Plant Diversity Enhances Soil Fungal Diversity and Microbial Resistance to Plant Invasion

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ABSTRACT Interactions and feedbacks between aboveground and belowground biomes are fundamental in controlling ecosystem functions and stability. However, the relationship between plant diversity and soil microbial diversity is elusive. Moreover, it remains unknown whether plant diversity loss will cause the stability of soil microbial communities to deteriorate. To shed light on these questions, we conducted a pot-based experiment to manipulate the plant richness gradient (1, 2, 4, or 8 species) and plant [*Symphyotrichum subulatum* (Michx.) G. L. Nesom] invasion status. We found that, in the noninvasion treatment, soil fungal diversity significantly and positively correlated with plant diversity, while the relationship between bacterial and plant diversity was not significant. Under plant invasion conditions, the coupling of plant-fungal alpha diversity relationship was enhanced, but the plant-fungal beta diversity relationship was decoupled. We also found significant positive relationships between plant diversity and soil microbial resistance. The observed positive relationships were determined by turnover (species substitution) and nestedness (species loss) processes for bacterial and fungal communities, respectively. Our study demonstrated that plant diversity enhanced soil fungal diversity and microbial resistance in response to plant invasion. This study expands our knowledge about the aboveground-belowground diversity relationship and the diversity-stability relationship.

IMPORTANCE Our study newly showed that plant invasion significantly altered relationships between aboveground and belowground diversity. Specifically, plant richness indirectly promoted soil fungal richness through the increase of soil total carbon (TC) without plant invasion, while plant richness had a direct positive effect on soil fungal richness under plant invasion conditions. Our study highlights the effect of plant diversity on soil fungal diversity, especially under plant invasion conditions, and the plant diversity effect on microbial resistance in response to plant invasion. These novel findings add important knowledge about the aboveground-belowground diversity relationship and the diversity-stability relationship.

KEYWORDS plant diversity, plant invasion, resistance, soil microbial diversity

Interactions and feedbacks between aboveground and belowground biomes play fundamental roles in controlling ecosystem functions and stability (1). There is a growing consensus that plants and soil microorganisms are closely linked through resources (root exudates and litter), physical microhabitats, nutrient dynamics, and other environmental conditions (2). The previous theoretical framework, known as the plant diversity hypothesis, assumed that greater plant diversity increases the range of organic substrates entering soil, thus creating niche space to promote the diversity of

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heterotrophic microorganisms (1, 3, 4). However, the evidence for correlation between plant diversity and soil microbial diversity, either from observations in natural ecosystems or from plant richness manipulation experiments, is limited (2, 4).

Relationships between plant diversity and soil microbial diversity are mixed. While some studies found positive or no effects of plant richness on soil microbial richness (5, 6), others observed that plant diversity predicted beta but not alpha diversity of soil microbes (7, 8). These inconsistent results may be partly attributed to the fact that relationships can differ at different spatial or temporal scales (9). For example, significant plant-fungal diversity relationships tend to occur at local and regional scales (10, 11). Yet, the plant-fungal diversity patterns fall apart at continental and global scales (6, 7). Although the relationship between plant richness and soil microbial richness was significant in an early stage of an alpine grassland, such coupling might gradually disappear with the succession of ecosystems (12). Also, the strength of relationships is probably different across taxonomic groups (e.g., bacteria and fungi), since microorganisms are distinct in their metabolic needs and symbiotic relationships with plants (13, 14). Few studies have concurrently tested the effect of plant richness on soil bacterial and fungal richness (but see references 12–15), with most observational studies focusing on soil fungal groups, especially for mycorrhizal fungi, which are more dependent on direct symbiotic relationships with plants (5, 10).

The relationship between plant diversity and ecosystem stability has long been a central topic in ecology (16). The stability can be classified generally into two categories—one is temporal stability, which is based on a system's response to time, and another is resistance and resilience stability, which is based on a system's response to disturbance (16). The diversity-stability relationship was first hypothesized by MacArthur (17), who predicted that increasing species richness should increase community stability. This hypothesis was then tested by a long-term field experiment in which plant richness was manipulated; the results showed that plant aboveground biomass in the more diverse treatment was less changed across years (18). After that, extensive experimental studies repeatedly found that plant species richness positively correlated with the temporal stability of plant community productivity (19–21). Despite great interest in exploring the relationship between plant diversity and the stability of plant community productivity, it remains largely unknown whether plant diversity loss will also cause the stability of belowground communities to deteriorate.

