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LETTER

Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide

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Abstract

Aboveground–belowground interactions exert critical controls on the composition and function of terrestrial ecosystems, yet the fundamental relationships between plant diversity and soil microbial diversity remain elusive. Theory predicts predominantly positive associations but tests within single sites have shown variable relationships, and associations between plant and microbial diversity across broad spatial scales remain largely unexplored. We compared the diversity of plant, bacterial, archaeal and fungal communities in one hundred and forty-five 1 m^2 plots across 25 temperate grassland sites from four continents. Across sites, the plant alpha diversity patterns were poorly related to those observed for any soil microbial group. However, plant beta diversity (compositional dissimilarity between sites) was significantly correlated with the beta diversity of bacterial and fungal communities, even after controlling for environmental factors. Thus, across a global range of temperate grasslands, plant diversity can predict patterns in the composition of soil microbial communities, but not patterns in alpha diversity.

Keywords

Aboveground–belowground interactions, archaea, bacteria, fungi, grasslands, microbial biogeography, soil biodiversity.

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INTRODUCTION

Soil microbes play a pivotal role in the functioning of terrestrial ecosystems and are increasingly recognized as important drivers of plant diversity (van der Heijden *et al*. 2008; Klironomos *et al*. 2011). Indeed, van der Heijden *et al*. (2008) estimate that at least 20 000 plant species need soil microbial symbionts to persist, they predict that free-living soil microbes can indirectly promote plant diversity by increasing the diversity of available nutrient pools, and they highlight evidence showing that symbiotic and pathogenic soil microbes can influence plant diversity by altering plant dominance. At the same time, plant diversity is predicted to promote the diversity of soil microbes by increasing the diversity of food resources (soil exudates and litter), physical microhabitats and environmental conditions, and by contributing a diversity of plant hosts for symbiotic and pathogenic microbes (Hooper *et al*. 2000; Wardle 2006; Millard & Singh 2010; Eisenhauer *et al*. 2011).

As a consequence of these direct and indirect functional relationships, the diversity of plant communities is widely

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expected to predict the diversity of soil microbial communities (Kardol & Wardle 2010; Mitchell *et al.* 2010; Hiiesalu *et al.* 2014). This is relevant to both alpha diversity, described by the number of taxa and their abundance within communities or habitats (typically measured at the plot scale; Whittaker 1972), and beta diversity, defined here as variation in community composition and measured in terms of pair-wise dissimilarity between plots (Whittaker 1972; Anderson *et al.* 2011). Given a predominance of positive functional associations between plants and soil microbes, more diverse plant communities would be expected to occur with more diverse soil microbial communities, and more distinct plant communities would be expected to occur with more distinct soil microbial communities. If these assumptions hold, information on plant communities and their responses to global change factors could be used to predict belowground communities and their responses to these factors (Hooper *et al.* 2000; De Deyn & Van der Putten 2005; Gao *et al.* 2013).

Despite theoretical support for associations between soil microbial and plant diversity, empirical evidence is inconclusive (Wardle 2006; Millard & Singh 2010; Gao *et al.* 2013). With respect to alpha diversity, only two of eight studies reviewed by Wardle (2006) showed positive relationships between soil microbial and plant diversity, leading Wardle to conclude that plant and soil biodiversity are somewhat uncoupled. A number of later studies have similarly found limited support for positive associations between above- and belowground alpha diversity (e.g. Culman *et al.* 2010; Millard & Singh 2010; McElroy *et al.* 2012). In contrast, evidence for positive relationships between soil and plant diversity has recently emerged from site-scale experimental (Eisenhauer *et al.* 2011; Milcu *et al.* 2013) and observational (Meadow & Zabinski 2012; Hiiesalu *et al.* 2014) studies, and from a meta-analysis indicating that ectomycorrhizal fungal richness is predicted by plant genus-level richness in forests around the world (Gao *et al.* 2013).

Direct tests of the relationships between the beta diversity of plants and soil microbes have been fewer, and results have been similarly mixed. Several studies have suggested significant correlations between vegetation type and soil microbial patterns across global (Öpik *et al.* 2006), regional (e.g. Griffiths *et al.* 2011) and local (e.g. Mitchell *et al.* 2010) scales, whereas others have found that plant communities and soil microbial communities are shaped by different environmental drivers (Fierer & Jackson 2006; Fierer *et al.* 2011; Soininen 2012).

A range of factors could account for the observed inconsistent relationships between soil microbial and plant diversity. First, relationships may depend on spatial scale (Hooper *et al.* 2000; De Deyn & Van der Putten 2005; Wardle 2006; Soininen 2012). Local-scale relationships are more likely governed by plant-soil interactions, but these may not persist across wide geographical scales if soil microbes and plants respond differently to broad-scale environmental drivers (De Deyn & Van der Putten 2005). As most studies have been conducted in single sites or microcosms, De Deyn & Van der Putten (2005) highlighted a need for assessment of aboveground–belowground diversity relationships across global gradients. Second, Bardgett *et al.* (2005) emphasize that soil organisms and plants can vary at different temporal scales, and consequently experimental approaches are vulnerable to lag effects which can obscure relationships between plant and soil communities (Eisenhauer *et al.* 2010, 2011; Milcu *et al.* 2013). Third, soil organisms themselves are likely to be diverse in their response to shifts in aboveground diversity, so the strength of relationships is likely to differ among taxonomic groups (Wardle 2006; Eisenhauer *et al.* 2011). In particular, evidence for aboveground–belowground relationships appears stronger for fungal than bacterial groups because fungi are often more directly dependent on plant products (Broeckling *et al.* 2008; Millard & Singh 2010) and mycorrhizal fungi are more dependent on direct symbiotic relationships with plants (Gao *et al.* 2013).

To address the need for comparisons of soil microbial and plant diversity across a range of soil microbial taxa and across broad geographical scales, we tested relationships between soil microbial diversity and plant diversity in one hundred and forty-five 1 m^2 plots across 25 temperate grassland sites on four continents. We focused on grasslands as widely used model systems for investigating aboveground–belowground relationships (Eisenhauer *et al.* 2011); and because there is already evidence that major biomes harbour distinct belowground communities (Fierer & Jackson 2006; Öpik *et al.* 2006; Fierer *et al.* 2012). We used a marker gene sequencing-based approach to characterize the alpha and beta diversity of archaea, bacteria, fungi, and major groupings therein and compared their diversity patterns across sites to the taxonomic and functional diversity of the corresponding plant communities.

