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Research

Soil fungal diversity in natural grasslands of the Tibetan Plateau: associations with plant diversity and productivity

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Summary

- Previous studies have revealed inconsistent correlations between fungal diversity and plant diversity from local to global scales, and there is a lack of information about the diversity–diversity and productivity–diversity relationships for fungi in alpine regions.
- Here we investigated the internal relationships between soil fungal diversity, plant diversity and productivity across 60 grassland sites on the Tibetan Plateau, using Illumina sequencing of the internal transcribed spacer 2 (ITS2) region for fungal identification.
- Fungal alpha and beta diversities were best explained by plant alpha and beta diversities, respectively, when accounting for environmental drivers and geographic distance. The best ordinary least squares (OLS) multiple regression models, partial least squares regression (PLSR) and variation partitioning analysis (VPA) indicated that plant richness was positively correlated with fungal richness. However, no correlation between plant richness and fungal richness was evident for fungal functional guilds when analyzed individually.
- Plant productivity showed a weaker relationship to fungal diversity which was intercorrelated with other factors such as plant diversity, and was thus excluded as a main driver. Our study points to a predominant effect of plant diversity, along with other factors such as carbon : nitrogen (C : N) ratio, soil phosphorus and dissolved organic carbon, on soil fungal richness.

Introduction

Soil fungi play an important role in decomposition and nutrient recycling (Setala & McLean, 2004; Voriskova & Baldrian, 2013) and as mutualists and pathogens of plants and animals (Redman *et al.*, 2002; Gilbert & Webb, 2007; Parniske, 2008; Dagenais & Keller, 2009). Until recently, studies of fungal diversity and community structure have been greatly limited by the problems of culturing and morphological identification. Novel high-throughput sequencing (HTS) methods have offered a radically new perspective on fungal ecology (Lindahl *et al.*, 2013; Balint *et al.*, 2016; Peay *et al.*, 2016). Depending on geographic scale and study system, fungal community structure and diversity may be affected by a wide range of environmental variables such as temperature (Newsham *et al.*, 2016), precipitation (Tedersoo *et al.*, 2014), altitude (Bahram *et al.*, 2012), soil pH (Rousk *et al.*, 2010), nutrient availability (Hanson *et al.*, 2008; He *et al.*, 2016), and plant community (Barberan *et al.*, 2015; Prober *et al.*, 2015; Tedersoo *et al.*, 2016).

Notably, spatial scale has a profound influence on the detection and relative importance of ecological patterns and processes, as well as the elucidation of the underlying mechanisms (Chase & Leibold, 2002; Martiny *et al.*, 2006; Sandel & Smith, 2009; Shi *et al.*, 2015). It has been suggested that correlation of fungal and plant diversities is stronger at the very broad scales, where plant and fungal diversities can covary along significant shared gradients (Hooper *et al.*, 2000), as indicated at the community level (Prober *et al.*, 2015). In addition, fungal alpha diversity is expected to increase along broad gradients of plant productivity based on environmental energy theory (Whittaker, 2006), which suggests that more abundant resources would facilitate coexistence of more fungal species.

In a global-scale study, Tedersoo *et al.* (2014) found only a weak, indirect relationship between soil fungal richness and plant taxonomic diversity, and no relation to productivity, whereas a weak correlation between plant productivity and fungal diversity was identified in global drylands (Delgado-Baquerizo *et al.*, 2016). It is plausible that on a global scale the fungal community

is strongly affected by climatic and edaphic predictors, and also by the historical influences of regional evolution and extinction, which could disguise the relationships of plant productivity or plant taxonomic diversity with fungal diversity. Thus, very large-scale (global or continental scale) studies may not be well suited to addressing subtle links among soil fungal diversity, plant diversity and plant productivity. Also, interactions and coevolution among groups of organisms are considered to occur mainly at local to regional scales (Gilbert & Webb, 2007; Kembel *et al.*, 2014; Toju *et al.*, 2014; Peay *et al.*, 2016), and thus the plant diversity effect may rather be expressed at these scales, rather than global and continental scales. For example, Peay *et al.* (2013) found that both tree and fungal diversities were low in poor sandy soils and relatively high in rich clayey soils in the western Amazon, whereas Tedersoo *et al.* (2016) reported greater fungal richness along with increasing tree species richness in an Estonian old forest site.

In this study, we aimed to disentangle the determinants of soil fungal diversity on a regional scale by intensive sampling along strong climatic, edaphic and floristic gradients in grasslands of the Tibetan Plateau (TP). We postulated the following working hypotheses. (1) Fungal alpha and beta diversities are strongly related to plant alpha and beta diversities, respectively, when accounting for confounding environmental variables (Hooper *et al.*, 2000; Wardle *et al.*, 2004; Gilbert & Webb, 2007). Here, fungal alpha diversity is defined as the number of observed operational taxonomic units (OTUs) at each site (i.e. fungal richness), while fungal beta diversity is defined as compositional dissimilarity between sites. (2) Fungal alpha diversity is positively related to plant productivity based on resource abundance and environmental energy theories (Whittaker, 2006). (3) Composition (rather than diversity) of fungi and their functional guilds is determined more by climatic and edaphic predictors than by floristic variables (such as plant diversity indices and productivity), because the effects of particular plant species are probably too weak in grassland plants as opposed to trees (Tedersoo *et al.*, 2016).

