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Research

Are plant–soil feedback responses explained by plant traits?

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Summary

- Plant–soil feedbacks can influence plant growth and community structure by modifying soil biota and nutrients. Because most research has been performed at the species level and in monoculture, our ability to predict responses across species and in mixed communities is limited. As plant traits have been linked to both soil properties and plant growth, they may provide a useful approach for an understanding of feedbacks at a generic level.
- We measured how monocultures and mixtures of grassland plant species with differing traits responded to soil that had been conditioned by model grassland plant communities dominated by either slow- or fast-growing species.
- Soils conditioned by the fast-growing community had higher nitrogen availability than those conditioned by the slow-growing community; these changes influenced future plant growth. Effects were stronger, and plant traits had greater predictive power, in mixtures than in monocultures. In monoculture, all species produced more above-ground biomass in soil conditioned by the fast-growing community. In mixtures, slow-growing species produced more above-ground biomass, and fast-growing species produced more below-ground biomass, in soils conditioned by species with similar traits.
- The use of a plant trait-based approach may therefore improve our understanding of differential plant species responses to plant–soil feedbacks, especially in a mixed-species environment.

Introduction

Plant–soil feedbacks (PSFs) occur when plant-induced changes in soil conditions influence the growth of future plants, either negatively via the promotion of pathogens, release of allelopathic molecules and/or reduced nutrient availability, or positively via the promotion of symbionts and/or nutrient availability (Bever *et al.*, 1997; Bever, 2003; Kulmatiski *et al.*, 2008; Popovici *et al.*, 2011; van der Putten *et al.*, 2013). By influencing the growth of individual plant species, PSFs can have an impact on plant competitive interactions, with implications for plant community dynamics and species invasions (van der Putten *et al.*, 2013). However, as most of this research has been carried out at the species level and in monocultures (Bever, 1994; Klironomos, 2002; Kulmatiski *et al.*, 2008; van de Voorde *et al.*, 2011), with few studies carried out at the community level (Kardol *et al.*, 2007; Harrison & Bardgett, 2010; Kulmatiski *et al.*, 2012), our ability to make predictions about feedback responses across species and in mixed communities is limited (van der Putten *et al.*, 2013). In view of this, there is a need for studies to investigate PSF responses in mixed communities and across a broad range of plant species with different life history strategies, functional traits (Kulmatiski *et al.*, 2008; Kardol *et al.*, 2013) and successional status (Kardol *et al.*, 2007).

A growing number of ecologists are using trait-based approaches to characterize plant strategies for nutrient acquisition and subsequent growth rates (i.e. the leaf economic spectrum (LES); Wright *et al.*, 2004), and to understand how plant species influence community dynamics and ecosystem processes (Diaz *et al.*, 2007; De Deyn *et al.*, 2008; Lavorel *et al.*, 2013). This includes studies that show that plant traits can explain variation in the composition and functioning of soil microbial communities at both the individual plant (Orwin *et al.*, 2010) and community (Laughlin, 2011; de Vries *et al.*, 2012; Grigulis *et al.*, 2013) scales. These effects are largely attributed to links between plant traits and the quality and/or quantity of resources entering the soil, which, in turn, influence the composition and activity of microbial communities (Bardgett & Wardle, 2010). For instance, slow-growing species with conservative traits, such as low tissue nitrogen (N) content and low specific leaf area (SLA), have been shown to promote fungal-based soil food webs, which are associated with slow rates of nutrient cycling (Orwin *et al.*, 2010; de Vries *et al.*, 2012). By contrast, fast-growing plant species with exploitative traits, such as high tissue N content and SLA, produce high-quality resource inputs to soil, which promote bacterial-based food webs associated with rapid recycling of nutrients (Orwin *et al.*, 2010; de Vries *et al.*, 2012). Differences in plant functional traits may also be linked to plant defence, and thus

influence the susceptibility of a species to pathogens (Kulmatiski *et al.*, 2008). Traits could therefore govern the outcome of PSFs by both determining how species modify the soil and how they respond to these changes (Kulmatiski *et al.*, 2008), which is consistent with the distinction between 'effect' traits, which affect ecosystem functioning, and 'response' traits, which affect the response of organisms to a change in their growth environment (Lavorel & Garnier, 2002). However, because past PSF studies have focused on conspecific responses (Bever, 1994; Klironomos, 2002; Kulmatiski *et al.*, 2008; van de Voorde *et al.*, 2011; van der Putten *et al.*, 2013), rather than assessing responses across a range of species with contrasting traits, this idea has not been fully tested. As a result, our understanding of how plant traits influence feedback responses remains poor.

As few studies have investigated PSF responses in multi-species communities (Kardol *et al.*, 2007; Harrison & Bardgett, 2010; Kulmatiski *et al.*, 2012), little attention has been given to the role of competition and species interactions in PSFs, and how these influence competitive interactions and plant community structure. It seems likely, however, that changes in the soil microbial community and nutrient availability caused by plants could modify competitive outcomes between subsequent plant species. Indeed, there is some evidence that PSF responses have a greater impact in mixed plant communities (Kardol *et al.*, 2007), and it has been proposed that negative feedbacks allow for species with contrasting nutritional strategies to co-exist in mixed communities as a result of the suppression of dominant species (Bever, 1994; Kulmatiski *et al.*, 2008). Given that plant traits can influence both competitive ability (Goldberg, 1990, 1996; Violle *et al.*, 2009; Wang *et al.*, 2010) and soil conditions, there is potential for them to play a role in determining how PSFs influence plant growth in mixed communities, as well as in monocultures (Reader, 1998; Wang *et al.*, 2010).

The overarching aim of this study was to test whether PSF responses across a broad range of grassland plant species could be explained on the basis of plant functional traits, in terms of both the effects of plants on soil nutrient conditions, mediated by 'effect' traits, and the feedback consequences for plant growth in monocultures and competitive mixtures, mediated via 'response' traits. In addition, we tested for the effects of feedbacks and competitive interactions on the plant traits themselves. We also tested the effects of soil conditioning on soil microbial structure and functioning. Our hypotheses were as follows. (a) Soil conditioning by plant communities dominated by fast- and slow-growing plant species would have a differential impact on soil conditions and the soil microbial community with feedback consequences for plant growth and competitive interactions; specifically, we predicted that the slow-growing conservative community would promote fungi over bacteria and decrease rates of N cycling, whereas the fast-growing exploitative community would promote bacteria and increase rates of N cycling. (b) The net effect of soil conditioning by plant communities dominated by species of contrasting traits would be dependent on the traits of the species in the feedback phase, and responses would be stronger in mixed species communities. (c) Changes in nutrient use, as a result of feedbacks and competition, would influence trait values of future plant communities. To test these hypotheses, we carried out a PSF experiment, using a range of grassland plant species covering a spectrum of functional traits, from fast-growing species with exploitative traits to slow-growing species with conservative traits. As we wanted to test for trait-based rather than species-based feedbacks, we measured responses to soil conditioned by two model plant communities of contrasting dominant traits.

Materials and Methods

Phase 1 – conditioning phase

In order to create different soil histories, we established two model plant communities representing two different ends of a community LES: a 'slow-growing community' and a 'fast-growing community'. This was performed by manipulating the dominance of four common plant species of temperate grassland. We chose to condition soils with mixed plant communities of the same species, but of varying dominance, to better reflect field conditions, where changes in the dominance of plant species varying in their functional traits typically occur in response to changes in land use and soil fertility (de Vries *et al.*, 2012), and to eliminate the effects of species richness and identity. Each community was replicated four times within blocks to give a total of eight experimental units (2 plant communities × 4 replicates = 8). The plant species used differed in their functional traits: a grass (*Anthoxanthum odoratum)* and herb (*Achillea millefolium*) with a slow growth rate, high leaf dry matter content (LDMC) and low SLA; and a grass (*Dactylis glomerata*) and herb (*Geranium sylvaticum*) with a fast growth rate, lower LDMC and higher SLA.