We hypothesized that for both alpha and beta diversity estimated at the plot level: (1) soil microbial and plant diversity are positively associated across our global range of grassland sites, (2) these relationships are at least partially explained by shared environmental drivers and (3) plant diversity is more strongly predictive of the diversity of fungi (especially mycorrhizal fungi) than the diversity of bacteria, due to a greater influence of plant root and litter characteristics on fungal communities, and direct symbiotic dependencies with mycorrhizal fungi. Contrasting with these expectations, we found that plant diversity is a poor predictor of the alpha diversity of all soil microbial groups across our globally–distributed sites. However, we found that beta diversity (compositional dissimilarity between sites) for plants can predict beta diversity for soil microbes even after controlling for environmental factors.

METHODS

We measured soil microbial and plant diversity at the 1 m^2 plot scale in 25 temperate-zone grassland sites of the global Nutrient Network experiment (Borer *et al.* 2014), including three in Africa, two in Australia, three in Europe and 17 in North America (Table S1). Sites ranged in absolute latitude from 28 to 54° and represented a wide range of environments, including mean annual precipitation (262–1898 mm $year^{-1}$), mean annual temperature (0–18°C), elevation (50–2320 m), soil pH (4.5–8.4), total soil P (1–253 ppm), soil %N (0.03–1.5%), soil texture (13–90% sand) and aboveground plant productivity (15–1482 gm^{-2} $year^{-1}$).

Plant species composition was sampled during 2011 or 2012, within six 1 m^2 unfertilized plots per site, arranged in a

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randomized complete block design with two plots in each of three blocks. Two sites had missing plots (Table S1), leading to a total of 145 plots. Each 1 m^2 plot was located within a relatively homogeneous, larger experimental plot of 25 m^2; for other purposes one of the 25 m^2 plots per block at most sites had been fenced to exclude vertebrate herbivores.

At peak aboveground plant biomass, per cent cover was visually estimated for all vascular plant species in each 1 m^2 plot. At sites with two distinct growth peaks, cover was estimated in each period and the maximum cover assigned. Plant species were classified into functional types (annual grasses, perennial grasses, other graminoids, annual/biennial forbs, perennial forbs, annual/biennial legumes, perennial legumes and woody species). We calculated the following plot-scale measures of plant alpha diversity: species richness, species Shannon diversity, generic richness, generic Shannon diversity and Shannon diversity of plant functional types (a weighted measure of plant trait diversity). Plant species and generic diversity measures correlated at $r^2 = 0.99$, so alpha diversity measures for plant genera are not presented further.

Five soil cores (2.5 cm diameter × 10 cm deep) were sampled from two 0.1 × 1 m strips adjacent to each floristic plot (and within the larger 25 m^2 experimental plot), homogenized and shipped on ice to a central processing laboratory (Corvallis, Oregon, USA), then distributed for further analyses (see below). To minimize contamination the soil corer was washed with alcohol wipes between plots, and gloves were worn.

We estimated a range of environmental variables that could potentially contribute to patterns in soil microbial and plant diversity. A pH probe (Fisher Scientific Waltham, Massachusetts, USA) was used to measure pH of a soil slurry (10 g dry soil, 25 mL deionized water). Total soil %C and %N were measured using dry combustion gas chromatography (COSTECH ESC 4010 Element Analyzer, Costech Analytical Technologies Inc., Valencia, California, USA), and total soil P using Mehlich-3 extraction and Inductively Coupled Plasma Mass Spectrometry (A&L Analytical Laboratory, Memphis, TN, USA). Soil texture was measured using the Buoycous method (Elliott *et al.* 1999) for soils collected in unfenced plots several years earlier. Climate variables [mean annual precipitation (MAP), mean annual temperature (MAT), precipitation seasonality and temperature seasonality] for each site were estimated using Bioclim V1.4 (Hijmans *et al.* 2005) at a 1 km^2 resolution. Land use histories were reflected in a cultivation score (18 sites never cultivated, three cultivated > 70 years ago, four cultivated < 15 years ago) and a subjective livestock grazing score (nil, low, moderate, high or very high in the period 5–100 years prior to the experiment), estimated by site leaders using local knowledge.

Microbial analyses

Microbial diversity was assessed using high-throughput sequencing to characterize the variation in marker gene sequences. We sequenced the V4 hypervariable region of the 16S rRNA gene for bacteria and archaea, and the first internal transcribed spacer (ITS1) region of the rRNA operon for fungi, following Fierer *et al.* (2012) and McGuire *et al.* (2013). Briefly, we extracted DNA from soils using the MoBio PowerSoil kit (Mo Bio Laboratories Inc., Carlsbad, California, USA) and conducted 35 cycles of polymerase chain reaction (PCR) amplification of the two marker genes separately. Error-correcting 12-bp barcoded primers specific to each sample were used to permit multiplexing of samples. PCR products from all samples were quantified using the PicoGreen dsDNA assay, and pooled together in equimolar concentrations. Samples from 19 sites were combined and sequenced on an Illumina HiSeq instrument, and the other six sites (see Table S1) were sequenced on an Illumina MiSeq instrument with separate sequencing runs for the 16S rRNA and ITS amplicon pools. All sequencing was undertaken at the University of Colorado Next Generation Sequencing Facility.

The HiSeq and MiSeq sequences were demultiplexed using a custom Python script ('prep_fastq_for_uparse.py', available at: https://github.com/leffj/helper-code-for-uparse) with quality filtering and phylotype (i.e. operational taxonomic unit) clustering conducted using the UPARSE pipeline (Edgar 2013). Sequences were trimmed to a consistent 100-bp length and a maximum per sequence expected error frequency value of 0.5 was used to quality-filter sequences. Sequences were dereplicated and singleton sequences were removed prior to phylotype determination. Representative sequences from the phylotypes that were not ≥ 75% similar over their entire length to sequences contained in either the Greengenes 13_5 database (McDonald *et al.* 2011) or the UNITE November 2012 database (Abarenkov *et al.* 2010) for 16S and ITS rRNA sequences, respectively, were discarded. Proportions discarded are shown in Table S2.