Materials and Methods

Soil sampling

We sampled soil at 60 grassland sites, scattered across a gradient stretching 815 km north to south and 960 km east to west, in the northeastern and central TP, situated in both Qinghai Province and the Tibetan Autonomous Region of China (Supporting Information Fig. S1). We sampled during the peak vegetation growing season (July–August) in 2011 to target maximum microbial activity and biomass. The survey area included a substantial range of vegetation types (alpine meadow, alpine steppe and desert steppe), climatic conditions (mean annual temperature (MAT) −5.2 to 4.7°C; mean annual precipitation (MAP) 66–560 mm) and soil properties (Table S1).

Across the whole transect, sixty 100 × 100 m sites were established to represent local vegetation types, avoiding sites with visible disturbance by grazing and anthropogenic activities (Shi *et al.*, 2012). Within each of the sites, three small plots – each 1 × 1 m in size – were randomly placed on the diagonals of the 1-ha site at least 40 m apart. Within each plot, seven randomly located soil cores with a diameter of 5 cm were collected. Plant litter was removed and 5 cm of topsoil was bulked and homogenized in the field by gently kneading the bag. The soil mainly comprised organic soil, or top mineral soil in some arid areas. The pretreatment of soil is described in Methods S1.

Vegetation survey

All vascular plant species and individuals were recorded at each of the 180 plots and summarized at the site level (Table S2). Most plant species were classified as forbs (44.1%), sedges (29.1%) and grasses (21.6%), with a low proportion of legumes (5.0%). Plant species were assigned mycorrhizal status based on the checklist of Wang & Qiu (2006). We used the normalized difference vegetation index (NDVI) as a metric for plant productivity. NDVI data were collected from the moderate resolution imaging spectroradiometer (MODIS) aboard NASA's Terra satellites (https://ladsweb.nascom.nasa.gov/data/search.html), which were updated once every 16 d with 250 m resolution. Specifically, we chose the average NDVI during our sampling dates (also the plant peak growing season on the TP) as a proxy for plant productivity at the site level (Paruelo *et al.*, 1997; Pettorelli *et al.*, 2005). This methodology was previously adopted in the studies by Delgado-Baquerizo *et al.* (2016) and Piao *et al.* (2014). In addition, a strong positive linear correlation between NDVI and aboveground biomass at site level was reported across the Tibetan grasslands (Yang *et al.*, 2008). Compilation of climatic metadata is described in Method S2.

Measurement of soil properties

All soil parameters were measured at the individual plot level following Jing *et al.* (2015), and averaged at the site level. Briefly, soil pH was measured using a pH meter (Thermo Orion-868; Thermo Orion Co., Waltham, MA, USA) after shaking a soil water (1 : 5 w/v) suspension. Soil moisture (SM) was measured gravimetrically. Total carbon (TC) and total nitrogen (TN) were determined with a carbon–hydrogen–nitrogen (CHN) elemental analyzer (2400 II CHN elemental analyzer; PerkinElmer, Boston, MA, USA). Soil total phosphorus (STP) was determined by the molybdenum blue method with an ultraviolet–visible spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan). Soil inorganic carbon (SIC) was calculated based on the content of soil $CaCO_3$, which was analyzed using a calcimeter (Eijkelkamp, Giesbeek, the Netherlands). Soil organic carbon (SOC) was calculated as the difference between TC and SIC. Dissolved total nitrogen (DTN; the sum of ammonium, nitrate and dissolved organic nitrogen) and dissolved organic carbon (DOC) were analyzed using a total organic carbon and total nitrogen analyzer (Shimadzu).

Sequencing and bioinformatics

Total soil DNA from each plot was extracted, stored and amplified by targeting the fungal internal transcribed spacer 2 (ITS2)

rDNA region using the primers ITS3 (5′-GCATCGATGAAGA ACGCAGC)/ITS4 (5′-TCCTCCGCTTATTGATATGC) (White *et al.*, 1990) equipped with unique identifier tags. The amplicons were sequenced using the Illumina MiSeq platform PE250 (Illumina Inc., San Diego, CA, USA). More details of the sequencing procedure are provided in Methods S3.

Starting with the original paired-end reads on the Illumina sequencer, we first merged them using FLASH (Magoc & Salzberg, 2011). QIIME v.1.9.0 (Caporaso *et al.*, 2010) and CUTADAPT v.1.9.1 (https://doi.org/10.14806/ej.17.1.200) were used for quality filtering, trimming and chimera checking. These procedures resulted in 11 851 579 high-quality reads after quality filtering (parameters: minlength = 280; maxambigs = 0, and phred quality threshold = 30). Then ITSX 1.0.11 (http://microbiology.se/software/itsx/) was used to remove the flanking large ribosomal subunit (LSU) and 5.8S genes according to the manual (Bengtsson-Palme *et al.*, 2013), and the putative chimeric sequences were removed using a combination of *de novo* and reference-based chimera checking, with the flag *–non_chimeras_rentention=union* (Edgar *et al.*, 2011). The remaining sequences were then clustered into operational taxonomic units (OTUs) at 97% similarity threshold using the USEARCH algorithm (Edgar, 2010). All singletons (clusters of size 1) were removed during the USEARCH clustering process, with the flag *–g 2*, because some singletons represent artifacts or contaminants, and would have inflated alpha diversity erroneously (Kunin *et al.*, 2010; Tedersoo *et al.*, 2010). Taxonomy was assigned to fungal OTUs using the rdp option in *parallel_assign_taxonomy_rdp.py* with minimum confidence of 0.8 (Wang *et al.*, 2007). The UNITE v.7 (http://unite.ut.ee) release for QIIME served as a reference database (Koljalg *et al.*, 2013). Altogether, 211 OTUs (comprising 128 374 sequences) not assigned to fungi were removed before subsequent analysis. The final data set included 11 576 489 fungal sequences covering 14 207 OTUs in 60 sites (minimum 123 753; maximum 341 014; mean 192 941 sequences per site). In order to analyze the alpha and beta diversities of soil fungi at the same sequencing depth, the data set was subsampled to 123 753 reads per site.