Plant invasion, as a major problem of global change, has the potential to alter native plant and soil community structure and affect ecosystem stability (16, 22). Prior work in plant invasion manipulation experiments mainly had a focus on the plant diversity effects on exotic plants. These studies found that both the biomass and the number of invasive plant species decreased significantly with the increasing richness of native plant communities (23–25), which confirmed Elton's hypothesis that more diverse communities should be more resistant to invasion (26). However, we know little about how the native belowground communities respond to plant invasion, whether the plant-soil microbial diversity relationship shifts under this disturbance, and whether plant diversity affects soil microbial community resistance stability to plant invasion, although soil microbes (e.g., mutualists and pathogens) are important actors in the process of plant invasion (27–29). This knowledge is important for understanding the aboveground diversity-belowground diversity and diversity-stability relationships and is necessary for advancing ecosystem ecology in a changing world (16).

In this study, we conducted a pot-based experiment to manipulate the plant richness gradient (1, 2, 4, or 8 species) for 2 years and plant [*Symphytotrichum subulatum* (Michx.) G. L. Nesom] invasion status for 1 year (Fig. 1). Our aims were to (i) test the plant-soil microbial diversity relationship and their potential influence by plant invasion; (ii) test the plant diversity-soil microbial resistance stability relationship; and (iii) identify the key factors influencing microbial diversity and uncover the ecological mechanisms underlying these relationships. We hypothesized that (i) relationships between plant and soil microbial diversity are significant and positive, but the

FIG 1 Experimental setup diagram. The grass shape represents native plant species. Different colors are used to differentiate the four plant richness levels (1, 2, 4, or 8 species). The orange triangles represent the invasive species *Symphyotrichum subulatum* (Michx.) G. L. Nesom.

correlation is stronger for fungi than for bacteria; (ii) plant invasion significantly alters plant diversity-soil microbial diversity relationships; and (iii) plant diversity has significant and positive effects on soil microbial resistance in response to plant invasion.

RESULTS

Bacterial communities. Across all soil samples in the noninvasion ($n=41$) and invasion ($n=41$) treatments, we obtained 3,109,314 quality sequences (minimum, 20,589; maximum, 60,324; mean, 37,918), which we assigned to 7,697 bacterial operational taxonomic units (OTUs). Based on the relative abundance of sequence reads, *Proteobacteria* (accounting for about 35%) was the most dominant taxonomic group of bacteria, followed by *Actinobacteria*, *Chloroflexi*, and *Acidobacteria* (see Table S2 and Fig. S1 in the supplemental material).

For the alpha diversity of the whole community, we found no correlation between plant richness and bacterial richness in the noninvasion and invasion treatments (Fig. 2; see also Table S3 in the supplemental material). When considering the alpha diversity of specific groups within bacterial communities, there was no significant relationship between plant richness and the richness of specific taxonomic groups in the noninvasion treatment, while the richness of *Acidobacteria* and *Alphaproteobacteria* taxa showed significant relationships with plant richness in the invasion treatment (see Tables S5 and S6 in the supplemental material). For beta diversity, there was also no significant relationship between bacterial beta diversity and plant beta diversity (Fig. 3).

The best ordinary least-squares (OLS) multiple regression models and Pearson correlation analyses indicated that the C/P ratio was the most important factor affecting bacterial richness in the noninvasion treatment, whereas in the invasion treatment, glomalin was the best predictor for bacterial richness (Table 1 and Table S3). There was no significant difference for bacterial community composition among plant richness levels in the noninvasion and invasion treatment, which were tested by canonical correspondence analysis (CCA) and permutational multivariate analysis of variance (PERMANOVA) (see Fig. S4 and Table S11 in the supplemental material). The distance-based multivariate analysis for a linear model (DistLM) indicated that total phosphorus (TP) was the major contributor to the variation of bacterial community composition in the noninvasion treatment (Table 2). Specifically, TP correlated significantly ($P < 0.05$) with the relative abundances of OTUs belonging to the bacterial phyla *Acidobacteria*,

FIG 2 Relationships of soil bacterial and fungal richness with plant species richness in the noninvasion and invasion treatments, respectively. The general linear model was selected. The dotted and solid lines indicate nonsignificant and significant relationships, respectively.

Planctomycetes, *Cyanobacteria*, and *Armatimonadetes* (Fig. 4; see also Table S12 in the supplemental material). In the invasion treatment, however, plant aboveground biomass was the most important factor affecting bacterial community composition (Table 2). Specifically, plant aboveground biomass correlated significantly ($P < 0.05$) with

FIG 3 Correlations of soil bacterial and fungal community dissimilarity with plant community dissimilarity in the noninvasion and invasion treatments, respectively. The dotted and solid lines indicate nonsignificant and significant correlations, respectively.