These sets of representative sequences were then used to categorize raw (trimmed to 100 bp) sequences into phylotypes at the 97% similarity threshold. Phylotypes were classified to taxonomic groups using the RDP classifier (Wang *et al.* 2007) against the respective databases. For each taxonomic group analysed (bacteria, archaea, fungi and sub-groups thereof, and putative mycorrhizal taxa), samples were rarefied to compare all samples at equivalent sequencing depths (shown in Table S3). Rarefied data were used to calculate phylotype richness and Shannon diversity for these groups. Maintenance of an adequate rarefaction depth led to exclusion of some sites for some microbial groups (Table S3). Putative mycorrhizal taxa were assigned using a script in the R statistical package (https://github.com/thebateslab/r_code/tree/master/htp_analyses, accessed Nov 2013; R Core Team 2013). This script searched phylotype classifications for genera of known or purported ectomycorrhizal fungi (Rinaldi *et al.* 2008; Tedersoo *et al.* 2010) or the phylum Glomeromycota. This yielded a mean of 20.7 putative mycorrhizal phylotypes per sample, although we acknowledge that the primers and classification methods employed may not capture all potential mycorrhizal diversity (e.g. Abarenkov *et al.* 2010). Taxonomy assignments, rarefaction and alpha diversity calculations were conducted in QIIME v. 1.7.0 (Caporaso *et al.* 2010).

To check for bias associated with Illumina HiSeq and MiSeq runs, all data analyses were conducted separately using data from all 25 sites, and using data from only the 19 sites processed using Illumina HiSeq. Results were comparable so only the full analysis is presented.

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Alpha diversity analyses

We used two approaches to analyse the alpha diversity data: using Genstat 13.0 we fitted linear mixed models to individual plot-level data, and regression models to site mean data (i.e. retaining the 1 m^2 plot scale but averaging across all plots per site). Linear mixed models employed restricted maximum likelihood estimation, and included site and block nested in site in the random model. Fencing was initially included in the fixed model, but this term was not significant so was removed. Where appropriate, data were log transformed to meet the assumptions of normality (see Results). We found few differences between the results of the two modelling approaches so, for simplicity, present only the regression models based on site means for 1 m^2 plot-level alpha diversity measures.

First we tested whether measures of microbial alpha diversity were significantly related to each other and to corresponding plant alpha diversity measures (i.e. microbial richness with plant richness, and microbial Shannon diversity with plant Shannon diversity or plant-functional Shannon diversity). Second, to compare environmental correlates of alpha diversity among microbial groups and plants, we tested the environmental attributes described above for their individual effectiveness in predicting plant, bacterial, archaeal and fungal richness and Shannon diversity (screening bivariate plots to check for apparent non-linear relationships).

Third, to test whether combinations of environmental variables explained alpha diversity better than single variables, we first checked for collinearity among variables. This led to removal of soil %C which correlated with soil %N ($r^2 = 0.94$); r^2 values among remaining environmental variables were mostly < 25% (maximum 53% for MAP vs. pH; variance inflation factors 1.1–4.5). We used all-subsets regression to test all possible additive models with combinations of up to four of the environment variables (only four were used to avoid over-fitting). We only accepted models where all terms were significant ($P < 0.05$) and of these, we tested whether interactions between model terms were also significant when added to the parent model. Of models meeting these criteria, we report those with the highest adjusted r^2 and the lowest Akaike Information Criterion (AIC), and models within two units of the lowest AIC models.

Finally, we investigated whether plant alpha diversity was a significant predictor of bacterial, archaeal and fungal alpha diversity beyond the variation explained by the environmental parameters, by including it and potential interaction terms as subsequent predictors in each of the best environment models identified above. The final model was selected to include only significant terms, unless they were parents to significant interaction terms.

Beta diversity analyses

To examine whether beta diversity (pair-wise dissimilarity in community composition) for plants predicts beta diversity of soil microbial communities, we compared Bray–Curtis dissimilarity matrices for plants with those for bacteria, archaea and fungi using Mantel tests and Spearman correlations (using Vegan v.2.0-8 in R Core Team 2013). In other words, we tested whether plots that were more dissimilar to each other in plant composition also harboured belowground microbial communities that were more dissimilar to each other. Dissimilarity matrices (pairwise comparisons between all plots) were assembled using Hellinger-transformed abundances (i.e. square-root transformed relative abundance per plot) of the microbial phylotypes or plant genera. We used plant genera to better detect relationships across our broad range of sites, due to low overlap at the plant species level. To avoid pseudo-replication, we then averaged the dissimilarities by site; hence, final pair-wise comparisons are between sites, averaged from plot-level dissimilarities.

To evaluate environmental correlations with the beta diversity of soil microbial and plant communities, we assembled dissimilarity matrices for pair-wise comparisons among sites (averaged from pair-wise distances between plots as above) using standardized environmental variables (transformed if required, see results) and Euclidean distances, as well as geographical distance. We tested relationships between matrices for community composition with matrices for environments, using Mantel tests with Spearman correlations. We assessed whether combinations of environmental factors explained differences in beta diversity better than single factors, by including multiple explanatory variables using the 'multiple regression on matrices' function (an extension of partial Mantel analysis, Lichstein 2007) in the 'ecodist' package in R (R Core Team 2013). Models were selected using backwards elimination ($P < 0.05$). We then tested whether plant beta diversity was a significant predictor of bacterial, archaeal and fungal beta diversity beyond the variation explained by the environmental parameters, by including it as a subsequent predictor in the best environment models.

RESULTS AND DISCUSSION

Soils harboured diverse microbial communities – we detected 39 780 bacterial, 219 archaeal and 4 747 fungal phylotypes, representing 284, 7 and 173 named families respectively. Soils were not equivalent in their rarefied alpha diversity levels; the mean number of bacterial, archaeal and fungal phylotypes per sample ranged from 2160 to 4786, 0–32 and 44–172 phylotypes respectively, across the 25 sites. There were 73 plant families, 369 plant genera and 673 plant species, with mean plant richness per plot ranging from 3.2 to 23.8 plant genera and 4.6–29.7 plant species across the 25 sites.