The sequence data associated with this study were submitted to the European Nucleotide Archive under the accession number PRJEB16010.

Statistics

All the analyses were performed at the site level ($n = 60$). Differences in climatic factors, soil properties and NDVI among different vegetation types were tested using Games–Howell tests. Pearson correlation analysis was used to detect multicollinearity among the 13 environmental variables (Table S3) and to recover raw trends in the relationships among fungal richness, plant richness and productivity (Table S4).

We calculated fungal alpha and beta diversities at the same sequencing depth (123 753 reads per site; Rarefied Fungal OTU-Table). The observed fungal OTU numbers and plant species richness were selected to represent fungal and plant alpha diversities, respectively. Bray–Curtis dissimilarity between each sample pair was used as a representation of fungal and plant beta diversities as calculated in the R package VEGAN 2.3-3 (Oksanen *et al.*, 2016). The community dissimilarity matrices of plants and soil fungi were linearized using PASSAGE2 (www.passagesoftware.net). To test our first hypothesis, Pearson correlation analysis and Mantel tests were used to examine the correlation of alpha and beta diversities between plants and fungi. After identifying strong individual environmental drivers of plant and fungal diversity (see later), we used partial Pearson correlation analysis and partial Mantel tests to further investigate this relationship after controlling for significant and shared environmental drivers and geographic distance (Yang *et al.*, 2016). All data were tested for normality and homogeneity of variance by Kolmogorov–Smirnov tests and Levene's tests, respectively, as implemented in SPSS STATISTICS 20.0 for windows (IBM-SPSS, Chicago, IL, USA). When necessary, data were logarithm-transformed before the analysis (see Table S5 for details).

To test the first and second hypotheses, we also used partial least squares regression (PLSR) to identify the richness−richness and productivity−richness relationships after accounting for the other environmental drivers, such as climatic and edaphic variables. Before setting up PLSR models, all environmental variables, productivity and alpha diversity indices were standardized (average = 0 and SD = 1). The 'optimal' number of components was chosen based on the so-called one-sigma heuristic (Hastie *et al.*, 2008), which was implemented by the function *selectNcomp* in the R package PLS 2.6-0. Then we used the function *plsr* to perform PLSR analysis and extracted the residuals, as implemented in the R package PLS 2.6-0. In addition, we carried out the same implementation (i.e. PLSR) to test the relationships between the richness of fungal functional guilds and plant richness/productivity, respectively.

As an alternative method of PLSR, the best ordinary least squares (OLS) multiple regression models of variation of fungal richness, plant richness and productivity were selected, respectively. All environmental variables, productivity and alpha diversity indices were standardized (average = 0 and SD = 1) before the OLS multiple regression analysis. Akaike's information criterion (AIC) was used to identify the best OLS model, as implemented in the R package MASS 7.3-45. The variance inflation factor (VIF) was calculated for OLS multiple regression models using the R package CAR 2.1-2. We used the criterion VIF < 3 to select suitable variables in the best multiple regression models to remove strongly multicollinear variables. Variation in the richness within fungal functional guilds was also analyzed using OLS multiple regression. In addition, we separately performed a variation partitioning analysis (VPA) for fungal richness using four categories, that is, plant richness, productivity, and edaphic and climatic variables, which enables us to understand the shared and independent contributions of these four categories (Tedersoo *et al.*, 2016).

To test the third hypothesis, distance-based linear model multivariate analysis (DISTLM) was used to determine the relative effects of spatial, climatic, edaphic and floristic variables on communities of soil fungi and functional guilds (McArdle & Anderson, 2001). Here, principal coordinates of neighbor matrices (PCNM) vectors with significant positive spatial autocorrelation

were regarded as proxies of spatial variables (Borcard *et al.*, 2011), and plant productivity, richness, plant NMDS1 and NMDS2 vectors were regarded as representations of floristic variables.

Fungal functional guilds were assigned according to Tedersoo *et al.* (2014) and Nguyen *et al.* (2016a). The functional guilds tested in this study included three major functional groups: pathogens, saprotrophs and symbionts. Among the last group, the variation of diversity and communities of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi were analyzed separately. For ECM (or AM) fungi, only sites with ECM (or AM) plants present were included.