Model plant communities were established in mesocosms at the Lancaster University Hazelrigg Field Station (UK) in autumn 2009. Mesocosms were $38 \times 38 \times 30\text{-cm}^3$ pots (Harrison & Bardgett, 2010; Orwin *et al.*, 2014) filled with 10 cm of gravel and 20 cm of low-nutrient topsoil (pH_{H2O} of 6.17, 0.26% N, 3.13% C) collected from a sandy loam pasture at the field site. Soil analysis for soil nutrients NO_3 and NH_4, pH, moisture content and microbial biomass N was carried out before planting, and soil properties were found to be consistent across treatments. Mesocosms were planted with tillers of the selected plant species obtained from field turfs taken from Colt Park, a traditionally managed hay meadow field site in the Yorkshire Dales, UK (De Deyn *et al.*, 2011). To minimize the transfer of soil organisms, roots were carefully washed after field collection, before transplanting in a standardized loam–sand mixture to homogenize the microbial communities. Mesocosms were planted with 36 individuals of the four species in a randomized planting design to prevent species clumping. The abundance of the four species was manipulated to produce a fast-growing plant community (termed 'fast-growing plant communities' henceforth) dominated by the two species with exploitative strategies (24 individuals of *D. glomerata* and four individuals of *G. sylvaticum)*, with the remainder made up with the two species with conservative strategies (six individuals of *A. odoratum* and two individuals of

A. millefolium). The slow-growing plant community (termed 'slow-growing plant communities' henceforth) was dominated by the two species with conservative growth strategies (24 individuals of *A. odoratum* and four individuals of *A. millefolium)*, with the remainder made up with the two species with exploitative strategies (six individuals of *D. glomerata* and two individuals of *G. sylvaticum*). These communities were designed to have contrasting community-weighted mean (CWM) trait values (e.g. SLA, LDMC and leaf nutrient content).

After conditioning for two growing seasons (18 months), the soil from the mesocosms was homogenized (per replicate ($n = 4$)) by sieving at 1 mm, before microcosm pots (diameter, 10 cm; depth, 9.5 cm) were filled with 450 g of soil.

Soil characterization following the conditioning phase

Above-ground biomass and leaf N content (LNC) were measured on plants grown in the soil conditioning phase using the methodology detailed below. Temperate grasslands are strongly limited by N and plant species are known to influence N cycling (Orwin *et al.*, 2010). We therefore focused on the effects of soil conditioning on N cycling rates and the microorganisms involved in N cycling, as these are likely to be strong drivers of feedback effects. However, we recognize that other nutrients, such as phosphorus (P) and potassium (K), are also likely to be important in these systems, and may also have influenced the responses. The soil N content and microbial community biomass and activity were analysed on subsamples before planting. Soil was dried at 105°C and ground to measure total soil % N using a Vario EL III (Elementar Analysensysteme GmbH, Hanau, Germany) elemental analyser. The dissolved inorganic N was measured in soil extracts using 0.5 M K_2SO_4 analysed using a colorimetric chain analyser (Bran and Luebbe AA 3; Seal Analytical, Southampton, UK). Microbial biomass was measured as microbial biomass N using the chloroform-fumigation extraction technique (Vance *et al.*, 1987). Briefly, paired soil samples (10 g fresh weight) were either fumigated with $CHCl_3$ for 7 d at 25°C in the dark, or not fumigated. Once the $CHCl_3$ had been evacuated, extractable N was measured on a colorimetric chain autoanalyser using 0.5 M K_2SO_4 extracts. Microbial biomass N was calculated from the microbial N flush (the difference in N between fumigated and unfumigated soil) using a k_{EN} factor of 0.54 (Brookes *et al.*, 1985). Microbial community structure, approximated by assessing the relative biomass of fungi to bacteria, was determined using phospholipid fatty acid (PLFA) analysis, employing the method of Bligh & Dyer (1959), adapted by White *et al.* (1979) and described by Bardgett *et al.* (1996). Briefly, this involved the extraction, fractionation and quantification of microbial PLFAs. The fatty acids i150:0, a150:0, 15:0, i16:0, 17:0, i17:0, cy17:0, cis18:1ω7 and cy19:0 were chosen to represent bacterial fatty acids, and 18:2ω6 to represent fungal fatty acids (Bardgett & McAlister, 1999). Microbial activity was measured using potential nitrification (NEA) and denitrification (DEA); NEA was measured using kinetic parameters (V_{max} and K_m), following the protocol described by Dassonville *et al.* (2011), and DEA was measured according to Attard *et al.* (2011).

Phase 2 – feedback phase

PSF effects on plant growth in monocultures To assess PSF effects on the growth of plants along the LES (Wright *et al.*, 2004), we used values of SLA and LDMC, taken from Grime *et al.* (2007) (Supporting Information Table S1), to select 16 common grassland species representing a spectrum from fast-growing species with exploitative traits (high SLA and low LDMC) to slow-growing species with conservative traits (low SLA and high LDMC). We focused on LDMC and SLA as key traits to describe plant species, as they are strongly associated with LES and plant growth rates (Wright *et al.*, 2004), and have been linked to many soil process rates (Orwin *et al.*, 2010). We carried out a principal component analysis (PCA) using values of SLA and LDMC to order the 16 species along a gradient from high LDMC and low SLA to low LDMC and high SLA (Fig. S1). The selected species used in the feedback phase were: *Filipendula ulmaria* (L.), *Festuca rubra* (L.), *Sanguisorba officinalis* (L.), *Alopecurus pratensis* (L.), *Cynosurus cristatus* (L.), *Anthoxanthum odoratum* (L.), *Achillea millefolium* (L.), *Ranunculus acris* (L.), *Dactylis glomerata* (L.), *Geranium sylvaticum* (L.), *Lolium perenne* (L.), *Trifolium pratense* (L.), *Plantago lanceolata* (L.), *Agrostis capillaris* (L.), *Holcus lanatus* (L.) and *Poa trivialis* (L.), and include the four species used in the conditioning phase.

Seeds of all species were sourced from Emorsgate Seeds (Kings Lynn, UK), sterilized using 1% bleach, sown in sterilized sand and germinated in a controlled environment room (temperature, 19°C day, 16°C night; light between 08:00 h and 20:00 h daily). After seedling establishment (*c.* 8 wk), individuals were planted in monocultures in pots, using one individual in the centre of each pot, to remove effects of intraspecific competition (Fig. 1). Pots were positioned in a controlled environment room in a randomized design and left to grow for 6 wk, to prevent the roots from becoming pot bound, before sampling. Pots were watered daily and checked weekly for weeds. In total, there were 128 monocultures (2 soil treatments (conditioned with fast- and slow-growing communities) × 16 species × 4 replicates = 128 experimental units).

PSF effects in interspecific competitive mixtures To assess the effect of PSFs on the competitive ability of species with contrasting traits ('species type' henceforth), we selected three species at each end of the trait spectrum to be grown in competitive mixtures. This was performed using PCA and cluster analysis to group the three fastest and three slowest growing species on the basis of their SLA and LDMC values (Fig. S1) (Grime *et al.*, 2007). The selected fast-growing species were three grasses, *A. capillaris*, *H. lanatus* and *P. trivialis*, and the slow-growing species were one herb, *S. officinalis*, and two grasses, *F. rubra* and *A. pratensis*. Competitive interactions were designed so that fast-growing species were competing with slow-growing species in mixtures containing two, four and six species (Fig. 1). Communities were established in the same sized pots as used for the monocultures with one individual per species planted in a randomized order to prevent species neighbour effects. As in the monocultures, pots were positioned in a controlled environment room in