The most abundant bacterial phyla were Acidobacteria, Bacterioidetes, Actinobacteria, Proteobacteria and Verrucomicrobia, with the two dominant phyla (Acidobacteria and Proteobacteria) varying in relative abundance from 12 to 37% and 11 to 27% respectively. The strongest distinctions were among continents, including very high relative abundances of Verrucomicrobia in two of the three African sites, and high relative abundance of Chloroflexi in the Australian sites (Fig. 1a). The archaeal communities had variable representation of the phyla Crenarchaeota and Euryarchaeota and the candidate phylum Parvarchaeota, but there were too few archaeal sequences from the African samples for their inclusion in analyses (Fig. 1b). The most abundant fungal phylum was Ascomycota which varied in relative abundance from 23

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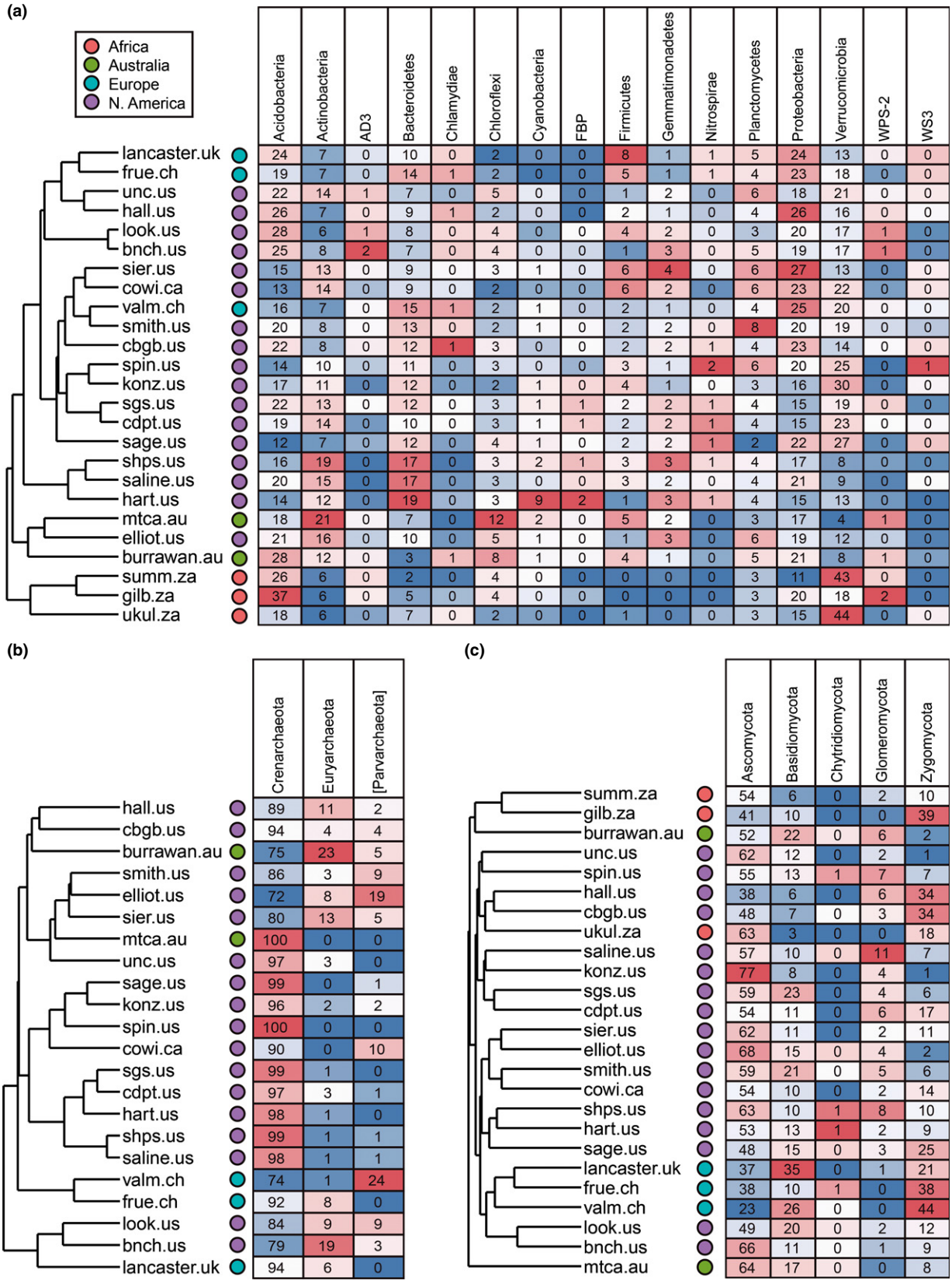

Figure 1 Hierarchical cluster diagrams (created using average linkage and truncated to show qualitative clustering of sites) and heatmaps for (a) bacterial, (b) archaeal and (c) fungal communities. Heatmap values reflect relative abundances (median percentage) of phyla among sites. Colours are scaled from highest (red) to lowest (blue) values within columns; the same values in a column may appear different colours owing to rounding. Only phyla with median percentages greater than or equal to 1 at any of the sites are shown, hence rows do not add to 100%.

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to 77% across sites, and some sites featured high relative abundances of Basidiomycota and/or Zygomycota. Four of the five Australian and African sites clustered apart from northern hemisphere sites, but the taxa driving this clustering were only apparent at the sub-phylum level (Fig. 1c).

Plant alpha diversity does not predict microbial alpha diversity

We found few significant relationships between mean bacterial, archaeal or fungal alpha diversity measures per site and any of their corresponding plant diversity measures, providing little support for our first hypothesis that the plot-scale alpha diversity of soil microbes would be positively associated with that of plants across our global range of grasslands (Fig. 2, Table S4). There was a weak correlation between bacterial and plant Shannon diversity ($r = -0.44$, $P = 0.016$), but this was negative and driven by three African sites with high plant diversity but low bacterial diversity. When African sites were removed, there were no significant relationships for any combination of alpha diversity measures.