Results

In total, 11 576 489 high-quality sequences at 60 sites were clustered into 14 207 fungal OTUs. Of the 14 207 fungal OTUs, 4936 OTUs were assigned to 10 fungal functional guilds, which accounted for 49.8% of total sequences (see Fig. S3 later). Soil fungal communities were strongly dominated by Ascomycota, which accounted for 90.9% of the sequences (Fig. S2). In terms of the functional composition of fungal communities, saprotrophs (30.5%) and plant pathogens (13.6%) were the dominant functional guilds (Fig. S3).

Diversity–diversity relationship

Fungal richness (alpha diversity) responded significantly to carbon : nitrogen (C : N) ratio, plant richness, STP, and DOC, which collectively explained 46.3% of variation in fungal richness in the best OLS multiple regression model (the highest R^2_{adj} and lowest AIC; Table 1). PLSR analysis also showed that increasing plant richness significantly enhanced soil fungal richness when accounting for the effects of plant productivity and edaphic and climatic variables (Fig. 1). Fungal beta diversity (compositional dissimilarity between sites) was most strongly correlated with plant beta diversity, MAP, C : N ratio and plant productivity (Fig. 2; Table S6). With increasing plant alpha and beta diversities, there was a corresponding increase in fungal alpha and beta diversities, respectively (Fig. 2). The logarithmic regression model showed a better fit for the relationship between plant and fungal alpha diversities compared with linear and quadratic models (Fig. S4).

Nevertheless, there were no shared environmental drivers of fungal and plant richness in the best OLS multiple regression models (Table 1), indicating that the richnesses of these two groups were constrained by different subsets of environmental predictors. Conversely, Mantel tests indicated that MAP, C : N ratio and productivity were the three strongest individual environmental drivers for both fungal and plant beta diversities (Table S6). After controlling for these shared environmental drivers and geographic distance, the strong beta diversity coupling of fungi and plants still persisted (Table S7).

Table 1 Summary of the best ordinary least squares (OLS) multiple regression models for the effects of environmental variables on fungal richness and plant richness

Variable	Estimate	SE	*t* value	*P*-value	VIF
Fungal richness: df = 55; R^2_{adj} = 0.463; SE_{resid} = 0.733; AIC = −32.5					
C : N ratio	−0.360	0.115	−3.140	0.003	1.446
Plant richness	0.237	0.110	2.158	0.035	1.326
STP	0.318	0.107	2.959	0.005	1.267
DOC	−0.279	0.098	−2.839	0.006	1.057
Plant richness: df = 57; R^2_{adj} = 0.435; SE_{resid} = 0.752; AIC = −31.3					
MAP	0.415	0.142	2.917	0.005	1.538
Productivity	0.309	0.142	2.169	0.034	1.247

AIC, Akaike's information criterion; VIF, variance inflation factor; MAP, mean annual precipitation; STP, soil total phosphorus; DOC, dissolved organic carbon; C : N, carbon : nitrogen. n = 60 sites.

Productivity–diversity relationship

The best OLS multiple regression model indicated that precipitation, SM and temperature were the best predictors of plant productivity, altogether explaining 68.7% of variation (Table S8). In addition, the best predictors in the multiple regression model for fungal richness (C : N ratio, DOC, plant richness and STP) also explained 65.9% of variation in plant productivity (adjusted R^2 in OLS multiple regression models). Adding productivity to the fungal richness model did not improve the model fit (not shown). PLSR analysis also corroborated that plant productivity did not directly affect soil fungal richness when accounting for the effects of plant richness and edaphic and climatic variables (Fig. 1). Further, the VPA showed that much of the plant productivity effects on fungal richness were shared with all other variable categories, such as plant richness and edaphic and climatic variables, and plant productivity did not have an exclusive effect on soil fungal richness (Fig. 1).

However, PLSR analysis showed that plant taxonomic richness significantly increased with increasing plant productivity, when accounting for the effects of soil fungal richness and edaphic and climatic variables (Fig. 1). The best OLS multiple regression model also demonstrated that plant productivity and precipitation were the best predictors of plant richness, altogether explaining 43.5% of variation (Table 1).

Community composition

The community composition of soil fungi was influenced by spatial, climatic, edaphic and floristic variables, as revealed by the best multivariate model (DISTLM; Table S9). Among 32 variables (15 PCNM vectors, 11 soil parameters, four floristic variables and two climatic factors), the 12 significant predictors taken together explained 43.0% of the variation in fungal composition. Specifically, spatial (PCNM1–PCNM6) and climatic factors (MAP) accumulatively explained 20.7% and 7.9% of the variation in fungal community, respectively, whereas edaphic (SM, pH and bulk density) and floristic factors (plant NMDS1 and NMDS2) explained 7.5% and 7.0% of the variation, respectively. For saprotrophs, spatial (PCNM1–PCNM7), climatic (MAP), edaphic (SM, pH and bulk density) and floristic factors