TABLE 1 Best ordinary least-squares multiple regression models for the effects of plant and edaphic properties on the richness of bacterial and fungal communities^f

Variable ^e	Estimate	SE	t	P	VIF
Noninvasion					
Bacterial richness ^a					
C/P	−0.494	0.161	−3.066	0.004	1.001
Nitrate reductase	−0.279	0.143	−1.997	0.053	1.001
Fungal richness ^b					
TC	0.331	0.144	2.288	0.027	1.104
Plant richness	0.324	0.144	2.241	0.031	1.104
Invasion					
Bacterial richness ^c					
Glomalin	−0.558	0.209	−2.668	0.011	2.167
Dehydrogenase	−0.300	0.154	−1.944	0.059	1.177
Plant richness	0.309	0.164	1.877	0.068	1.339
TP	0.286	0.206	1.387	0.174	2.109
Nitrate reductase	−0.218	0.161	−1.350	0.185	1.288
Fungal richness ^d					
Plant richness	0.702	0.120	5.840	<0.001	1.071
Plant root biomass	0.441	0.162	2.716	0.009	1.951
Plant aboveground biomass	−0.309	0.164	−1.890	0.066	1.986

^adf = 37; $R^2_{adj} = 0.277$; SE_{resid} (residual standard error [SE]) = 0.883; Akaike's information criterion (AIC) = −6.36.

^bdf = 38; $R^2_{adj} = 0.281$; $SE_{resid} = 0.870$; AIC = −8.5.

^cdf = 35; $R^2_{adj} = 0.292$; $SE_{resid} = 0.899$; AIC = −3.18.

^ddf = 37; $R^2_{adj} = 0.499$; $SE_{resid} = 0.735$; AIC = −21.41.

^eTC, total carbon; TP, total phosphorus.

^fEstimate, estimated value of the coefficient; t, t statistic value; VIF, variance inflation factor criterion.

the relative abundances of OTUs belonging to the bacterial phyla *Deltaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Cyanobacteria*, *Armatimonadetes*, *Nitrospirae*, *Chlorobi*, and "*Candidatus* Latescibacteria" (Fig. 4; see also Table S13 in the supplemental material). Compared with noninvasion conditions, five bacterial phyla, including *Armatimonadetes*, *Cyanobacteria*, *Chloroflexi*, *Alphaproteobacteria*, and *Chlorobi*, had significantly lower abundances in response to plant invasion, while *Gammaproteobacteria* had significantly higher abundances in response to plant invasion. In total, 19 bacterial functional groups were found to be significantly altered by plant invasion, with most of them having significantly lower abundances in response to plant invasion, except for groups

TABLE 2 Results of distance-based linear model analysis^a

Variable ^b	SS ^c (trace)	Pseudo-F	P	Proportion
Bacterial community composition				
Noninvasion				
TP	2,909.944	1.3974	0.021	0.0346
Dehydrogenase	2,765.9906	1.3259	0.044	0.0329
Invasion				
Plant aboveground biomass	2,913.6559	1.3714	0.015	0.034
Plant root biomass	2,842.4061	1.3367	0.037	0.0331
Fungal community composition				
Noninvasion				
TC	4,263.145	1.479	0.011	0.0365
pH	4,142.0494	1.4354	0.03	0.0355
Invasion				
Plant aboveground biomass	5,278.1057	1.8941	0.004	0.0463
Plant root biomass	4,689.2893	1.6738	0.005	0.0412

^aDetermining the suite of environmental variables that describe significant and independent proportions of the variation in bacterial and fungal community composition.

^bOnly significant ($P < 0.05$) variables are listed in order of importance, and they are added to the model. TC, total carbon; TP, total phosphorus.

^cSS, sum of squares.

FIG 4 Relationships showing the relative abundance of bacterial phyla and fungal phyla/classes that correlated significantly (Spearman; $P < 0.05$) with TP and TC in the noninvasion treatment, respectively. Relationships showing the relative abundance of bacterial phyla and fungal phyla/classes that significantly (Spearman; $P < 0.05$) correlated with plant aboveground biomass in the invasion treatment. TC, total carbon; TP, total phosphorus; PAB, plant aboveground biomass.

involved in ureolysis, xylanolysis, and chitinolysis, as well as plant pathogens and intracellular parasites (see Fig. S5 in the supplemental material).

We found that bacterial community dissimilarities between the noninvasion and invasion treatments significantly decreased with increasing plant richness ($R^2 = 0.026$; $P < 0.001$). Furthermore, plant richness correlated significantly ($R^2 = 0.021$; $P = 0.002$) with the turnover component of bacterial community dissimilarities, but did not correlate significantly ($R^2 = 0.003$; $P = 0.256$) with the nestedness component (Fig. 5).

Fungal communities. A total of 7,153,468 quality sequences with 35,896–132,066 sequences per sample (mean 87,237) were assigned to 4,178 fungal OTUs (Table S2). Fungal sequences were dominated by *Ascomycota*, which accounted for more than 80% of the total sequences, followed by *Basidiomycota*, *Zygomycota*, *Glomeromycota*, and *Chytridiomycota* (Fig. S1).