The lack of relationships between soil microbial and plant alpha diversity extended to individual bacterial and fungal groups. At the phylum level, we observed only one significant positive correlation of 24 comparisons (for Ascomycetes) and two significant negative correlations (Table S4). Importantly, there were no significant relationships between plant alpha diversity and the alpha diversity of fungi classified as mycorrhizal. These results do not support our third hypothesis in which we proposed stronger correlations with plant diversity for fungal and mycorrhizal groups due to their greater dependence on plant-host relationships and products of rhizodeposition (Millard & Singh 2010; Gao *et al.* 2013). Furthermore, they are not consistent with the meta-analysis of Gao *et al.* (2013) that suggested ectomycorrhizal fungal richness is positively correlated with genus-level richness of ectomycorrhizal plants in forests. However, a re-compilation and re-analysis of the data used in Gao *et al.* (2013) corrected for proposed data inconsistencies and subsequently found no significant relationship between ectomycorrhizal fungal richness and host richness at any taxonomic level (Tedersoo *et al.* 2014), a result that is more in line with our findings.

Taken together, our alpha diversity results most strongly support the conclusion drawn by Wardle's (2006) review, that plant and soil biodiversity are largely uncoupled. Given recent strong evidence supporting positive relationships between plant and soil microbial alpha diversity within single sites from experimental and observational studies (Meadow & Zabinski 2012; Milcu *et al.* 2013; Hiiesalu *et al.* 2014), we propose that these apparently conflicting results are explained by differences in the spatial scale of enquiry. In particular, significant relationships between plant and soil microbial alpha diversity observed within sites or microcosms may not persist across broader spatial scales as they become overridden by differences in the environmental factors driving plant vs. microbial diversity patterns (Tedersoo *et al.* 2014).

Indeed, bacterial, archaeal, fungal and plant alpha diversity were not significantly related to one another ($P > 0.05$), nor were they correlated with similar environmental variables (Table 1). The strongest individual predictor of plant richness

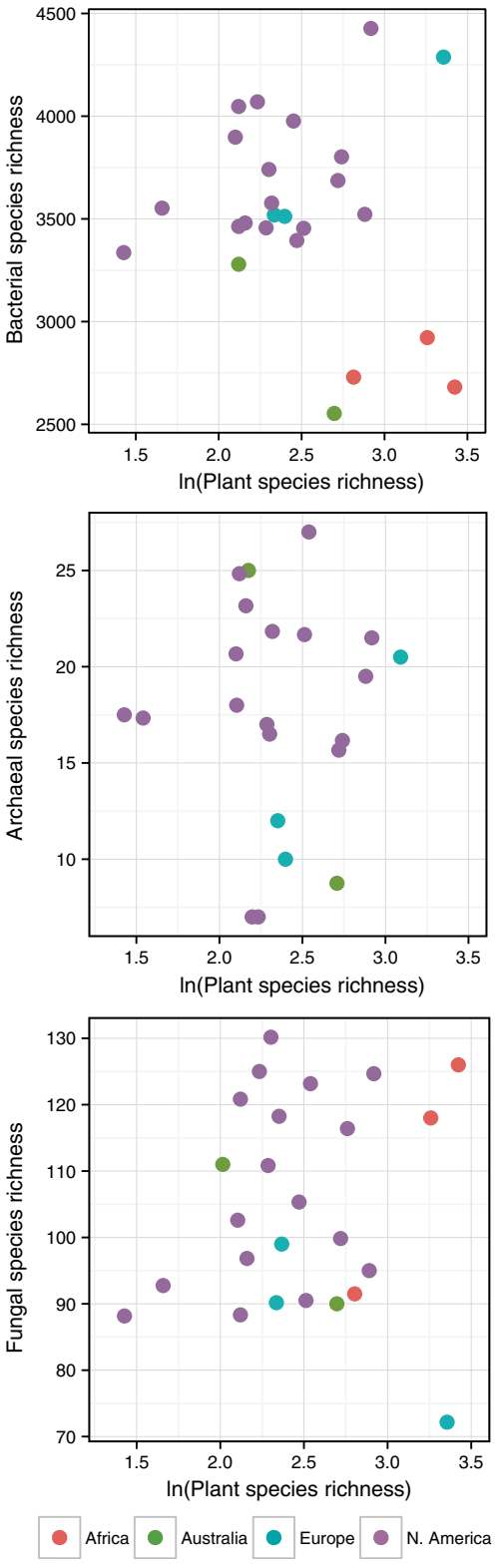

Figure 2 Relationships between plant species richness and phylotype richness for bacteria, archaea and fungi, measured at the 1 m^2 plot scale and averaged over plots in each site. No relationships were significant ($P > 0.1$ in all cases). Plot-scale richness for microbes was estimated after rarefaction to equal sequence numbers (see Table S3). Different colours reflect sites from different continents. See Table S4 for correlations among all microbial groups and plant alpha diversity measures.

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Table 1 Relationships of plant, bacterial, archaeal and fungal richness with individual environmental variables (r and P values), estimated for plot-scale richness averaged at the site level

	Plants		Bacteria		Archaea		Fungi	
Variable	r	P	r	P	r	P	r	P
MAP	–	n.s.	–	n.s.	−0.3	0.094	–	n.s. **(0.025)**
MAT	–	n.s.	**−0.55**	**0.003**	–	n.s.	**0.12**	n.s. **(0.001)**
Precipitation seasonality	–	n.s.	–	n.s.	0.26	n.s.	0.02	n.s.
Temperature seasonality	−0.25	n.s.	0.41	0.025	0.11	n.s.	–	n.s.
Total soil %N (ln)	0.16	n.s.	–	n.s.	–	n.s.	**0.25**	n.s. **(0.008)**
C : N (ln)	0.33	0.057	0.48	0.009	–	n.s.	0.19	n.s.
Total soil P (ln)	–	n.s.	0.53	0.004	–	n.s.	–	n.s.
Soil pH	–	n.s.	0.09	n.s.	**0.44**	**0.024**	–	n.s. **(0.004)**
Soil % sand	–	n.s.	–	n.s.	0.36	0.065	0.12	n.s.
Cultivation	**−0.39**	**0.031**	0.18	n.s.	−0.23	n.s.	–	n.s.
Livestock grazing history	–	n.s.	–	n.s.	–	n.s.	–	n.s.
r^2 and P of best model	**15.2%**	**0.031**	**29.8%**	**0.003**	**19.1%**	**0.024**	**41.4%***	**0.005**