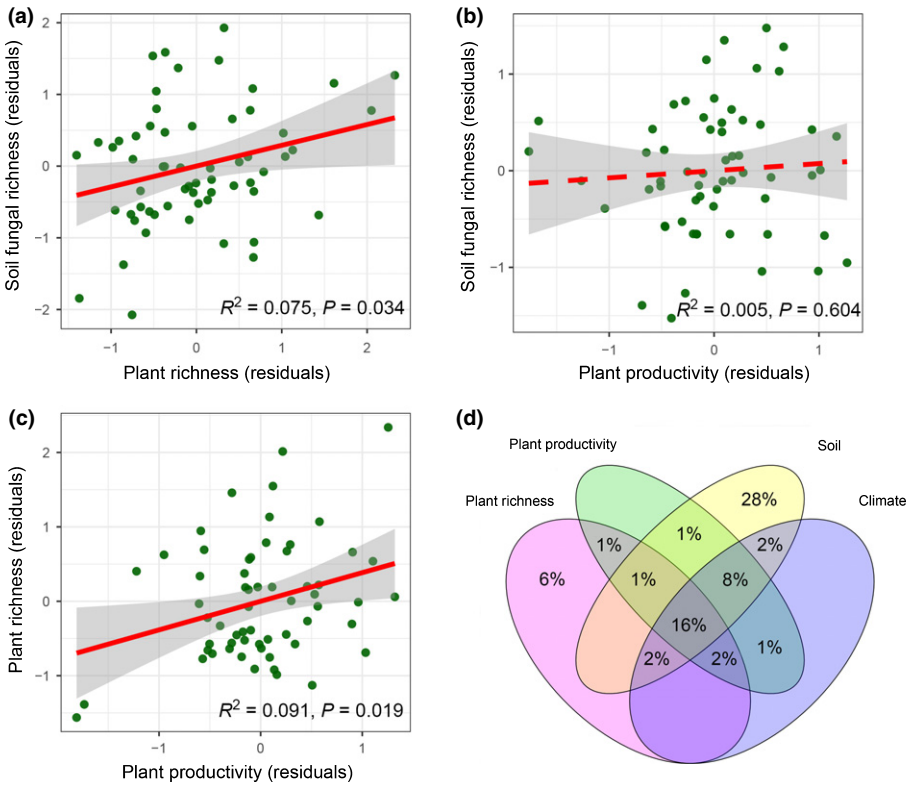

Fig. 1 The internal relationships among fungal richness, plant richness and productivity based on (a–c) partial least square regression and (d) variation partitioning analysis. (a) Relationship between soil fungal richness and plant richness when accounting for the effects of climatic factors, edaphic variables and plant productivity. (b) Relationship between soil fungal richness and plant productivity when accounting for the effects of climatic factors, edaphic variables and plant richness. (c) Relationship between plant richness and plant productivity when accounting for the effects of climatic factors, edaphic variables and soil fungal richness. (d) A Venn diagram of variation partitioning analysis, illustrating the shared and exclusive effects of plant richness, productivity, edaphic variables and climatic factors on soil fungal richness. Note that the fraction of unexplained variation and values < 1% are not shown for simplicity. The solid red lines indicate statistical significance for the relationships, while the dashed lines indicate no statistical significance for the relationships. The shaded areas show the 95% confidence interval of the fit.

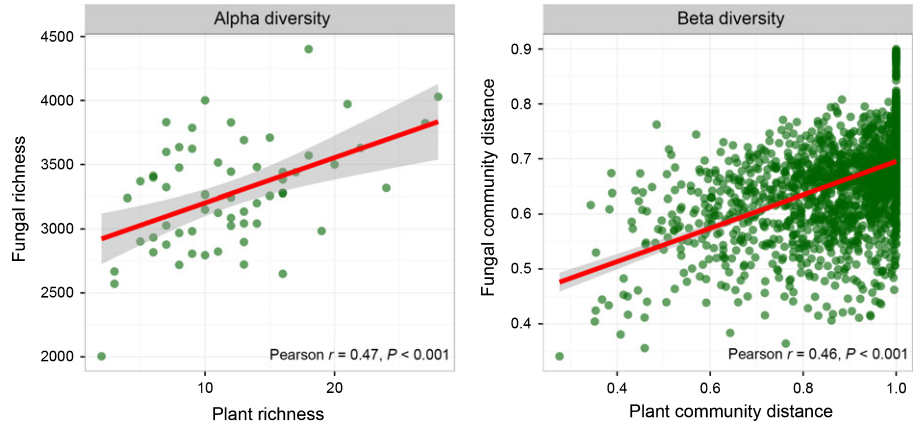

Fig. 2 Coupling of fungal and plant diversities across the Tibetan Plateau (TP) (n = 60 sites). Alpha diversity shows linear regression of soil fungal richness against plant species richness, and beta diversity shows linear regression of the pairwise Bray–Curtis distances for fungal and plant communities. The results of Pearson correlation analyses are shown, and for beta diversity coupling, Mantel tests also showed a significant positive correlation, with more similar plant communities having more similar fungal communities (Mantel r = 0.4563; P = 0.001). The shaded areas show the 95% confidence interval of the fit.

(plant NMDS1 and NMDS2) explained 22.1%, 7.2%, 8.3% and 7.5% of the variation in community composition, respectively. For pathogens, MAP alone explained 8.1% of the variation. Spatial factors (PCNM1–PCNM2 and PCNM4), edaphic variables (pH, SM and C : N ratio) and floristic factors (plant NMDS1, NMDS2 and productivity) explained 11.0%, 18.5% and 10.7% of the variation in pathogen composition, respectively. For all symbionts taken together, spatial factors

(PCNM1–PCNM3 and PCNM7) explained 24.8% of the variation in community composition, followed by climatic predictors (MAP), edaphic variables (SM) and floristic factors (productivity) which explained 7.0%, 2.9% and 2.3% of community variation, respectively. For AM fungi, spatial (PCNM1–PCNM3 and PCNM5) and climatic factors (MAP) explained 25.7% and 4.9% of the variation in community composition, respectively. For ECM fungi, spatial (PCNM1–PCNM3, PCNM9 and PCNM13) and climatic factors (MAT and MAP) explained 29.7% and 9.3% of community variation, respectively.