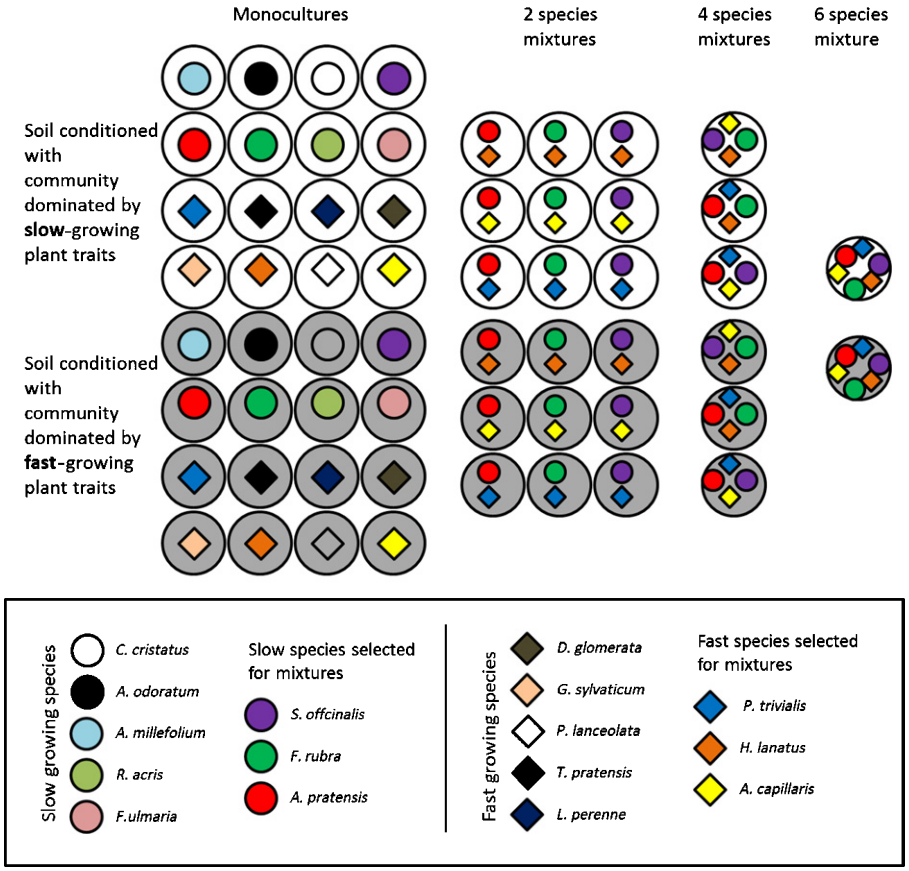

Fig. 1 Experimental design of monocultures and planting scheme of plant competitive mixtures. Competitive mixtures were set up in a fast- vs slow-growing species design. Half of the microcosms were planted in soil conditioned with a slow-growing plant community (open circles), whereas the other half were planted in soil conditioned with a fast-growing plant community (grey closed circles). Sixteen species in total were grown in monocultures (colours show species ID), with eight of these species displaying traits associated with slow-growing species (circles) and the other eight species displaying traits associated with fast-growing species (diamonds). Three slow species and three fast species were selected for the two, four and six species competitive mixtures. One individual per species was placed in each pot, following an additive design. The diagrammatic microcosms of one of the four replicates are shown, and the arrangement of all the microcosm pots was randomized. Species abbreviations: *A. capillaris*, *Agrostis capillaris*; *A. millefolium*, *Achillea millefolium*; *A. odoratum*, *Anthoxanthum odoratum*; *A. pratensis*, *Alopecurus pratensis*; *C. cristatus*, *Cynosurus cristatus*; *D. glomerata*, *Dactylis glomerata*; *F. rubra*, *Festuca rubra*; *F. ulmaria*, *Filipendula ulmaria*; *G. sylvaticum*, *Geranium sylvaticum*; *H. lanatus*, *Holcus lanatus*; *L. perenne*, *Lolium perenne*; *P. lanceolata*, *Plantago lanceolata*; *P. trivialis*, *Poa trivialis*; *R. acris*, *Ranunculus acris*; *S. officinalis*, *Sanguisorba officinalis*; *T. pratense*, *Trifolium pratense*.

a randomized design and left to grow for 6 wk before harvesting. In total, there were 144 multi-species mixtures (2 soil treatments (conditioned with fast- and slow-growing communities) × 6 species × 3 mixed communities (2, 4 and 6 species) × 4 replicates = 144 experimental units).

Plant biomass and trait sampling

At harvest, above-ground biomass per species was measured after drying plant material at 60°C for 1 wk. Below-ground biomass per species was measured on all roots after washing and drying at 60°C for 1 wk. To investigate the influence of soil feedbacks and competition on plant trait expression, above-ground and below-ground plant traits were measured on individuals grown in monocultures and six species competitive mixtures at the time of harvest. In mixtures, the roots were sorted to the species level, which was performed by carefully separating the roots of individual plant species with their above-ground biomass still attached to aid identification. Above-ground traits, namely plant height, SLA, LDMC, LNC, leaf C content (LCC) and leaf C : N ratio, were measured using standard protocols; all traits measured, their abbreviations and units are listed in Table 1 (Cornelissen *et al.*, 2003; Perez-Harguindeguy *et al.*, 2013). Plant height was measured for each individual before harvest, and leaf traits were measured on one leaf per individual, which was rehydrated overnight below 6°C (Garnier *et al.*, 2001). Root traits, namely root diameter, specific root length (SRL), root dry matter content (RDMC), root N content (RNC), root C content (RCC) and root C : N ratio, were measured on washed roots stored in 10% ethanol solution until analysis. The root : shoot ratio was calculated by dividing the root biomass by the shoot biomass. Analysis for length and diameter was carried out using WinRhizo® root analysis software (Regent Instruments Inc., Sainte-Foy-Sillery-Cap-Rouge, QC, Canada) and an Epson flatbed scanner.

Table 1 List of all plant functional traits measured and their abbreviations and units

Abbreviation	Plant trait	Unit
Leaf traits		
LNC	Leaf N content	mg g^{-1}
LCC	Leaf C content	mg g^{-1}
Leaf C : N	Leaf C : N ratio	No unit
SLA	Specific leaf area	cm^2 g^{-1}
LDMC	Leaf dry matter content	mg g^{-1}
Root traits		
RNC	Root N content	mg g^{-1}
RCC	Root C content	mg g^{-1}
Root C : N	Root C : N ratio	No unit
Root : shoot	Root : shoot ratio	No unit
SRL	Specific root length	m^2 g^{-1}
Root diameter	Root diameter	mm
RDMC	Root dry matter content	mg g^{-1}

Statistical analysis

Above-ground biomass (termed shoot biomass henceforth) and below-ground biomass (termed root biomass henceforth) and total biomass (sum of shoot and root biomass) were used as measures of plant growth in monocultures and competitive mixtures in the soil conditioning treatments. PSF responses to the soil conditioning were calculated for paired replicates (paired block replicates from the soil conditioning phase):

$$\text{PSF index} = \frac{(\text{Biomass}_1 - \text{Biomass}_2)}{\text{Biomass}_2} \quad \text{Eqn 1}$$

(Biomass_1, total biomass of a species in soil conditioned with plants with similar functional traits; Biomass_2, total biomass of the same species in soil conditioned with plants of contrasting functional traits). PSF responses of species grown in multi-species mixtures were calculated in the same way for each species, in each multi-species mixture.

To investigate the effect of soil conditioning on species competitive ability, the relative competition index (RCI) of shoot, root and total biomass of each species was calculated using the following equation:

$$\text{RCI} = \frac{(\text{biomass in monoculture} - \text{biomass in competitive mixture})}{\text{biomass in monocultures}} \quad \text{Eqn 2}$$

To investigate the effects of soil conditioning and competition on plant growth and trait expression, ANOVAs were carried out using species, soil conditioning, competitive mixture, neighbour biomass (total biomass of other species in competitive mixture) and their interactions as fixed factors and replicate as a random effect. To further investigate species effects, we decomposed responses into species ID and species type (slow-growing or fast-growing effects). Competitive mixture effects were also decomposed into species richness (two, four or six) and composition effects (identity of competitive neighbours). We used sequential ANOVAs to determine the effect of these decomposed variables; that is, for species effects, we ran the model including species ID alone, species type alone, and then with both in the same model to test for species type effect additional to species identity effects (Hector *et al.*, 2010; Wilby & Orwin, 2013). We calculated the percentage variation explained by each decomposed effect from the sums of squares (SS) from these sequential ANOVAs. We also tested for correlations between PSF responses and trait values of SLA and LDMC, using the scores from the PCA used to define species as slow or fast growing.

Results

Plant and soil analysis after the conditioning phase

The abundance of the four species used in the conditioning phase changed over the 2-yr conditioning period; however, the communities differed significantly in conservative : exploitative species ratio ($F_{1,3} = 8.78$, $P = 0.05$) at the end of the conditioning phase, being greater in the slow-growing communities than in the fast-growing communities. The total above-ground biomass ($F_{1,3} = 371$, $P = 0.0003$), LNC ($F_{1,3} = 13.84$, $P = 0.03$), soil N ($F_{1,7} = 13.36$, $P = 0.04$) and the concentration of dissolved inorganic N ($F_{1,7} = 7.74$, $P = 0.07$) were greater in soil conditioned by the fast-growing plant community than in the soil conditioned by the slow-growing plant community, as were two microbial parameters, namely DEA ($F_{1,7} = 20.29$, $P = 0.02$) and microbial biomass N ($F_{1,7} = 58.33$, $P = 0.005$). NEA and the F : B (Fungal : Bacterial) ratio were not affected by soil conditioning.