See Methods for description of cultivation and grazing indices. Terms in the lowest AIC multivariate models for each response group are bolded (with P values in parentheses if different from individual models; see text for alternative models). Best model r^2 and P are indicated in the final row; no interaction terms were significant in the best models. Relationships with Shannon diversity were similar to those with richness but were typically weaker (not shown).n.s.: not significant; ln: natural log transformation; –: residual variance exceeds variance of response variable.
*All terms show a positive trend.

was cultivation history (AIC 28.2, $r^2 = 15.2\%$); a second potential model involved MAP + soil %N (AIC 28. 7, adjusted $r^2 = 17.1\%$) although these terms did not correlate significantly individually (Table 1). By contrast, bacterial richness was best explained by a negative relationship with MAT (AIC 25.4, $r^2 = 29.8\%$, Fig. 3a); or a positive relationship with soil P (AIC 25.9, $r^2 = 28.3\%$, Table 1). There was a single potential model for archaeal richness, involving a positive relationship with soil pH ($r^2 = 19.1\%$, Fig. 3b, Table 1). None of the climate or soil variables significantly explained variation in fungal richness when considered individually ($P > 0.05$; Table 1); but one model (MAP + MAT + soil %N + soil pH) had all terms significant (adjusted $r^2 = 41.4\%$, $P = 0.005$; Table 1). The differing models among groups are consistent with earlier studies suggesting that the environmental drivers of plant alpha diversity typically differ from drivers of alpha diversity in belowground microbial communities (Fierer & Jackson 2006; Fierer *et al.* 2011; Soininen 2012).

Adding plant richness to the best environment model (MAT) for bacterial richness improved model fit. The main effect of plant richness remained non-significant ($P = 0.15$), but the interaction was significant ($P < 0.001$), adjusted r^2 increased from 29.8 to 39.8% (Fig. 3a), and AIC was reduced by two units. This model indicated that bacterial richness decreased with MAT, but the decline was strongest at high plant richness. This highlights the potential for more complex, environmentally dependent relationships between plant and bacterial alpha diversity at the global scale, as might be expected if local-scale relationships become obscured by differing environmental drivers of plant vs. bacterial alpha diversity across larger spatial scales. Nevertheless, we did not detect significant relationships between alpha diversity of plants and archaea or fungi even after controlling for environmental factors.

Our focus on aboveground rather than belowground measures of plant diversity (see Hiiesalu *et al.* 2014), and differences in the temporal scales of community assembly between plants and soil microbes, may also have contributed to the lack of relationships in alpha diversity patterns across our sites. With regard to the latter, disturbance events or changes in land-use may lead to changes in plant diversity that are not synchronous with changes in microbial diversity (Bardgett *et al.* 2005). Our study sites have had similar management in place for at least 5 years (often > 50 years), reducing legacy effects that may impact shorter term experimental manipulations of plant diversity (Eisenhauer *et al.* 2010, 2011). Nevertheless, plant and microbial communities are likely to change at different rates in response to legacies such as historical cultivation, which was observed to affect plant richness in our analysis.

Plant beta diversity predicts beta diversity of soil microbes

In contrast to the lack of relationships for alpha diversity, our first hypothesis predicting significant positive relationships between soil microbes and plants across sites was supported for beta diversity; i.e. sites that were more distinct in the composition of their plant communities also harboured more distinct soil microbial communities. This was consistent for bacteria ($\rho = 0.43$; $P = 0.001$), fungi ($\rho = 0.45$; $P = 0.001$) and to a lesser extent, archaea ($\rho = 0.18$; $P = 0.03$; Fig. 4), despite constraints associated with high dissimilarities in plant composition among some sites (Fig. 4).

Furthermore, consistent with our second hypothesis that shared environmental drivers contribute to relationships between soil microbial and plant diversity, MAT was a significant predictor in the best models for beta diversity of plant communities as well as bacterial, archaeal and fungal communities (Table 2). This finding is supported by recent analyses suggesting the composition of some microbial groups can be predicted by temperature (e.g. Garcia-Pichel *et al.* 2013), as well as more fundamental concepts such as metabolic theory,

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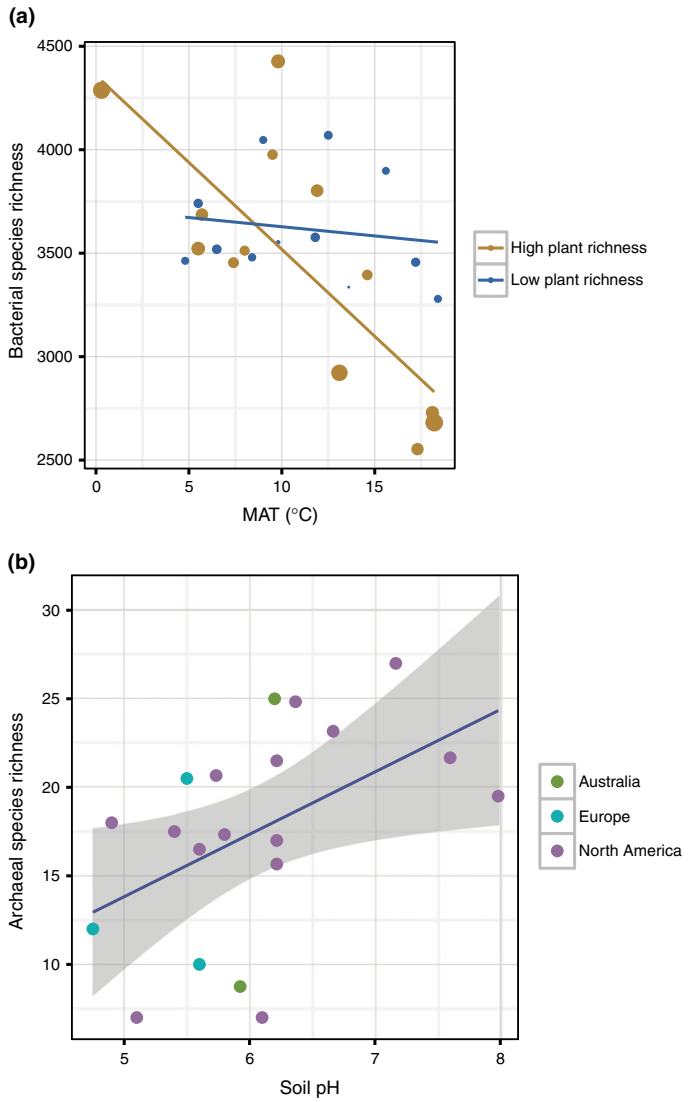

