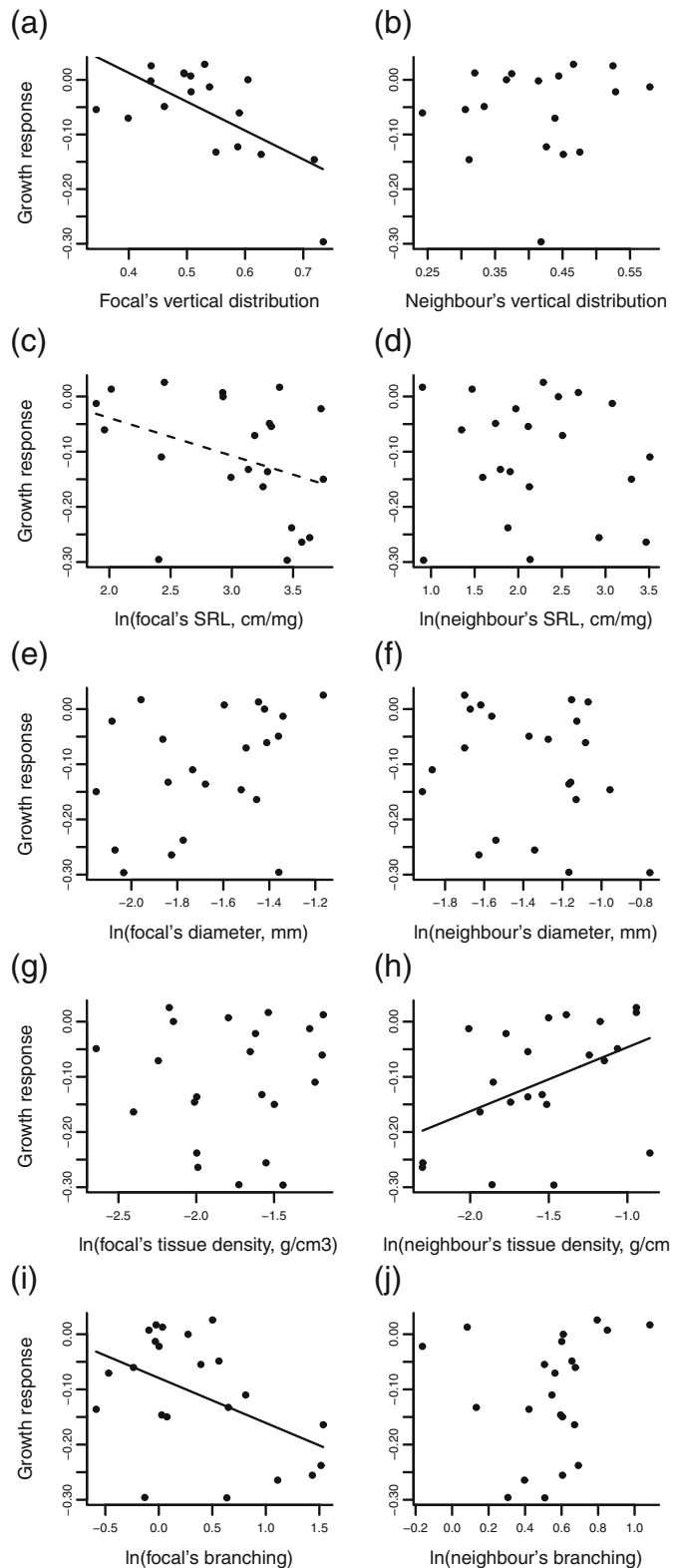
competition and the distance between the trait values of focal and neighbour species; Table 3). Differences in root vertical distribution significantly affected growth response to competition but in the opposite direction to that predicted by niche theory: focal species were least

Table 2 Summary of multiple regression models examining the relationship between growth response to competition (response variable) and mean trait values of the focal and neighbour species (explanatory variables). Model coefficients, standard errors and statistical significance (NS - $P > 0.1$; \$ - $P < 0.1$; * - $P < 0.05$; ** - $P < 0.01$;

*** - $P < 0.001$) are presented. Negative coefficients mean greater competitive suppression with increasing trait value. Phyl. signal denotes likelihood ratio tests comparing models with and without phylogenetic correlation structure. Phylogenetic structure was included in the models if significant signal was detected. N – number of species

Trait	Focal's trait	Neighbour's trait	Phyl.signal	N
Root mass (g)	-0.02(0.02) NS	-0.06(0.01) ***	NS	22
Horizontal spread	0.001(0.07) NS	-0.22(0.06) **	**	26
Vertical distribution	-0.52(0.16) **	0.06(0.19) NS	NS	18
SRL (cm/mg)	-0.07(0.04) \$	-0.01(0.03) NS	NS	22
Diameter (mm)	0.13(0.08) NS	-0.06(0.07) NS	NS	22
Tissue density (g/cm ³)	0.04(0.05) NS	0.12(0.05) *	NS	22
Branching frequency(1/cm)	-0.08(0.03) *	0.08(0.08) NS	NS	22