For taxonomic richness of each functional guild of fungi, different subsets of climatic, edaphic and floristic variables constituted the strongest predictors (Table 2). Specifically, the richness of saprotrophs was best explained by C : N ratio, DOC and STP, which accumulatively explained 30.7% of its variation. C : N ratio, DOC and STP accumulatively explained 42.3% of the variation in symbiont richness, while MAT, DOC and STP explained 34.0% of the variation in pathogen richness. The richness of AM fungi was best explained by AM plant coverage, SM, C : N ratio and STP, which accumulatively explained 52.1% of the variation. The richness of ECM fungi was best explained by SM, DOC and productivity, which cumulatively explained 48.8% of the variation. Consistently, PLSR models showed that there were no significant relationships between plant richness and the taxonomic richness of each functional guild of fungi, whereas with enhanced AM plant coverage and site productivity, AM and ECM fungal richnesses increased, respectively (Fig. S5).

Table 2 Summary of the best ordinary least squares (OLS) multiple regression models for the effects of environmental variables on the richness of functional guilds

Variable	Estimate	SE	*t* value	*P*-value	VIF
Saprotrophs: df = 56; R^2_{adj} = 0.307; SE_{resid} = 0.833; AIC = −18.1					
C : N ratio	−0.343	0.121	−2.846	0.006	1.238
DOC	−0.361	0.111	−3.239	0.002	1.054
STP	0.308	0.120	2.555	0.013	1.234
Symbionts: df = 56; R^2_{adj} = 0.423; SE_{resid} = 0.760; AIC = −29.1					
C : N ratio	−0.364	0.110	−3.304	0.002	1.238
DOC	−0.320	0.102	−3.150	0.003	1.054
STP	0.428	0.110	3.892	< 0.001	1.234
Pathogens: df = 56; R^2_{adj} = 0.340; SE_{resid} = 0.813; AIC = −21.1					
MAT	0.257	0.119	2.160	0.035	1.263
DOC	−0.386	0.109	−3.530	< 0.001	1.068
STP	0.343	0.121	2.839	0.006	1.301
AM fungi: df = 53; R^2_{adj} = 0.521; SE_{resid} = 0.692; AIC = −37.9					
AM plant coverage	0.347	0.105	3.322	0.002	1.301
SM (log_e)	−0.489	0.130	−3.775	< 0.001	1.999
C : N ratio	−0.371	0.107	−3.471	0.001	1.358
STP	0.537	0.117	4.594	< 0.001	1.626
ECM fungi: df = 34; R^2_{adj} = 0.488; SE_{resid} = 0.716; AIC = −21.7					
SM (log_e)	0.470	0.140	3.362	0.002	1.412
DOC	−0.471	0.126	−3.725	< 0.001	1.154
Productivity	0.385	0.132	2.907	0.006	1.226

AIC, Akaike's information criterion; ECM, ectomycorrhizal; VIF, variance inflation factor; C : N, carbon : nitrogen; DOC, dissolved organic carbon; STP, soil total phosphorus; MAT, mean annual temperature; AM, arbuscular mycorrhizal; SM, soil moisture; log_e, natural log transformation. n = 60 sites.

Discussion

Diversity begets diversity

The overall fungal alpha and beta diversities were positively related to plant alpha and beta diversities, respectively, when accounting for important environmental predictors (Tables S7, S10). In terms of alpha diversity, the best OLS multiple regression models accounted for 46.3% and 43.5% of the variation in fungal and plant richnesses, respectively, and plant richness was included as one of the strongest predictors of fungal richness in the best OLS model. The corresponding PLSR model also corroborated that plant richness was significantly positively related to soil fungal richness when accounting for the effects of plant productivity and edaphic and climatic variables (Fig. 1). The observed diversity–diversity relationship was stronger and more consistent than those observed on a global scale (Tedersoo *et al.*, 2014; Prober *et al.*, 2015) or local scale (Shen *et al.*, 2014; Barberan *et al.*, 2015). Hiiesalu *et al.* (2014) also found a strong positive relationship between AM fungal richness and plant richness, when accounting for the effects of environmental variables. Site-level plant richness varied from two to 28 species in our study, which was a more pronounced gradient than in previous studies, potentially contributing to the greater plant diversity effect.

In their synthesis, Hooper *et al.* (2000) found that a positive correlation between plant and microbial diversities may occur when both taxa respond similarly to the same environmental driving factors. Later, this mechanism was corroborated by a series of empirical studies (Landis *et al.*, 2004; Barberan *et al.*, 2015; Wang *et al.*, 2016). In our study, the simple Pearson correlation coefficient between fungal richness and plant richness was 0.47 (Table S4), but after controlling for the significant and shared environmental variables, the partial Pearson correlation coefficient decreased sharply to 0.286 (Table S10). Accordingly, the richness−richness R^2 was 0.208 in the simple linear regression model (Table S5), but decreased to 0.075 in the PLSR model (Fig. 1), which indicated that the plant richness–fungal richness coupling was partly due to their similar responses to the shared environmental drivers.