Soil feedbacks in monocultures

As expected, plant biomass differed significantly between the 16 species grown in monoculture (Table 2), although only a small amount of this variation was explained by the classification of the species as fast or slow growing (12% of total biomass, 25% of shoot biomass and 0% of root biomass). The large residual variation in biomass was explained by differences between individual species. Shoot biomass differed weakly between soil conditioning treatments ($F_{1,51} = 3.7$, $P = 0.06$), with most species having greater biomass in soil conditioned by the fast- than the slow-growing plant community (Fig. 2a). The PSF index calculated using shoot biomass differed between species types ($F_{1,39} = 3.32$, $P = 0.05$), but not individual species: fast-growing plant species performed better in soil conditioned by communities dominated by plant species with similar traits, resulting in neutral or positive feedbacks, whereas slow-growing species performed better in soil conditioned by plant species with contrasting traits, resulting in neutral or negative feedbacks (Table 2, Fig. 3b). The PSF index calculated using the total plant biomass differed weakly between individual species ($F_{14,46} = 3.6$, $P = 0.06$) (Table 2), and the index calculated using root biomass did not differ significantly between plant species or species types (Fig. 3a,c). Additional analysis using the PCA scores to classify species by their trait values showed a weak positive correlation between trait values and PSF index calculated using shoot biomass.

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Table 2 Impact of species effect (decomposed into species type (fast or slow growing) and residual species identity effect), soil conditioning (by fast- or slow-growing community) and interactions on biomass and plant–soil feedback (PSF) index of total plant, shoot and root separately of 16 species grown in monocultures

	Total plant								Shoots								Roots							
	Biomass				PSF index				Biomass				PSF index				Biomass				PSF index			
	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
Species effect	15	15.8	8.02	***	15	0.9	3.86	0.19	15	7.2	12.02	***	15	1.0	7.73	0.11	15	3.7	7.06	***	15	7.7	0.99	0.48
Species type	*1*	*1.9*	*16.78*	***	*1*	*0.3*	*1.02*	*0.32*	*1*	*1.8*	*48.24*	***	*1*	*0.7*	*3.32*	*	*1*	*0.0*	*0.07*	*0.79*	*1*	*0.1*	*0.17*	*0.69*
Residual species identity	*14*	*13.9*	*8.66*	***	*14*	*0.9*	*3.62*	*0.06*	*14*	*5.4*	*10.31*	***	*14*	*0.3*	*1.54*	*0.22*	*14*	*3.7*	*8.32*	***	*14*	*7.6*	*1.05*	*0.43*
Soil	1	0.3	2.57	0.11					1	0.1	3.66	0.06					1	0.0	1.09	0.30				
Species effect × soil	15	0.9	0.47	0.95					15	0.0	0.55	0.9					15	0.2	0.4	0.98				
Species type × soil	1	0.0	0.08	0.78					1	0.0	0.03	0.86					1	0.0	0.11	0.75				
Residuals	99	11.3			46	11.4			99	3.7			39	11.5			99	3.2			33	17.1		

Data analysed using ANOVA with contrasts; terms in italics are contrasts within the main species identity factor. Replicate was used as random effect in the model. Degrees of freedom (df), sums of squares (SS), *F* values and *P* values from ANOVA are presented. Significance: *, 0.05; ***, < 0.0001.

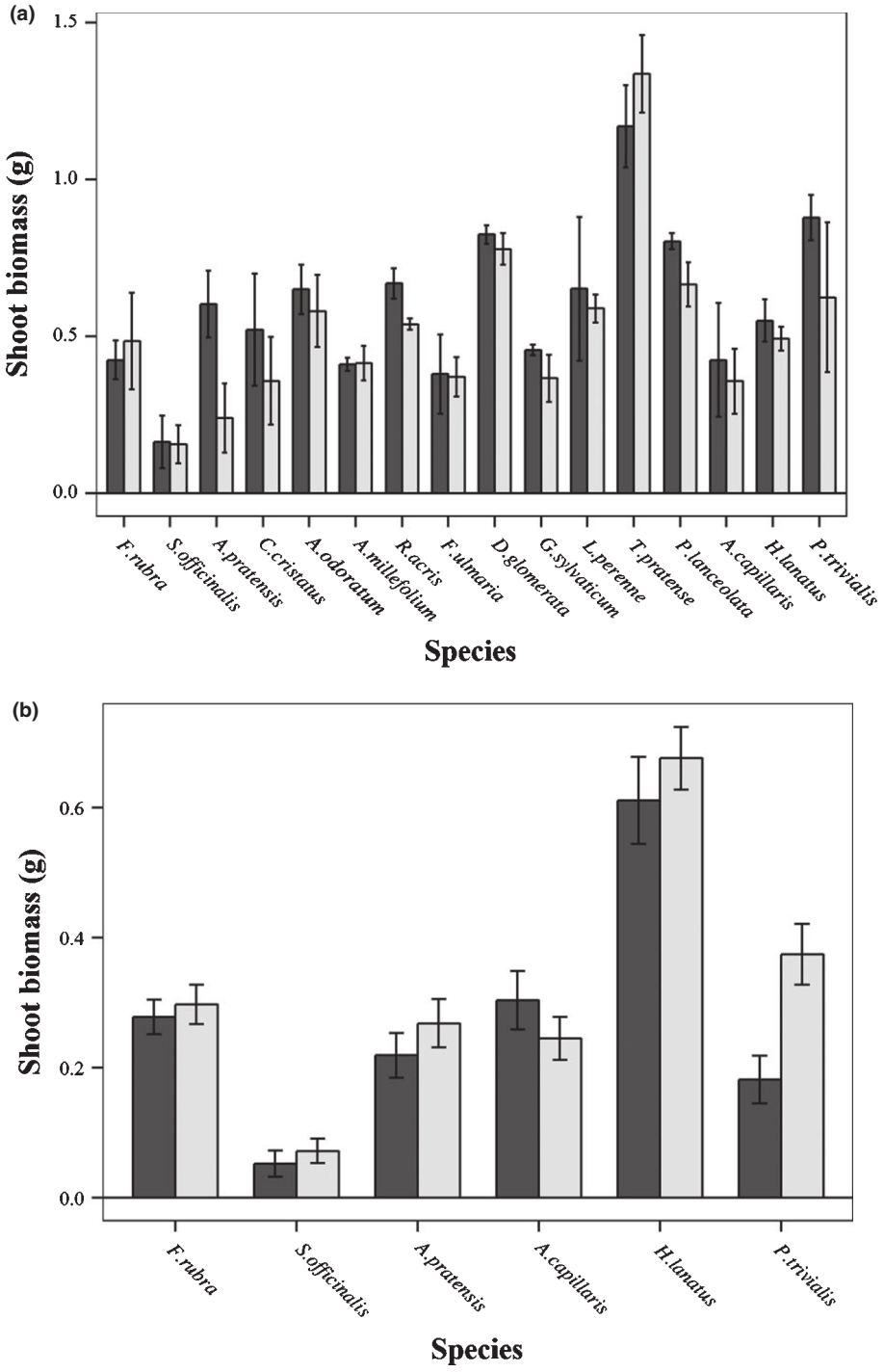

Fig. 2 Shoot biomass of 16 species grown in monocultures (a) and six species grown in multi-species mixtures (b) in two soils conditioned with plant communities of contrasting dominant plant traits: slow-growing plants (light grey bars) and fast-growing plants (dark grey bars). Error bars, ± 1SE ($n = 4$). Species abbreviations: *A. capillaris*, *Agrostis capillaris*; *A. millefolium*, *Achillea millefolium*; *A. odoratum*, *Anthoxanthum odoratum*; *A. pratensis*, *Alopecurus pratensis*; *C. cristatus*, *Cynosurus cristatus*; *D. glomerata*, *Dactylis glomerata*; *F. rubra*, *Festuca rubra*; *F. ulmaria*, *Filipendula ulmaria*; *G. sylvaticum*, *Geranium sylvaticum*; *H. lanatus*, *Holcus lanatus*; *L. perenne*, *Lolium perenne*; *P. lanceolata*, *Plantago lanceolata*; *P. trivialis*, *Poa trivialis*; *R. acris*, *Ranunculus acris*; *S. officinalis*, *Sanguisorba officinalis*; *T. pratense*, *Trifolium pratense*.