Fungal richness was correlated significantly ($R^2 = 0.181$; $P = 0.005$) with plant richness in the noninvasion treatment, and this relationship was stronger ($R^2 = 0.4$; $P < 0.001$) in the invasion treatment (Fig. 2 and Table S3). When considering the alpha diversity of specific groups within fungal communities, the richness of the *Basidiomycota* showed significant relationships with plant richness in the invasion treatment (Table S6). Fungal beta diversity correlated significantly (Mantel test $r = 0.187$; $P = 0.005$) with plant beta diversity in the noninvasion treatment. However, the relationship between plant and fungal beta diversity was nonsignificant (Mantel test $r = 0.003$; $P = 0.161$) in the invasion treatment (Fig. 3).

The best OLS multiple regression models and Pearson correlation analyses indicated that TC was the most important factor affecting bacterial and fungal richness in the noninvasion treatment, respectively, whereas in the invasion treatment, plant richness was the best predictor for fungal richness (Table 1 and Table S3). Partial Pearson

FIG 5 Soil bacterial and fungal community dissimilarities between the noninvasion and invasion treatments significantly decreased with plant richness, indicating a significant and positive plant diversity-soil microbial resistance stability relationship. The turnover and nestedness components between the noninvasion and invasion treatments were related to plant richness. The dotted and solid lines indicate nonsignificant and significant correlations, respectively.

correlation analyses showed that the relationship between plant richness and fungal richness was nonsignificant when the shared edaphic properties in the noninvasion treatment were controlled, whereas the relationship was still significant when the shared edaphic properties in the invasion treatment were controlled (see Table S8 in the supplemental material). Path analysis of structural equation modeling (SEM) was further used to infer the direct and indirect effect of plant richness on fungal richness. We found that plant richness had a positive effect on soil TC (0.643; $P < 0.001$) and that soil TC explained 28.7% variation of fungal richness (0.536; $P < 0.001$) in the noninvasion treatment. However, in the invasion treatment, plant richness had no effect on soil TC (0.204; $P > 0.05$) but directly (0.633; $P < 0.001$) affected fungal richness, and plant richness explained 40% of the variation of fungal richness (Fig. 6).

There was no significant difference for fungal community composition among plant richness levels in the noninvasion and invasion treatment, which were tested by CCA and PERMANOVA (Fig. S4 and Table S11). The DistLM analysis indicated that TC was the major contributor to the variation of fungal community composition in the noninvasion treatment (Table 2). Specifically, TC correlated significantly ($P < 0.05$) with the relative abundances of OTUs belonging to the fungal phyla or classes Glomeromycota, Sordariomycetes, Leotiomyces, Glomeromycetes, and Ustilaginomycetes (Fig. 4; see also Table S14 in the supplemental material). In the invasion treatment, however, plant aboveground biomass was the most important factor affecting fungal community

FIG 6 Structural equation models showing the direct and indirect effects of plant species richness on soil fungal richness in the noninvasion and invasion treatments. Gray and black arrows indicate statistically nonsignificant and significant (***, $P < 0.001$) relationships, respectively. R^2 denotes the proportion of variance explained for endogenous variables. GFI, goodness-of-fit index; RMSEA, root mean square error of approximation.

composition (Table 2). Specifically, plant aboveground biomass correlated significantly ($P < 0.05$) with the relative abundances of OTUs belonging to the fungal phyla or classes of Zygomycota, Glomeromycota, Sordariomycetes, Leotiomyces, Ustilaginomycetes, Glomeromycetes, "Incertae_sedis_10," "Incertae_sedis_14," and "Unclassified" (Fig. 4; see also Table S15 in the supplemental material). Compared with the noninvasion treatment, six phyla/classes and four functional groups showed significant variation in relative abundance, and most of them, including Ascomycota, Chytridiomycetes, Zygomycota, Leotiomyces, the arbuscular mycorrhizal group, and the wood saprotroph and lichenized group, had significantly greater abundances in response to plant invasion (Fig. S5).

We found that fungal community dissimilarities between the noninvasion and invasion treatments significantly decreased with increasing plant richness ($R^2 = 0.053$; $P < 0.001$). Furthermore, plant richness correlated significantly ($R^2 = 0.035$; $P < 0.001$) with the nestedness component of fungal community dissimilarities, but did not correlate significantly ($R^2 < 0.001$; $P = 0.764$) with the turnover component (Fig. 5).