The VPA clearly showed that plant richness had an exclusive effect on fungal richness after accounting for confounding soil, climate and productivity effects, which was also corroborated by the PLSR and OLS multiple regression models (Table 1; Fig. 1). In addition, in terms of the associations of fungal beta diversity with other environmental variables, the strongest correlation existed between fungal beta diversity and plant beta diversity (the highest Mantel r value), and the positive relationship between fungal and plant beta diversities was still significant after controlling for the shared environmental drivers and geographic distance (Table S7). This provides strong support for the resource diversity and niche differentiation hypothesis which is applicable to saprotrophic, pathogenic and mutualistic fungi (Wardle *et al.*, 2004; Lewis, 2010; Peay *et al.*, 2013; Nguyen *et al.*, 2016b). In contrast to the overall fungal richness, diversity of saprotrophs, mycorrhizal symbionts and pathogens was not driven by plant richness. The corresponding host plant cover and plant

productivity (instead of plant richness) were among the strongest predictors of the richness of AM and EcM fungi, respectively, in the best OLS multiple regression models and PLSR models (Table 2; Fig. S5). These results point to the importance of resource quantity in determining the richness of mycorrhizal fungi.

The strong effect of plant richness on total fungal richness *per se* is of great ecological importance, highlighting that even small individuals of grassland plant species may generate complementary belowground niches by providing differential qualities of root environment, exudates, and root and leaf litter (Waring *et al.*, 2015), which may support greater diversity of various guilds of biotrophic and saprotrophic fungi. In addition, genetic compatibility between fungi and host plants is another potential mechanism for diversity effects in biotrophic fungi (Gilbert & Webb, 2007; Saikkonen *et al.*, 2010). Although this relationship is cumulative rather than linear (Fig. S4), it demonstrates that aboveground plant diversity may represent a good proxy for conservation planning with respect to belowground organisms.

Of multiple tested variables, there was a lack of shared predictors of fungal and plant richnesses in the best OLS multiple regression models (Table 1), which suggested that the alpha diversity of plants and soil fungi is constrained by the different subsets of environmental predictors. MAP was the strongest predictor of plant richness, individually explaining 39.8% of the variation in this study. Previous studies also determined water availability to be the strongest predictor for plant richness across the TP (Ma *et al.*, 2010; Yan *et al.*, 2013; Wu *et al.*, 2014), and on a global scale (Kreft & Jetz, 2007). Soil C : N ratio was the strongest predictor of fungal richness, individually explaining 29.2% of the variation. Recently, Newsham *et al.* (2016) also found that soil C : N ratio was one of the strongest predictors of fungal Chao1 richness in the maritime Antarctic.

Productivity–diversity relationship

Species number often increases with an increase in total available energy, which is the so-called species-energy theory (Whittaker, 2006). Although the species-energy theory is widely accepted for plants and animals (Hawkins *et al.*, 2003; Phillips *et al.*, 2010), a few empirical studies have demonstrated its applicability in fungal ecology (Schmit, 2005; Yang *et al.*, 2016). In our study, soil fungal richness was significantly positively related to plant productivity in the simple linear regression models (Table S5); however, we did not find a significant relationship between fungal richness and plant productivity in the PLSR and OLS multiple regression models (Table 1; Fig. 1). Furthermore, while the VPA showed that plant productivity explained *c.* 30% of variation of soil fungal richness, all the effects were shared with other variable categories, such as plant richness, soil and climate. These results indicated that there is a lack of direct correlation between soil fungal richness and plant productivity *per se*, and that productivity and other environmental drivers' effects on fungal richness may confound each other and have a synergistic effect. For example, Maestre *et al.* (2015) found that plant coverage indirectly affected soil fungal diversity by enhancing soil organic carbon in global drylands. It is possible that plant productivity may affect soil fungal richness indirectly through the modification of the soil C : N ratio, which was one of the strongest predictors of fungal richness (Table 1). Related to productivity, soil C : N ratio represents a proxy for nutrient availability (Cleveland & Liptzin, 2007) that may constrain build-up of fungal biomass and the activity of exoenzymes (Prevost-Boure *et al.*, 2011; Drake *et al.*, 2013; Grosso *et al.*, 2016). Soil C : N ratio varied by an order of magnitude in our study, and increasing C : N ratio had a strong negative effect on the richness of fungi and all functional groups, except pathogens and ECM fungi (Tables 1, 2).

It is also suggested that plant richness and productivity effects on fungal richness may confound each other, given that *c.* 20% of fungal richness variation was attributed to the combined effects of plant richness and productivity (Fig. 1). With enhanced plant productivity, plant richness significantly increased in the PLSR and OLS multiple regression models, suggesting the direct effect of productivity on plant richness *per se*. Similarly, in light of the results of their critical meta-analyses, Gillman & Wright (2006) proposed that almost all the productivity–plant species richness relationships were positive at the regional scale. Taken together, our study also indicated that the productivity–diversity relationship is inconsistent for plants and soil fungi in the same region, although they are strongly correlated with respect to diversity.