Figure 3 Best environment – richness models for bacteria (a; MAT, $P = 0.003$) and archaea (b; pH, $P = 0.024$), including interaction with plant richness for bacteria (circle size is proportional to plant richness; low richness ≤ 10 plant species m^{-2}). Richness was measured at the 1 m^2 plot scale and averaged over plots in each site; plot-scale richness for microbes was estimated after rarefaction to equal sequence numbers (see Table S3). In (b) different colours reflect sites from different continents (insufficient Archaea were detected in African sites for inclusion, see Methods).

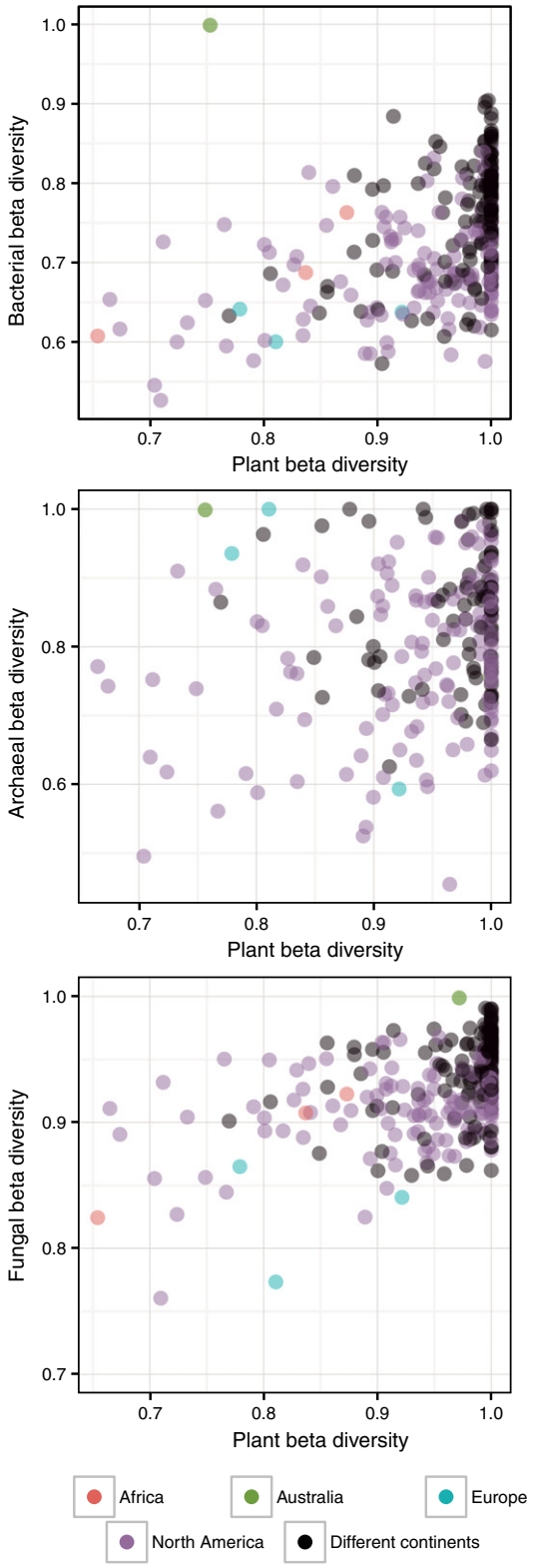

Figure 4 Relationships between beta diversity for plants and (a) bacteria ($\rho = 0.43$; $P = 0.001$), (b) archaea ($\rho = 0.18$; $P = 0.03$) and (c) fungi ($\rho = 0.45$; $P = 0.001$). Each point represents the dissimilarity in taxonomic composition between a pair of sites, calculated as the average of Bray–Curtis dissimilarities for all comparisons (typically 36) between plots from those two sites. Different colours represent comparisons between sites from within a continent (Africa, Australia, Europe or North America) or from two different continents (e.g. a European and an African site).

which may apply to microbes as well as plants (De Deyn & Van der Putten 2005; Stegen *et al.* 2009). The C : N ratio was similarly a common predictor in best models for all soil microbe groups and was the third best predictor for plants ($\rho = 0.26$, $P < 0.003$). The potential importance of C : N is consistent with links between the stoichiometry of plant litter and of soil microbial biomass; and with the contribution of shifts in microbial composition to the stoichiometric plasticity of microbial communities (Mooshammer *et al.* 2014).

However, not all of the environmental correlates with beta diversity were shared between plant and microbial communities. Plant beta diversity itself was best explained by MAT

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Table 2 Correlations (Spearman's rho) of plant, bacterial, archaeal and fungal beta diversity (plot-scale dissimilarities across sites, averaged at the site level) with geographical and environmental distances (averaged at the site level), estimated using Mantel tests

	Plants		Bacteria		Archaea		Fungi	
Variable	ρ	*P*	ρ	*P*	ρ	*P*	ρ	*P*
Geographical distance	0.38	0.001	0.47	0.001	0.24	0.021	0.49	0.001
MAP	0.13	0.05	**0.28**	**0.003**	0.31	0.002	0.21	0.02
MAT	**0.28**	**0.001**	**0.25**	**0.002**	**0.26**	**0.004**	**0.31**	**< 0.001**
MAP seasonality	0.11	0.06	−0.06	n.s.	0.07	n.s.	−0.06	n.s.
Temperature seasonality	0.13	0.03	0.18	0.01	0.11	0.09	0.23	0.003
Total soil %N (ln)	0.00	n.s.	0.20	0.04	**0.32**	**0.003**	0.06	n.s.
C : N (ln)	0.26	0.003	**0.35**	**0.002**	**0.28**	**0.02**	**0.40**	**< 0.001**
Total soil P (ln)	0.18	0.008	0.12	n.s.	0.12	0.10	0.20	0.02
Soil pH	−0.05	n.s.	**0.35**	**< 0.001**	**0.38**	**< 0.001**	**0.23**	**0.02**
Soil % sand	**0.28**	**0.002**	0.14	0.07	0.04	n.s.	0.14	0.06
Cultivation	0.09	n.s.	−0.15	n.s.	0.14	0.10	−0.11	n.s.
Livestock grazing history	0.00	n.s.	0.09	n.s.	0.14	0.09	0.08	n.s.
ρ^2 and *P* of best model	**16.0%**	**0.002**	**35.0%**	**0.001**	**37.4%**	**0.001**	**31.0%**	**0.001**