DISCUSSION

Plant diversity-soil microbial diversity relationships. (i) Generalization of the plant-soil fungal alpha diversity relationship. In this study, we hypothesized a significant and positive relationship between plant richness and soil microbial richness. In partial support of our hypothesis, we found that plant richness was significantly correlated with soil fungal richness, whereas the correlation with soil bacterial richness was not significant. This finding is consistent with most previous studies, as revealed by our literature analysis, which have observed significant plant-soil fungal alpha diversity relationships (11, 14, 30–32). Specifically, based on the statistics of 25 observational

and experimental studies, 56% of the studies found a coupling relationship between plant and soil fungal richness (see Table S10 in the supplemental material). This result, together with those of the experimental study described here, suggests that the plant-soil fungal alpha diversity relationship might be generalized. Notably, there are still a few studies (accounting for 32%) that found a nonsignificant relationship between plant and soil microbial richness; this case especially occurs in observational studies focusing on forest ecosystem. This phenomenon may result from two causes, namely, the huge soil heterogeneity in forest ecosystems and the taxonomic resolution. For example, we found that there was no significant relationship between plant richness and the richness of bacterial or fungal specific phyla/classes/groups in the noninvasion treatment, highlighting the potential influence of taxonomic resolution in community relationships (33, 34).

(ii) Plant invasion significantly altered plant-soil fungal diversity relationships.

It is well known that plant invasion has impacts on native plant and soil microbial community structure (24, 27, 28, 35). However, the knowledge of whether plant invasion would influence the plant-soil microbial diversity relationship is missing. Here, supporting our second hypothesis, we found that the plant-soil fungal diversity relationship was significantly altered under plant invasion conditions. Specifically, the Pearson correlation coefficient between plant richness and fungal richness was 0.426 ($P=0.005$) in the noninvasion treatment, and it increased to 0.633 ($P<0.001$) in the invasion treatment (Fig. 2; see also Table S3 in the supplemental material). In contrast, the relationship between plant and fungal beta diversity is significant ($r=0.187$; $P=0.005$) in the noninvasion treatment, whereas the correlation is nonsignificant ($r=0.033$; $P=0.161$) in the invasion treatment (Fig. 3). These results suggest that plant invasion enhanced the coupling of plant-fungal alpha diversity relationship, but broke up the coupling of the plant-fungal beta diversity relationship.

In contrast with the significant plant-fungal diversity relationship, we found no significant correlation between plant and bacterial diversity. This may be the case that bacteria experience more stringent top-down forces (regulation by their consumers) and bottom-up forces (resource quantity and quality) than fungi (2). It should be noted that very limited studies have found significant plant richness effects on bacterial richness (12, 36, 37). Interestingly, Schlatter et al. (37) found that bacterial richness was negatively correlated with plant richness, and the authors concluded that monoculture promoted resource competition, which would favor antagonistic communities, and then contributed higher bacterial richness.

(iii) Mechanisms underlying the plant-soil fungal diversity relationship. Our results demonstrated that ecological mechanisms underlying the plant-soil fungal diversity relationship are fundamentally different between circumstances without plant invasion and with plant invasion. In the noninvasion condition described here, the primary mechanism underlying the plant-fungal alpha diversity relationship is that plant richness indirectly promoted soil fungal richness through the increase of resource quantity (i.e., soil TC). Simple Pearson correlation analysis showed that both plant richness and soil fungal richness had the highest correlation with soil TC (see Tables S3 and S7 in the supplemental material). Although the simple Pearson correlation between plant and soil fungal richness was significant ($r=0.426$; $P<0.05$), the partial Pearson correlation was not significant ($r=0.126$; $P=0.44$) after controlling for soil TC (see Table S8 in the supplemental material). The best OLS multiple regression models indicated that soil TC was the best predictor for fungal richness (Table 1). This indirect effect of plant richness on soil fungal richness was also verified by our path analysis of SEM models (Fig. 6). Despite the large effect of soil TC, two other potential mechanisms should not be neglected, namely, that plant richness indirectly promotes soil fungal richness via the regulation of substrate richness and plant species identity (2, 4, 32). For example, Cline et al. (32) concluded that plant richness enhanced soil fungal richness by increasing the richness of organic substrates. Increased plant richness has a higher chance to contain key plant species, and these certain species or functional groups can strongly affect soil fungal richness (14, 15, 31).

Under plant invasion conditions, the enhanced plant-fungal alpha diversity relationship resulted from the direct effect of plants on soil fungi. The best OLS multiple regression models indicated that plant richness most explained the variation of fungal richness (Table 1). The simple Pearson correlation coefficient between plant richness and soil fungal richness was the highest ($r = 0.633$; $P < 0.001$) among all correlations, and the partial Pearson correlation remained strong ($r = 0.422$; $P = 0.008$) even after controlling for soil TC and pH (Table S8). Our path analysis of SEM models showed that plant richness had a positive direct effect (0.633) on fungal richness and explained the 40% variation of fungal richness (Fig. 6). In fact, plant invasion always involves multiple mechanisms, which depend largely on direct interactions of plants with soil mutualists (e.g., mycorrhizal fungi) and pathogens (e.g., oomycetes) (27). For instance, mutualistic microbes such as arbuscular mycorrhizal fungi may provide novel or enhanced mutualistic advantages to exotics over natives (termed the “enhanced mutualisms hypothesis”) (38). Alternatively, accumulated pathogens such as *Fusarium* spp. in invader-occupied soils may have a strong negative effect on native plant fitness (termed the “accumulation of pathogens hypothesis”) (39).