Fig. 2 Relationship between the growth response of focal species to competition and (a–b) root vertical distribution; (c–d) SRL (specific root length); (e–f) diameter; (g–h) tissue density and (i–j) branching frequency. Relationships with trait values of focal and neighbour species are shown in the left-hand and right-hand column, respectively. The y-axis shows the slope of the relationship between focal plant mass and neighbour density, with more negative values indicating stronger growth suppression by neighbours. The y-axis reflects competitive response in the left-hand column and competitive effect in the right-hand column of the panels. Root vertical distribution was measured as the proportion of root tips located at 5 cm soil depth out of the total located at 5 cm and 10 cm depths. Solid and dashed lines indicate relationships that were significant at $P < 0.05$ or $P < 0.1$, respectively. See Fig. S4 for partial regression plots



suppressed by their neighbours when their root vertical distributions were similar (shown by a negative model coefficient in Table 3). The inclusion of pot volume and soil N content did not significantly improve model fit for any of the traits (likelihood ratio test $P > 0.05$). Similarly, there were only significant negative relationships between growth response to competition and trait distances in multidimensional trait space, i.e. competitive suppression was weaker in species pairs with similar trait combinations (Table S3). Interestingly, the strength of competitive suppression was most successfully predicted by a combination of morphological and architectural traits rather than by differences in root biomass (Table S3). Very similar negative relationships were detected between the relative growth response to heterospecific versus conspecific competition and trait distances in multidimensional space. Plants were more suppressed by conspecific than heterospecific competition only in species pairs with very similar trait values, while heterospecific competition outweighed conspecific competition in species pairs with dissimilar trait values (Fig. S5).

Correlations between measured root traits

There was a significant positive correlation between root mass and horizontal spread ($r = 0.47$, $P = 0.029$). The vertical distributions and morphological traits of roots of focal species were not significantly related to their mass or horizontal spread ($P > 0.05$), except for a positive

Table 3 Summary of models examining the relationship between growth response to competition (response variable) and trait distance between the focal and neighbour species (explanatory variable). Model coefficients, standard errors and statistical significance (NS – $P > 0.05$; * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$) are presented. Negative coefficients indicate greater competitive suppression with increasing trait distance. Phyl. signal denotes likelihood ratio tests comparing models with and without phylogenetic correlation structure. Phylogenetic structure was included in the models if significant signal was detected. N – number of species

Trait	Trait distance	Phyl. Signal	N
Root mass (g)	0.02(0.02) NS	NS	22
Horizontal spread	0.14(0.13) NS	*	26
Vertical distribution	-0.42(0.17) *	NS	18
SRL (cm/mg)	-0.01(0.03) NS	NS	22
Diameter (mm)	-0.07(0.08) NS	NS	22
Tissue density (g/cm ³)	0.09(0.05) NS	NS	22
Branching frequency(1/cm)	0.01(0.09) NS	NS	22

relationship between SRL and horizontal spread ($r = 0.44$, $P = 0.038$; Fig. S6). In addition, root vertical distribution showed no significant correlations with root morphological traits ($P > 0.05$) and root morphological traits were not correlated with each other except for a negative correlation between SRL and root diameter ($r = 0.73$, $P = 0.0001$; Fig. S6). A significant negative correlation was found between root branching frequency and the mycorrhization index estimated as the prevalence of mycorrhizal colonisation in previous studies ($r = -0.58$, $P = 0.0057$; Fig. S7). Mycorrhization was not significantly correlated with specific root length ($r = -0.26$, $P = 0.2518$) and diameter ($r = 0.32$, $P = 0.155$; Fig. S7).

For neighbour species, there was a strong correlation between root horizontal spread and root mass ($r = 0.74$, $P < 0.0001$; Fig. S8). There was also marginally non-significant correlation between root tissue density and root mass ($r = -0.42$, $P = 0.0501$) and root tissue density and root horizontal spread ($r = -0.36$, $P = 0.0991$; Fig. S8).

Discussion

Belowground niche differentiation, particularly segregation by rooting depth, has been predicted to promote species co-existence and to increase productivity in diverse communities (Parrish and Bazzaz 1976; Berendse 1982). In this study, we examined a wide range of temperate grassland species and found no evidence for niche differentiation based on analysis of seven root traits. This is in agreement with other studies that have sought evidence for vertical niche segregation (von Felten and Schmid 2008; Mommer et al. 2010). It has been shown that weak stabilising niche differences could be detected by using a combination of below- and aboveground traits, although the same traits contributed more strongly to competitive hierarchies (Kraft et al. 2015). In this study, competitive suppression was stronger, rather than significantly weaker, between species with more dissimilar phenotypes, regardless of whether phenotypes were characterised by single traits or multidimensional trait space. Root traits contributed to the competitive hierarchy between species, with different sets of traits enhancing suppression and tolerance of competitors. These traits were only weakly correlated with each other, suggesting that species may possess similar competitive ability conferred through a diversity of trait combinations. It is possible that trade-offs with other functions prevent the

evolution of superior competitors that would combine the benefits of all traits conferring high competitive ability in a single phenotype.