Soil feedbacks in competitive mixtures

As in the monocultures, plant biomass differed significantly between the six species grown in multi-species mixtures (Table 3), although only a small amount of this variation was explained by species type (11% of total biomass, 35% of shoot biomass and 0% of root biomass); the remaining variation was explained by differences between individual species. As in monocultures, individual plant shoot biomass was affected significantly by soil conditioning. Although the magnitude of the effect of soil conditioning on growth was species specific, all species, with the exception of *A. capillaris*, had greater shoot biomass in soil conditioned by the slow-growing relative to the fast-growing plant community (Fig. 2b). This increase in shoot biomass was also seen at the community level ($F_{1,99} = 7.82$, $P = 0.006$). Total, shoot and root biomass were also significantly and negatively correlated with neighbour biomass, suggesting that the competitive ability of neighbouring plants influences plant growth (Table 3). The PSF index, calculated using the shoot and root biomass

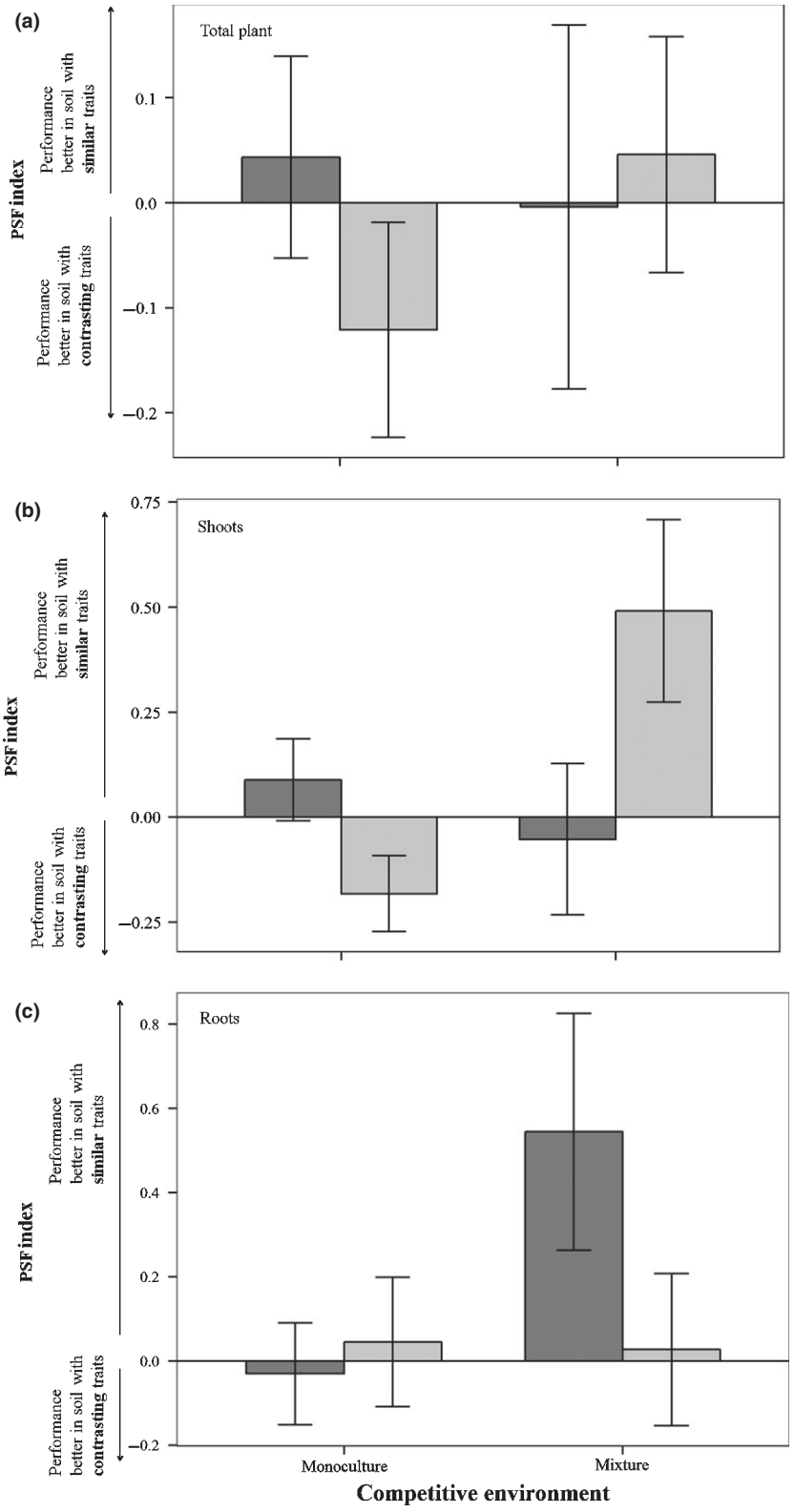

Fig. 3 Plant–soil feedback (PSF) indices calculated using total (a), shoot (b) and root (c) biomass of slow-growing (light grey bars) and fast-growing (dark grey bars) plant species, grown individually in monocultures and in multi-species mixtures, in soil conditioned by slow-growing or fast-growing communities. Plant–soil feedback values give an indication of the effect of soil conditioning on plant growth, with positive values indicating better performance in soil conditioned with a plant community dominated by similar plant traits, and negative values indicating better performance in soil conditioned with a plant community dominated by contrasting plant traits. Error bars, ± 1SE (monocultures, $n = 32$; mixtures, $n = 12$).

of the six species grown in competitive mixtures, differed significantly between species types ($F_{1,101} = 1.51$, $P = 0.022$ and $F_{1,101} = 6.37$, $P = 0.013$, respectively), but not between individual species (Table 3, Fig. 3b,c). Above ground, slow-growing species displayed more positive PSF indices than fast-growing species, whereas, below ground, fast-growing species displayed more positive PSF indices than slow-growing species (Fig. 3b,c). The PSF index calculated using the total plant biomass did not differ significantly between individual plant species or species types.

Table 3 Impact of species effect (decomposed into species type (fast or slow growing) and residual species identity effect), soil conditioning (by fast- or slow-growing community), neighbour biomass and interactions on biomass and the plant–soil feedback (PSF) index of total plant, shoot and root separately of six species grown in multi-species mixtures

	Total plant								Shoots								Roots							
	Biomass				PSF index				Biomass				PSF index				Biomass				PSF index			
	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
Species effect	5	11.4	27.0	***	5	4.6	1.2	0.31	5	7.6	51.3	***	5	16.8	0.9	*	5	1.3	12.8	***	5	21.9	2.9	*
Species type	*1*	*1.3*	*15.0*	***	*1*	*0.1*	*0.1*	*0.78*	*1*	*2.7*	*87.5*	***	*1*	*5.7*	*1.5*	*	*1*	*0.3*	*12.2*	***	*1*	*9.6*	*6.4*	*
Residual species identity	*4*	*10.1*	*29.7*	***	*4*	*4.6*	*1.5*	*0.21*	*4*	*4.9*	*40.8*	***	*4*	*11.1*	*0.7*	*0.5*	*4*	*1.0*	*12.7*	***	*4*	*12.4*	*2.1*	*0.09*
Soil	1	0.2	2.7	0.1					1	0.2	7.9	**					1	0.0	0.0	0.96				
Neighbour biomass	1	5.0	11.7	***					1	1.6	6.6	*					1	0.9	17.3	***				
Species effect × soil	5	0.6	1.4	0.23					5	0.3	2.2	*					5	0.2	1.6	0.17				
Species type × soil	1	0.0	0.1	0.82					1	0.0	0.7	0.4					1	0.0	0.5	0.5				
Neighbour biomass × species type	1	0.2	2.4	0.12					1	0.2	1.5	0.22					1	0.1	3.2	0.07				
Neighbour biomass × soil	1	0.1	3.1	0.08					1	0.4	2.9	0.09					1	0.0	2.5	0.11				
Neighbour biomass × species type × soil	1	0.8	0.0	0.88					1	0.1	0.1	0.76					1	0.4	0.7	0.41				
Residuals	242	20.4			101	78.0			242	7.1			101	382			242	4.8			101	151.8		

Data analysed using ANOVA with contrasts; terms in italics are contrasts within the main species identity factor. Replicate was used as random effect in the model. Degrees of freedom (df), sums of squares (SS), *F* values and *P* values from ANOVA are presented. Significance: *, 0.05; **, 0.01; ***, <0.0001.

Table 4 Impact of species effect (decomposed into species type (fast or slow growing) and residual species identity effect), competitive composition (decomposed into species richness and residual composition effects), soil conditioning treatment (by fast- or slow-growing community) and interactions on relative competitive index (RCI) calculated for total biomass and shoot and root biomass separately

	Total RCI				Shoot RCI				Root RCI			
	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
Species effect	1	45.0	4.38	*	1	14.5	1.56	0.21	1	4	0.23	0.63
Species type	*1*	*39.0*	*3.84*	*	*1*	*12.2*	*1.28*	*0.26*	*1*	*6*	*0.34*	*0.56*
Residual Species identity	*1*	*0.4*	*0.04*	*0.85*	*1*	*2.8*	*0.30*	*0.58*	*1*	*9*	*0.51*	*0.48*
Composition	12	156.9	1.27	0.24	12	208.2	1.87	*	12	374	1.90	*
Species richness	*2*	*42.8*	*2.11*	*0.12*	*2*	*41.3*	*2.17*	*0.12*	*2*	*92*	*2.75*	*0.07*
Residual composition	*10*	*120.4*	*1.19*	*0.30*	*10*	*169.9*	*1.79*	*0.06*	*10*	*275*	*1.64*	*0.09*
Soil	1	6.2	0.60	0.44	1	31.3	3.38	0.07	1	4	0.29	0.59
Composition × soil	12	68.0	0.56	0.86	12	66.5	0.59	0.85	12	184	0.93	0.51
Species effect × soil	1	18.7	1.82	0.18	1	32.1	3.47	0.06	1	40	2.45	0.12
Species type × soil	1	29.3	2.87	0.09	1	46.0	4.72	*	1	20	1.18	0.56
Composition × species effect	12	61.7	0.50	0.91	12	80.5	0.72	0.73	12	255	1.29	0.23
Species effect × soil × composition	12	191.7	1.55	0.11	12	217.4	1.96	*	12	179	0.91	0.54
Residuals	238	2415.5			238	2265			231	3861		

Data analysed using ANOVA with contrasts; terms in italics are contrasts within the main species identity factor. Replicate was used as random effect in the model. Degrees of freedom (df), sums of squares (SS), *F* values and *P* values from ANOVA are presented. Significance: *, 0.05.