We infer that the primary mechanism underlying the plant-fungal beta diversity relationship might be the host specificity of fungal functional groups. For instance, mycorrhizal fungi and fungal pathogens are tightly linked to plants by forming symbiotic and parasitic relationships (10, 31). In the noninvasion treatment, the host specificity was robust. Our partial Mantel test showed that plant beta diversity and soil fungal beta diversity remain significant even after controlling for other plant and edaphic properties (see Table S9 in the supplemental material). Under plant invasion conditions, the host specificity was broken, which led to decoupling of the relationship between plant and fungal beta diversity.

Plant diversity-soil microbial resistance relationship. The diversity-stability relationship has long been a central topic in ecology (16). Traditional research studies have put great efforts into testing the effect of plant diversity on the temporal stability of plant aboveground biomass and illuminating its underlying mechanisms (19–21). As an important component of stability, community resistance stability is worthy of more attention due to increasing global change (e.g., warming, drought, and plant invasion) (40, 41). In this study, we found significantly decreased patterns of soil microbial community dissimilarities between noninvasion and invasion treatments with increasing plant richness, which suggests a positive plant diversity-soil microbial resistance stability relationship (Fig. 5). To better understand the ecological processes underlying the stability relationships, we partitioned the community dissimilarity into turnover and nestedness components and related them to plant richness. The results indicated that the bacterial stability relationship was relevant to turnover processes, whereas the fungal stability relationship was primarily determined by nestedness processes (Fig. 5). “Turnover” refers to species substitution processes reflecting the niche breadth of species resulting from environmental filtering or competition, and “nestedness” reflects species loss processes across communities (42). Our results suggest that species loss resulting from plant invasion across soil fungal communities was more serious in less diverse plant communities. This seems to correspond to the above-described finding that plant invasion enhanced the coupling of the plant-fungal richness relationship. It has been previously reported that a variety of invasive plants could produce allelochemicals that directly or indirectly degrade local mycorrhizal fungi to promote invasion (termed the “mycorrhizal degradation hypothesis”) (43). Unlike the significant species loss across fungal communities under plant invasion conditions, bacterial communities mainly encountered species substitution processes. This may be attributed to the wider niche breadth of bacterial species or to their life history strategy. For instance, *r*-strategy species may rapidly occupy the niche space of the lost fungal species under plant invasion conditions (44).

Linkages of soil microbial community with plant and edaphic properties. Bacterial richness has been widely demonstrated to be determined by soil pH (45). In this study, however, we found that pH had no influence on the richness of the whole

bacterial community, yet it had a significant effect on the richness of specific phyla, such as *Acidobacteria*, *Bacteroidetes*, *Alphaproteobacteria*, and *Deltaproteobacteria* (see Tables S5 and S6 in the supplemental material). Our results showed that bacterial richness was mainly correlated with soil C/P in the noninvasion treatment, and soil TP was significantly correlated with the richness of the most dominant phylum, *Alphaproteobacteria* (Table 1; see also Tables S4 and S5 in the supplemental material). This finding is identical with that of a global grassland study, which observed that bacterial richness was not effectively explained by pH but was best predicted by soil TP, indicating that soil phosphorus could be a limiting factor for bacterial richness in grasslands (7). Under plant invasion conditions, bacterial richness was mainly affected by soil glomalin (Table 1). Glomalin, a soil protein which is released by arbuscular mycorrhizal fungi, is greatly influenced by plant photosynthate and carbon resources (46). Pearson correlation analysis showed that soil glomalin was negatively correlated with bacterial richness but positively correlated with fungal richness in the invasion treatment (Table S3), indicating that bacterial communities might be indirectly constrained by fungal communities via the release of soil glomalin.