We found that neighbour species with large root systems and extensive root horizontal spread were most effective at suppressing focal plants, as were species with low root tissue density. Root mass and horizontal spread were correlated, meaning that large root mass allowed greater occupation of soil space and more extensive overlap with competitors' root systems. Root tissue density was negatively related to root system size, suggesting a common link with growth rate (Ryser 1996; Kramer-Walter et al. 2016). This finding is in agreement with other studies showing that large plant size and acquisitive traits in the leaf economics spectrum translate into high competitive ability (Goldberg and Landa 1991; Keddy et al. 2002; Herben and Goldberg 2014). However, we found that size-related root traits were not related to the ability to tolerate competition (i.e. competitive response ability), suggesting that fast growth provides only partial success in competition.

While it is widely recognised that large size and fast growth are important for competitive suppression, traits conferring tolerance to competition remain largely unknown (Wang et al. 2010). We found that species with deep root systems, low specific root length and limited branching were most tolerant of competition, i.e. they possessed high competitive response. Competitive response could simply reflect general stress tolerance. However, some evidence suggests a trade-off between tolerance of competition and tolerance of abiotic stress (Liancourt et al. 2005; Campitelli et al. 2016). Species with deep root systems may tolerate competition with neighbours by having access to nutrients in deeper soil layers, while low SRL and branching may be associated with high levels of mycorrhizal colonisation, which could compensate for nutrient shortages under competition (Maherali 2014; Koziol and Bever 2015; Cortois et al. 2016). By combining our root trait data with published data on mycorrhizal prevalence (Hempel et al. 2013), we found that plant species with lower branching frequencies in our study were indeed associated with a higher degree of mycorrhizal colonisation. Grassland species with deeper root systems and lower SRL have also been shown to achieve higher stand productivity (Schröder-Georgi et al. 2016), supporting the suggestion that these traits provide an additional mechanism by which plants can maintain growth under competitive conditions.

In addition to differences in trait means, the outcome of competition may be influenced by phenotypic plasticity, i.e. the ability to modify phenotype in response to environmental conditions, including competitors (Berg and Ellers 2010; Bennett et al. 2016). We found that the grassland species we examined exhibited a wide range of responses to interspecific competition. Species differed in the traits that were affected by competition, and in whether specific traits exhibited positive or negative responses to the density of competitors. Roots of different species were shown to become more or less branched, either thinner or thicker, and either shallower or deeper in response to increasing competitor density. The direction and magnitude of these plastic responses was not significantly correlated with competitive ability. These results support findings showing that plant species exhibit a wide range of behavioural strategies to deal with belowground competition (Semchenko et al. 2007, 2010; Valverde-Barrantes et al. 2013; Belter and Cahill 2015). Therefore, although plasticity in functional traits in response to competition may play an important role in community assembly, the apparent diversity of possible responses makes it difficult to predict the way in which it operates.

In agreement with previous studies highlighting the importance of growth rate for competitive ability, we found that traits related to root system size strongly contributed to competitive effects. However, a set of traits unrelated to size explained the ability to tolerate competitors. It is notable that the best multi-trait model explaining variation in competitive outcomes did not include root system size but only root morphology, branching and vertical distribution – traits that were linked to competitive tolerance rather than the ability to suppress neighbours. Traits related to competitive tolerance may be even more critical in natural vegetation where seedlings have to establish among larger adult plants and there would be limited potential for exerting a strong competitive effect (Goldberg 1996; Howard and Goldberg 2001). The situation may be different in early-successional or annual communities, where size effects may dominate (Goldberg 1996). However, trade-offs with other functions, such as tolerance of herbivore damage (Rose et al. 2009), timing of reproduction (Chaney and Baucom 2014; Tracey and Aarssen 2014) and resource use efficiency (Ryser 1996; Campitelli et al. 2016) may still limit the selective advantage of large size. We therefore need to move beyond the paradigm of fast growth being equivalent to competitive superiority to recognize the importance of traits conferring competitive

tolerance. Moreover, our results demonstrate that some functional traits may reflect plant reliance on microbial associations. More effort should be directed towards uncovering such links. Explicit consideration of the functional traits that are associated with competitive suppression and tolerance is likely to result in better understanding of key ecological topics such as the factors governing plant community assembly (Wang et al. 2010; Baron et al. 2015; Kraft et al. 2015), biological invasions (Suding et al. 2004; Gruntman et al. 2014) and response to global change (Pakeman 2011).

Data accessibility The dataset analysed during the current study available from the corresponding author on reasonable request.

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