Values for relative competitive outcome (RCI), a measure of species growth in competitive mixtures relative to growth in monocultures, were mostly negative, indicating that plant growth was greater in competitive mixtures than in monocultures. However, this response was not consistent across all species or for total, shoot and root biomass. The RCI of total biomass differed significantly between species ($F_{1,238} = 3.84$, $P = 0.05$), with 13% of this variation explained by differences in biomass between fast- and slow-growing species, and the residual 87% variation explained by differences in individual species (Table 4). The RCI of roots differed significantly between competitive mixtures ($F_{12,232} = 1.9$, $P = 0.04$); the species richness of competitive mixtures explained 24% of this variation, with the residual 76% variation explained by species composition (neighbour effects). This suggests that the identity of the neighbours is more important than species richness in influencing root competitive ability.

The RCI of shoots was significantly affected by soil conditioning, but this response varied with species type, with slow-growing species having significantly better competitive performance (more negative RCI) in soil conditioned by the slow- rather than the fast-growing plant community (Fig. 4). Shoot RCI responses to soil conditioning were also influenced by species identity and the competitive environment, suggesting that neighbour identity influences feedback effects, which influence shoot competitive ability at a species level ($F_{1,239} = 1.96$, $P = 0.03$). In general, two of the slow-growing species, *F. rubra* and *A. pratensis*, were more competitive in soil conditioned by the slow-growing community, suggesting a similar trait advantage. By contrast, *S. officinalis*, the

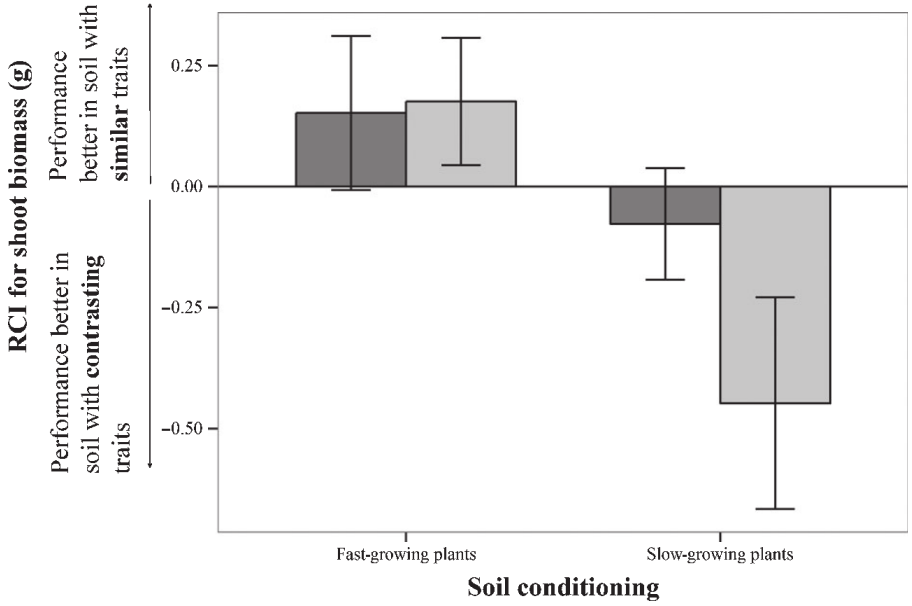

Fig. 4 Relative competitive outcome (RCI) of shoot biomass of slow-growing (light grey bars) and fast-growing (dark grey bars) species grown in competitive mixtures in soil conditioned with slow-growing and fast-growing plant communities. RCI values give an indication of plant competitive ability, with positive values indicating greater total biomass in monocultures and negative values indicating better growth in multi-species communities. Error bars, ± 1SE ($n = 4$).

Table 5 Impact of species effect (decomposed into species type and residual species identity effects), treatment (monoculture or mixture), soil conditioning (by fast- or slow-growing community) and interactions on expression of leaf and root chemical and structural traits, sampled from individuals grown in mixtures and monocultures

	Leaf N				Leaf C				Leaf C : N				Root N				Root C				Root C : N			
	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
Species effect	5	1379	4.6	**	5	13 372	15.6	***	5	2916	10.0	***	5	207	11.5	***	5	14 227	3.2	*	5	22 050	7.9	***
Species type	*1*	*223*	4.3	*	*1*	*599*	1.7	*0.2*	*1*	*917*	3.8	*0.1*	*1*	*98*	25.1	***	*1*	*3625*	4.5	*	*1*	*9834*	17.3	***
Residual species identity	*5*	*663*	3.2	*	*5*	*13 935*	10.0	***	*5*	*1149*	1.2	*0.3*	*5*	*94*	6.0	***	*5*	*11 075*	3.4	*	*5*	*11 174*	4.9	**
Treatment	1	900	15.2	***	1	1274	7.4	**	1	6473	110.6	***	1	57	15.8	***	1	1890	2.1	0.2	1	1989	3.6	0.1
Soil	1	101	1.7	0.2	1	5	0.0	0.9	1	5	0.1	0.8	1	3	0.8	0.4	1	88	0.1	0.8	1	2363	4.2	*
Species effect × soil	5	203	0.7	0.6	5	2668	3.1	*	5	2005	6.8	***	5	26	1.4	0.2	5	3275	0.7	0.6	5	3550	1.3	0.3
Species type × soil	1	22	0.3	0.6	1	135	0.2	0.6	1	97	0.3	0.6	1	2.4	0.5	0.5	1	247	0.3	0.6	1	6	0.0	0.9
Soil × treatment	1	55	0.9	0.3	1	310	1.8	0.2	1	618	10.5	**	1	1	0.3	0.6	1	8	0.0	0.9	1	11	0.0	0.9
Species effect × treatment	5	266	0.9	0.5	5	5730	6.7	***	5	4737	16.2	***	5	44	2.5	*	5	1940	0.4	0.8	5	2897	1.0	0.4
Species type × treatment	1	1	0.1	0.9	1	1123	2.2	0.2	1	5	0.1	0.9	1	7.5	1.5	0.2	1	216	0.2	0.6	1	184	0.3	0.6
Soil × species effect × treatment	5	57	0.2	1	5	2808	3.3	*	5	2854	9.7	***	5	9	0.5	0.8	5	3026	0.7	0.6	5	3257	1.2	0.3
Soil × species type × treatment	1	0.1	0.0	1	1	922	1.6	0.2	1	260	1.1	0.3	1	0.7	0.1	0.7	1	1086	1.2	0.3	1	506	0.7	0.4
Residuals		2600				17 334				12 204				282				58 523				41 031		

	SLA				LDMC				Root : shoot				Root diameter				RDMC				SRL			
	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
Species effect	5	319 037	4.0	**	5	0.06	2.8	*	5	50	35.5	***	5	0.21	10.9	***	5	0.34	1.6	0.2	5	10 023 666	4.8	***
Species type	*1*	*66 843*	*4.4*	*	*1*	*0.03*	7.2	**	*1*	*27*	*72.7*	***	*1*	*0.01*	*3.6*	*0.1*	*1*	*0*	*0.1*	*0.7*	*1*	*4 339 209*	*9.8*	**
Residual species identity	*5*	*256 494*	*4.2*	**	*5*	*0.04*	2.1	*0.1*	*5*	*25*	*17.0*	***	*5*	*0.19*	*11.9*	***	*5*	*0.32*	*2.2*	*0.1*	*5*	*8 318 107*	*4.7*	**
Treatment	1	51 029	3.2	0.1	1	0.01	2.2	0.1	1	5	16.5	***	1	0	0.1	0.8	1	1.1	27.0	***	1	52 702 819	127	***
Soil	1	22 374	1.4	0.2	1	0.03	7.0	*	1	1	2.5	0.1	1	0.01	2.5	0.1	1	0.02	0.4	0.5	1	64 630	0.2	0.7
Species effect × soil	5	64 350	0.8	0.6	5	0.02	1.1	0.4	5	1	0.7	0.6	5	0.02	1.0	0.4	5	0.06	0.3	0.9	5	1 348 483	0.6	0.7
Species type × soil	1	4472	0.2	0.6	1	0	0.3	0.6	1	0	0.3	0.6	1	0	0.0	0.9	1	0.06	1.2	0.3	1	61 249	0.1	0.8
Soil × treatment	1	6843	0.4	0.5	1	0	0.9	0.4	1	1	3.5	0.07	1	0.02	5.9	*	1	0.05	1.2	0.3	1	69 887	0.2	0.7