Likewise, productivity strongly affected the richness of ECM fungi in the PLSR and OLS multiple regression models (Table 2; Fig. S5), which could be attributed to the increasing relative abundance of ECM *Kobresia* spp. in more productive sites. AM plant coverage, an alternative proxy for productivity, was the strongest predictor of AM fungal richness (Fig. S5), suggesting that both mycorrhizal guilds are predominantly affected by host plant growth in grasslands of the TP. This also confirmed that plant correlates with soil fungal diversity were to a large extent guild-specific (Peay *et al.*, 2013; Nguyen *et al.*, 2016b; Tedersoo *et al.*, 2016).

Community composition

Surveys of fungal and plant biodiversity have seldom addressed the community composition of plants, fungi and specific functional guilds at the same time. In agreement with Tedersoo *et al.* (2016), we found that the community composition of the various different guilds of soil fungi was driven by various spatial, climatic, edaphic and floristic variables that differed in relative importance among functional groups. The community composition of plants was partly driven by similar environmental predictors, such as spatial (PCNM1–PCNM4), climatic (MAP) and edaphic factors (SM and bulk density), which also strongly affected the community composition of soil fungi and their functional guilds (Tables S9, S11). Since nearly all of the significant predictors we found here have been shown to be biologically important in some studies, the combination of deep sequencing and 60 sampling units provides ample material for a powerful multivariate analysis that is able to detect responses as weak as 1.7% of explained variation in fungal community composition.

Consistent with the third hypothesis, the composition of the total fungal community was more strongly determined by spatial, climatic and edaphic predictors, which accumulatively explained 36.1% of the variation, compared with 7.0% of the variation explained by floristic variables. Although individual tree species effects are stronger in forest ecosystems, Tedersoo *et al.* (2016) found that the composition of the forest soil fungal community was more strongly driven by spatial and edaphic variables than by floristic variables. Here, MAP was the strongest individual driver of the community composition of total fungi and soil saprotrophs, which is consistent with previous studies on the TP (Zhang *et al.*, 2016) and elsewhere (Bahram *et al.*, 2012; Shi *et al.*, 2014; Timling *et al.*, 2014).

Conclusions

In contrast to previous findings at the global scale (Tedersoo *et al.*, 2014; Prober *et al.*, 2015), in this regional-scale study there were strong and consistent associations between plants and soil fungi with respect to alpha and beta diversity, after accounting for a suite of environmental predictors and plant productivity effects. This demonstrates the potential importance of such coupling in maintaining biological diversity – even though the directions of cause and effect between plant and fungal diversities were not discernible. Additionally, aboveground plant diversity may represent a good proxy of soil fungal resources for use in regional conservation planning. Plant richness was significantly positively related to plant productivity, whereas fungal richness did not vary in relation to productivity, after accounting for the effects of plant richness and edaphic and climatic variables. This suggests that the productivity–diversity relationship is complex and shows different patterns for aboveground plants and belowground organisms. Our results also highlight the importance of recording soil and floristic variables, as well as the choice of suitable statistical methods. Further local- to regional-scale studies in other ecosystems and higher productivity habitats are required to determine whether the findings of this study are more generally applicable to natural ecosystems.

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Author contributions

H.C. and J-S.H. designed the research. T.Y., Y.S., X.J. and L.C. performed experiments and conducted fieldwork. T.Y., Y.S. and H.C. analyzed data. T.Y., J.M.A., L.T., X.J., Y.S., J-S.H. and H.C. wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Locations of sampling sites across the TP.

Fig. S2 Taxonomic composition of fungal communities recovered from alpine grassland soil on the TP.

Fig. S3 Functional composition of fungal communities recovered from alpine grassland soil on the TP.

Fig. S4 Coupling of fungal and plant diversity across the TP ($n = 60$ sites).

Fig. S5 Partial correlation between the richness of AM fungi and AM plant coverage and partial correlation between the richness of ECM fungi and plant productivity.

Table S1 Description of the geographic, climatic, soil and plant variables among different vegetation types

Table S2 A list of plant species inventoried across the 60 studied vegetation sites on the TP

Table S3 The Pearson's correlation within the 13 environmental variables ($n = 60$ sites)

Table S4 The Pearson's correlation among fungal richness, plant richness and productivity ($n = 60$ sites)

Table S5 Summary of linear regression models for the effects of individual environmental variables on fungal richness, plant richness and productivity

Table S6 The Spearman's product-moment correlation (r) between fungal/plant community structure (Bray–Curtis distances) and environmental variables determined by Mantel tests

Table S7 Partial Mantel tests on the correlation between fungal and plant community turnover when controlling for the shared environmental drivers and geographic distances

Table S8 Summary of the best ordinary least squares (OLS) multiple regression model for the effects of climatic and edaphic variables on plant productivity

Table S9 Best multivariate models (DISTLM) for community composition of soil fungi and representative functional guilds

Table S10 Partial Pearson's correlation analyses between fungal and plant richness when controlling for the shared environmental drivers

Table S11 Best multivariate model (DISTLM) for community composition of aboveground plants

Method S1 Pretreatment of soil samples.

Method S2 Compilation of climatic metadata.

Method S3 Sequencing procedure.

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