Similarly to the richness pattern, soil TP was identified as the most relevant factor for bacterial community composition in the noninvasion treatment (Table 2). Specifically, increased TP was associated with higher relative abundances of *Acidobacteria* and *Planctomycetes* and with lower relative abundance of *Cyanobacteria* and *Armatimonadetes* (Fig. 4). Nutrient addition experiments have revealed that phosphorus had a significant influence on bacterial community compositions, especially for phosphate-solubilizing bacteria (47, 48). We infer that the higher abundance of *Acidobacteria* with increasing TP was due to the decrease of soil pH, because pH was negatively correlated with soil TP (see Fig. S3 in the supplemental material). This is expected from previous studies showing that the dissolving of inorganic P is usually associated with the release of organic acid by microorganisms (e.g., *Pseudomonas* spp.) (49, 50). Meanwhile, we found that soil TC mainly influenced fungal community composition in the noninvasion treatment, with the relative abundances of Glomeromycota, Sordariomycetes, Glomeromycetes, Ustilaginomycetes, and Leotiomycetes significantly varying with TC. Undoubtedly, fungal community variations are always associated with soil carbon dynamics, although there still exists debate about whether symbiotic arbuscular mycorrhizal fungi facilitate or reduce soil carbon sequestration (51, 52). Under plant invasion conditions, plant aboveground biomass was identified as the major contributor to variation in both bacterial and fungal community composition (Fig. 4 and Table 2). In fact, there exists an invasion mechanism hypothesis positing that invasive plants may indirectly affect decomposer communities and nutrient availability to promote invasion (termed the “novel weapons hypothesis”) (22, 27). Our response ratio analysis showed that the relative abundance of bacterial functional groups involved in chitinolysis, xylanolysis, and ureolysis significantly increased after plant invasion, which partly corroborates the novel weapons hypothesis (see Fig. S5 in the supplemental material). In addition, previous studies suggest that plant aboveground biomass strongly influence the rhizosphere priming effect on soil organic matter decomposition (53, 54). For example, plants that have high leaf biomass and photosynthesis capacity may exude a greater total amount of labile carbon into the soil, and labile carbon inputs can regulate decomposition of more recalcitrant carbon by controlling the relative abundances of bacteria and fungi (54).

Conclusion. In summary, we found that plant diversity was coupled with soil fungal diversity, and that this relationship was significantly altered under plant invasion conditions. Plant invasion enhanced the coupling of the plant-fungal alpha diversity relationship, but broke up the coupling of plant-fungal beta diversity relationship. Our results revealed the mechanisms—indirect and direct plant richness effects—underlying the plant-soil fungal diversity relationship. Specifically, plant richness indirectly promoted soil fungal richness through the increase of soil TC without plant invasion, whereas plant richness had a direct positive effect on soil fungal richness under plant invasion conditions. We also found significant positive plant diversity-soil

microbial resistance relationships. Stability relationships were determined by turnover processes and nestedness processes for bacterial and fungal communities, respectively. Taken together, these results demonstrated that plant diversity enhanced soil fungal diversity and microbial resistance in response to plant invasion. Further study is needed to evaluate how plant invasion influences relationships between aboveground and belowground diversity and to better understand the ecological mechanisms underlying the diversity-diversity and diversity-stability relationships.

MATERIALS AND METHODS

Experiment design and sampling. The experiment was conducted on the campus of Taizhou University (121°23' E, 28°39' N) in southeastern China. In March 2013, a plant richness gradient (1, 2, 4, and 8 species) was established on 90 pots (72 cm × 64 cm × 42 cm per pot; see Table S1 in the supplemental material). The species pool of the experiment consisted of 16 perennial grassland native species (Table S1). The 16 species were grown in monocultures as 16 replicates for 1-species richness. For each of the species richness levels of 2, 4, 8, and 10 pots, each with a different species composition, were initially established to represent 10 experimental replicates, but one replicate of the 8-species treatment was finally abandoned due to very low coverage of the target species. The species assigned to each pot were randomly chosen from the species pool. In December 2013, we introduced a total of 800 seeds into each pot based on the designed species composition. After germination, excess seedlings were removed to maintain an equal plant density of 32 seedlings in each pot. Seedlings of the same species were not adjacent, and the 32 seedlings were evenly distributed in the pot. Weeds were periodically removed from each pot to maintain the original species composition. The plant richness treatments were replicated twice for noninvasion and invasion experiments (Fig. 1). In December 2015, 50 seeds of *Symphyotrichum subulatum* (Michx.) G. L. Nesom (an invasive species) were added to each pot of the invasion experiment. To avoid the influence of seeds of other species, all of the experiments (noninvasion and invasion) were conducted in a vinyl house (each pot was surrounded by ventilating nets, and plastic film was on the top). The same amount of water through sprinkler irrigation was added into each pot every 2 days.

Plants were harvested in October 2016. Before harvesting, five soil cores (0 to 10 cm depth) were randomly collected in each pot and composited together as a single sample. In total, we obtained 90 soil samples. The fresh soil samples were sieved through a 2-mm sieve and divided into two subsamples. One was stored at 4°C to determine the soil properties, and the other was stored at −40°C prior to DNA extraction.