Table 5 (Continued)

	SLA				LDMC				Root : shoot				Root diameter				RDMC				SRL			
	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
Species effect × treatment	5	69 869	0.9	0.5	5	0.01	0.7	0.7	5	3	5.4	*	5	0.02	1.0	0.5	5	0.17	0.9	0.5	5	4 633 018	3.0	*
Species type × treatment	1	1667	0.1	0.8	1	0	0.2	0.7	1	5	6.9	***	1	0	0.1	0.8	1	0.08	2.1	0.2	1	1 953 853	4.5	*
Soil × species effect × treatment	5	61 673	0.8	0.6	5	0.01	0.6	0.7	5	1	0.6	0.7	5	0.02	1.0	0.4	5	0.04	0.2	1	5	1 022 803	0.5	0.8
Soil × species type × treatment	1	667	0.0	0.9	1	0	0.5	0.5	1	1	1.6	0.2	1	0	0.5	0.5	1	0.49	1.3	0.3	1	74 179	0.1	0.7
Residuals		1 200 362				0.34				16				0.31				2.78				33 673 193		

Data analysed using ANOVA with contrasts; terms in italic are contrasts within the main species identity factor. Replicate was used as random effect in the model. Degrees of freedom (df), sums of squares (SS), *F* values and *P* values from ANOVA are presented. Significance: *, 0.05; **, 0.01; ***, <0.0001.

other slow-growing species, was more competitive in soil conditioned by the fast-growing community, suggesting a contrasting trait advantage. The three fast-growing species showed individual responses to competition in the soil conditioning treatments, with *A. capillaris* being more competitive in soil conditioned by the fast-growing community, suggesting a similar trait advantage. By contrast, *H. lanatus* was more competitive in soil conditioned by the slow-growing community, and *P. trivialis* had positive values for total and shoot RCI in both soils, indicating that it grew better in monocultures than mixtures and that it is a poor competitor in both soil treatments. However, these responses were strongly influenced by competitive neighbour identity, albeit with no clear patterns emerging.

Soil feedbacks and competitive interaction effects on plant trait expression

Apart from RDMC, all 11 plant traits sampled from species grown in monocultures and six species competitive mixtures differed significantly between species compositions (Tables 5, S2). Differences between fast- and slow-growing species explained a small amount of variation in traits (from 2% to 30%), except for root N concentration, root C concentration and LDMC, which were more strongly related to species type (i.e. 47%, 44% and 43% of this variation, respectively). The remaining residual variation in traits (45–98%) was explained by differences between individual species. Soil conditioning significantly affected the expression of root C : N and LDMC, which were both greater in soil conditioned by slow- than fast-growing plant communities (Table 5).

The competition treatment (monoculture vs mixture) significantly affected the expression of leaf N and RDMC (Table 5), with greater leaf N concentration and lower RDMC in individuals grown in monoculture than in competitive mixtures. Expression of root SRL was significantly greater in individuals grown in monocultures than in those grown in mixtures, although this effect was greater in fast-growing species (Table 5, Fig. 5a). By contrast, the root : shoot ratio of slow-growing species was significantly greater for individuals grown in mixtures than in monocultures, but, for fast-growing species, this measure did not differ between competitive treatments (Table 5, Fig. 5b). The competition treatment affected leaf C : N, LDMC and root diameter, but responses varied with soil conditioning: leaf C : N was greater, but LDMC and root diameter were lower, in individuals grown in mixtures in soil conditioned by the fast- than the slow-growing plant community (Table 5, Fig. 5c,d,e).

Discussion

The main goal of this study was to test whether PSF responses across a broad range of grassland plant species can be explained on the basis of plant functional traits in monoculture and competitive mixtures. To address this, we measured PSF responses of grassland species along an LES to soil conditioned with model plant communities dominated by slow- or fast-growing traits. In addition, we measured the effects of PSFs and competitive interactions on plant trait values. Our findings support our prediction

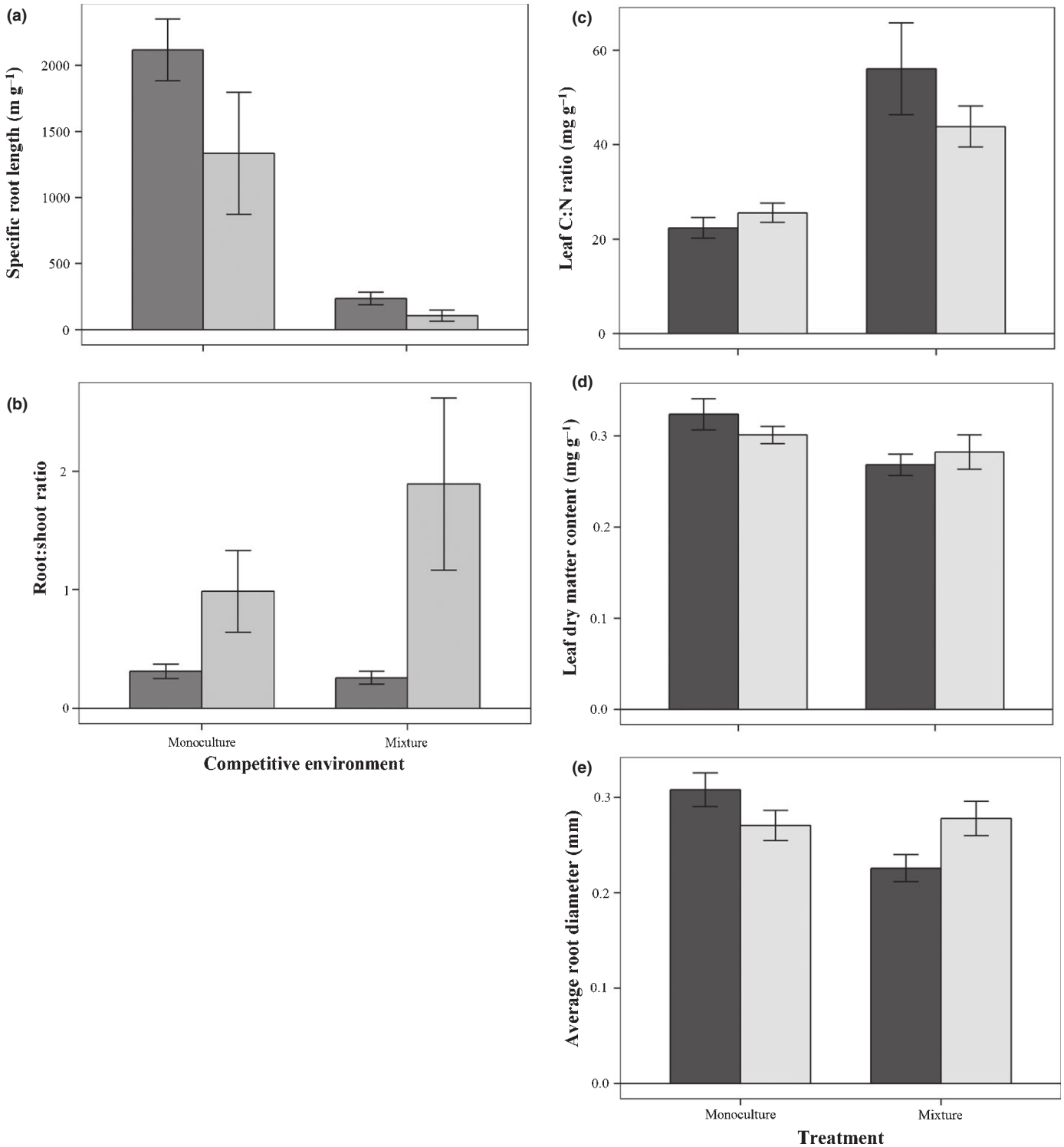

Fig. 5 Expression of (a) specific root length (SRL) and (b) root : shoot ratio of slow-growing (light grey bars) and fast-growing (dark grey bars) plants in monocultures and mixtures, and (c) leaf C : N ratio, (d) leaf dry matter content (LDMC) and (e) root diameter in plants grown in monocultures and mixtures in soil conditioned with slow-growing (light grey bars) or fast-growing (dark grey bars) plants. Error bars, ± 1SE ($n = 24$).