See Methods for description of cultivation and grazing indices. Terms in the best multivariate models (not including geographical distance) are bolded (see text). ρ: rho correlation; n.s.: not significant; ln: natural log transformation.

and soil % sand ($\rho^2 = 16.0\%$; $P < 0.01$ for all terms; Table 2), whereas models for microbial beta diversity all included soil pH as well as MAT and C : N. The importance of pH is consistent with previous work demonstrating it is a strong driver of soil bacterial community composition (e.g. Fierer & Jackson 2006; Rousk *et al*. 2010); our results indicate it is important for archaeal and fungal composition as well. The best model predicting bacterial beta diversity also included MAP and soil % sand ($\rho^2 = 35.0\%$; $P < 0.001$ for all four terms, Table 2), whereas the best model for archaeal beta diversity also included soil %N ($\rho^2 = 37.4\%$; $P < 0.001$ for all four terms; Table 2). The contribution of N corroborates previous observations that soil archaeal communities are structured across diverse ecosystems by N availability (Bates *et al*. 2010), likely reflecting the important role of soil archaea in N-cycling (Leininger *et al*. 2006). The best model predicting fungal beta diversity did not include any additional terms other than MAT, C : N and soil pH ($\rho^2 = 31.0\%$; $P < 0.001$ for all factors, Table 2). These contrasting environmental correlates among models suggest that, as we found for alpha diversity, relationships between the beta diversity of soil microbes and plants are likely to be weakened across broad scales by the distinct responses of soil microbes and plants to environmental drivers.

Finally, for bacteria and fungi, plant beta diversity predicted the beta diversity of soil microbes even beyond the predictive capacity of the strongest environmental explanatory variables. In particular, adding plant beta diversity as a predictor after the best environmental variables in models increased their power to predict bacterial beta diversity from $\rho^2 = 35.0\%$ to 43.3% ($\rho = 0.66$, $P = 0.001$) and for fungal beta diversity from $\rho^2 = 31\%$ to 40.2% ($\rho = 0.63$, $P = 0.001$). This suggests that factors other than shared relationships with MAT and C : N also contribute to the relationships between the beta diversity of plants and soil microbes. These potentially include common environmental drivers not analysed in our study, shared evolutionary histories (potentially reflected in continent groupings and correlations with geographical distance; Fig. 1, Table 2, Martiny *et al*. 2006), and direct linkages between plants and microbes mediated by plant species-specific symbioses or rhizodeposition of C compounds (Broeckling *et al*. 2008).

Although direct linkages remain a potential driver, relationships between plants and fungi ($\rho = 0.45$; $P = 0.001$) were not notably stronger than between plants and bacteria ($\rho = 0.43$; $P = 0.001$), and relationships between plants and mycorrhizal fungi ($\rho = 0.27$; $P = 0.003$) were not stronger than for plants and fungi in general ($\rho = 0.45$; $P = 0.001$). This again fails to support our third hypothesis that the stronger general dependence of fungi (and especially mycorrhizal fungi) on plants and plant products would lead to stronger relationships with plant diversity.

Taken together, the beta diversity patterns indicate that the beta diversity of bacteria and fungi across sites is partly predictable from commonly measured soil and climatic variables across temperate grasslands worldwide, but that knowledge of patterns in plant community composition can increase predictability. This finding corroborates previous work at finer scales, which has demonstrated that plant and soil microbial community members can influence one another (e.g. Carney & Matson 2006; Broeckling *et al*. 2008).

CONCLUSIONS

Our study spanning four continents shows that, counter to expectations, alpha diversity of soil microbes is poorly correlated with that for plants when comparing across sites, regardless of the microbial group in question. This lack of significant relationships may be driven by differences in the biogeographical drivers of alpha diversity for soil microbes vs. plants, that mask potential plant richness effects at more local scales (e.g. Eisenhauer *et al*. 2011; Milcu *et al*. 2013). This is consistent with recent conclusions of Tedersoo *et al*. (2014) who suggest climatic factors, disturbance and age of communities have stronger effects than richness of hosts on ectomycorrhizal richness.

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In contrast, the beta diversity (compositional dissimilarity between sites) of soil microbes does correspond with the beta diversity of plants, potentially due to a combination of shared environmental drivers, common evolutionary histories and direct functional associations. Greater consistency of patterns in beta than alpha diversity concurs with findings for comparisons across other (aboveground) groups of taxa (e.g. Gossner *et al*. 2013). We conclude that focusing on plant communities and their responses to global change factors is insufficient to predict responses of belowground microbial alpha diversity at the global scale, but the concordance between the beta diversity of plants and soil microbes does enhance our ability to predict turnover in the composition of soil microbial communities.

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AUTHOR CONTRIBUTIONS

E.T.B., W.S.H., E.L. and E.W.S. are Nutrient Network coordinators. N.F., J.W.L. and S.M.P. developed and framed research questions. P.B.A., J.D.B., E.T.B., E.E.C., N.M.D., E.D., N.F., J.F., N.H., W.S.H., Y.H., K.S.H., K.P.K., J.M.H.K., J.W.L., E.L., K.J.L.P., A.S.M., R.L.M., C.E.M., S.M.P, A.C.R., M.S., E.W.S., C.S. collected data used in this analysis. J.W.L., S.M.P. and N.F. led the data analyses with help from S.T.B., J.F., W.S.H., E.L., J.D.B., Y.H. and R.J.W. The paper was written by S.M.P., J.W.L. and N.F. with input from all authors.

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