that soil conditioning by plant communities dominated by contrasting plant traits influences future plant growth. However, the strength and direction of the responses of slow-growing and fast-growing species varied depending on whether they were growing in monoculture or mixtures. In monoculture, all species, regardless of their growth strategy, responded in the same way to soil conditioning, with greater biomass in soil conditioned by the fast-growing community, suggesting that plant traits have limited value in predicting PSF outcomes in monoculture. By contrast, in competitive mixtures, species with different traits differed in

their responses to soil conditioning, suggesting that traits could provide an insight into the mechanisms that underpin PSFs and allow broad-scale prediction of their outcomes. We also found that PSFs and the biomass of neighbouring plants influenced the competitive ability of plants, as well as PSF influences on the values of a range of leaf and root traits, the effects of which were largely species specific. Collectively, our results suggest that a trait-based approach has the potential to be a useful tool to explain PSF outcomes in mixed communities, but is less effective in monoculture responses.

Soil conditioning by model plant communities of the same species, but different relative abundances, had significant effects on a range of soil properties related to N cycling, resulting in N availability being higher in soils conditioned by fast-growing species, supporting our hypothesis. This is consistent with other studies which have shown that changing the relative abundance of species within model communities can have strong effects on ecosystem functioning (Orwin *et al.*, 2014). In addition, it agrees with field studies (de Vries *et al.*, 2012; Grigulis *et al.*, 2013) which have shown that communities dominated by fast-growing species with exploitative traits can enhance soil N availability, and the abundance and activity of soil microbes involved in the N cycle, relative to those dominated by slow-growing species with conservative traits. The greater total N pool and availability of inorganic N in soil conditioned by fast-growing species were probably a result of the input and decomposition of comparatively large amounts of high-quality litter to soil and, potentially, the greater release of root exudates, which can stimulate microorganisms involved in N cycling (Dijkstra *et al.*, 2013). It is also possible that the faster growth of these species resulted in less leaching of nutrients in these systems early in community development, leading to reduced N loss and a greater pool of available nutrients.

Soil conditioning by the two model plant communities also resulted in differential growth responses of a broad range of plant species, consistent with our first hypothesis. As we did not use the exact same species in both the conditioning and feedback phases, the probability of strong species-specific pathogen or symbiont effects commonly found in other PSF studies (Mills & Bever, 1998; Bever, 2003; Mangan *et al.*, 2010; Mordecai, 2011) would have been reduced. This is supported by the lack of negative feedback on the growth of the four species used in both the conditioning and feedback phases. Although pathogen or symbiont effects on other species cannot be ruled out, this suggests that changes in nutrient cycling were probably the main mechanism behind the effects seen here. This is the first time, as far as we are aware, that plant communities of the same composition, but dominated by species with different traits, have been shown to cause sufficient changes in soil properties to result in significant changes in the growth of subsequent plant communities.

Soil conditioning effects were generally weak in monoculture, with only a marginally significant effect on shoot biomass production: all species produced more shoot biomass in monoculture when grown in soil conditioned by fast-growing communities than in soil conditioned by slow-growing communities, probably because of the enhanced nutrient availability in these soils. Although this resulted in a significant species type effect on the PSF index for shoot biomass, traits may be of limited use in explaining shoot biomass responses to soil conditioning in monoculture, as all species, regardless of growth strategy, responded in the same direction. Soil conditioning did, however, influence significantly plant growth and competitive ability in mixtures, suggesting that soil feedbacks are more important in mixed communities. This is consistent with our hypothesis and also with the findings of Kardol *et al.* (2007), who showed that PSF responses were increased significantly when grown in competitive mixtures. In addition, species with different growth strategies responded differently to soil conditioning, both in terms of the magnitude of their response, and whether above-ground or below-ground biomass responded. This suggests that plant traits may be a useful tool for understanding the mechanisms behind PSFs in mixtures.

The shoots of slow-growing species responded positively to soil conditioned by species with similar traits in mixtures, in contrast with their negative response in monoculture. This suggests that these species respond positively to the higher N availability of soil conditioned by fast-growing species in monoculture, but are unable to utilize these nutrients to the same extent when competing with fast-growing species. Instead, they do better in the low-nutrient soil conditioned by slow-growing communities, in which the slow cycling of nutrients may inhibit the growth rate of fast-growing plants. This interpretation is supported by the RCI results for shoots, as slow-growing species had a much higher competitive ability in soil conditioned by the slow-growing community than in soil conditioned by the fast-growing community. Our findings support the theory that soil conditioned with slow-growing communities promotes slow-growing plants within a competitive environment, and that PSFs may help promote the co-existence of at least some slow- and fast-growing species (Bever, 2003; Gross *et al.*, 2007). Interestingly, a different pattern was seen for fast-growing species, with root biomass, rather than shoot biomass, being promoted by soil conditioned by similar species. In the longer term, this may translate into greater shoot biomass in soil conditioned by fast-growing species, and promote the persistence of fast-growing species in a similar way to that seen for slow-growing species. The different responses to soil conditioning by species with different growth strategies may result from differences in the requirements of these species in the establishment phase, that is light and space for slow-growing species and nutrients for fast-growing species, and therefore underpin the effects of soil conditioning on competitive outcomes between species of differing traits in the longer term.

As well as modifying plant biomass responses, soil conditioning and competition altered plant trait values. The lower nutrient availability of the soil conditioned by the slow-growing community was associated with higher C : N ratios and LDMC of all individuals and higher root diameter of individuals grown in multi-species mixtures (Al Haj Khaled *et al.*, 2005; Duru *et al.*, 2009). Such changes in plant traits may contribute to the persistence of legacy effects in the longer term. The remainder of the variation in leaf and root trait responses to experimental conditions was dependent on whether plants were grown in monoculture or mixture, and the identity of species and their neighbours,

suggesting a stronger influence of plant community structure than soil conditioning on the expression of most traits. Of particular interest was the finding that the root : shoot ratio of slow-growing species was modified in the competitive mixture, whereas that of fast-growing species did not change; by contrast, however, the reduction in SRL in mixtures was greater in fast-growing species. This suggests that these individuals were experiencing below-ground competition for resources (Casper & Jackson, 1997; Wardle & Peltzer, 2003; Dybzinski & Tilman, 2007), resulting in modifications to trait values and possibly to root foraging behaviour (Mommer *et al.*, 2012). However, these differences in resource allocation resulting from competition are likely to be caused by the different nutrient strategies used by these species types (Violle *et al.*, 2009; Wang *et al.*, 2010).

In conclusion, our results provide new insights into the role of plant traits in explaining PSF responses in grasslands. Specifically, we show that soil conditioning by plant communities dominated by contrasting plant trait composition influences future plant growth via changes in soil nutrient availability, but that the strength and direction of these responses vary depending on the competitive environment. The classification of plants in the feedback phase into fast growing and slow growing revealed a fundamental difference in how these two groups respond to PSFs in mixtures, with the shoots of slow-growing plants, and the roots of fast-growing plants, responding positively to soil conditioned by species with similar traits. Although we found that species with broadly similar traits responded similarly to PSFs in mixtures, there was significant variation in responses, which probably reflects the coarse nature of the trait groupings used, or that traits based on the LES are not the only traits that influence PSFs. For example, above-ground traits, such as SLA, have been shown to correlate poorly with root traits (Mommer & Weemstra, 2012), which may have a stronger influence on below-ground interactions and nutrient cycling than on above-ground traits. Further research is clearly needed to identify particular plant traits that are useful predictors of such plant responses to PSFs. However, our results suggest that a trait-based approach has the potential to enhance our knowledge of the mechanisms behind differential plant species responses to PSFs, especially in mixed communities.

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Supporting Information

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

Fig. S1 The 16 grassland species ordered by leaf economic traits as analysed by principal component analysis using trait values of specific leaf area (SLA) and leaf dry matter content (LDMC).

Table S1 Species trait values for specific leaf area (SLA) and leaf dry matter content (LDMC) used in the species selection process; values taken from Grime *et al.* (2007)

Table S2 Mean values for shoot, root and total plant biomass and 12 chemical and structural leaf and root traits for 16 species grown in two different soils

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