Measurement of plant and soil properties. Plants from each pot were placed on gauze and washed with water. The roots of each plant were carefully separated. All of the aboveground and root biomass (living plants) were sorted to species, dried to constant mass at 80°C for 48 h, and weighed separately.

Soil total carbon (TC) contents were measured by the elemental analyzer (vario Max; Elementar, Germany). Soil total phosphorus (TP) was determined by the molybdenum blue method with an UV-visible spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan). Soil pH was measured after shaking a soil water (1:5 wt/vol) suspension for 30 min (AB15 pH meter; Accumet, Fisher Scientific). Soil total glomalin was extracted from a 1-g air-dried soil sample by four repeated cycles with 8 ml 50 mmol/liter sodium citrate buffer (pH 8.0) by autoclaving for 60 min at 121°C, as described by Wright and Upadhyaya (46). The extract was centrifuged at 10,000 × *g* for 10 min to remove soil particles. The content of total glomalin in the supernatant was quantified by the spectrophotometric method.

Soil enzyme activity was determined by different methods, respectively. β -Glucosidase activity was assayed by the method of Eivazi and Tabatabai (55), using the substrate analogue *p*-nitrophenyl- β -D-glucoside (pNPG). Protease activity was determined as described by Ladd and Butler (56). Dehydrogenase activities were measured by the method reported by Tabatabai (57). Urease activity was measured using colorimetric determination of ammonium (58). Nitrate reductase activity was assayed by the method of MacGregor et al. (59).

DNA sequencing and bioinformatics. We extracted soil DNA using the PowerSoil DNA isolation kit (MoBio, Carlsbad, CA) following the manufacturer's instructions. Two primer sets, 515f/806r and ITS1f/ITS2, were used to amplify (in triplicate reactions for each sample) the bacterial 16S rRNA gene and the fungal ITS1 region of the rRNA gene (<http://www.earthmicrobiome.org/protocols-and-standards/>). Amplicons were pooled and sequenced with a HiSeq2500 PE250 instrument (Illumina, San Diego, CA). The QIIME v1.9 pipeline was used to process the raw sequence data, including barcode extraction, paired read assembly, and quality filtering (*Q* > 30). The FASTA format data were then uploaded to the Galaxy/DengLab platform (<http://mem.rcees.ac.cn:8080/>) for downstream analysis (60). The UPARSE method was utilized to conduct chimera detection and OTU clustering (97% similarity). Taxonomy was identified for each OTU using RDP Classifier trained on the Greengenes and UNITE databases for bacterial and fungal sequences. Samples were rarefied to 20,000 and 35,000 sequences per sample for bacteria (20,589 to 60,324 sequences) and fungi (35,896 to 132,066 sequences), respectively. Functions were predicted based on bacterial and fungal taxa using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database (<http://www.zoology.ubc.ca/louca/FAPROTAX/>) and the FUNGuild database (<http://www.stbates.org/guilds/app.php>). Four samples (two replicates of the 1-species level, one replicate of the 2-species level, and one replicate of the 4-species level) in the noninvasion treatment and

the four corresponding samples in the invasion treatment were abandoned due to low numbers of sequences. Finally, a total of 82 samples was used to perform statistical analyses.

Statistical analyses. To test for the relationship between plant alpha diversity and soil microbial alpha diversity, we conducted general linear regression models. The Chao1 richness and plant richness (i.e., number of plant species) were selected to represent microbial and plant alpha diversity, respectively. The nonparametric Chao1 index (an estimate of the true OTU richness based on the frequencies of singletons and doubletons) was selected to represent the richness of soil bacterial and fungal communities because it is sensitive to rare species (61). In addition, we also estimated the Chao1 richness for 10 bacterial phyla and 10 fungal phyla/classes/guilds based on the same sequencing depth (see Table S4 in the supplemental material). Pearson's correlations were performed to examine the relationships between plant/microbial richness and other factors. Partial Pearson's correlation analyses were further performed to identify the relationship between plant and soil fungal richness after controlling the shared edaphic factors. Multiple ordinary least-squares (OLS) regression models were used to identify the best predictors of soil microbial richness. Specifically, when the detected variables were centralized and standardized (average = 0; standard deviation [SD] = 1), the best regression models were determined by Akaike's information criterion (AIC) and adjusted R^2 (R^2_{adj}) values. Also, we used the variance inflation factor criterion (VIF, <3) to eliminate multicollinear variables. These analyses were performed using the R packages *MASS* and *car*. Structural equation modeling (SEM) was applied to investigate the direct and indirect effect of plant richness on soil microbial richness. The SEM analysis was performed via the robust maximum-likelihood evaluation method using the software AMOS 22.0 (IBM, USA). The overall fitness of the final model was evaluated on the basis of a nonsignificant chi-square test ($P > 0.05$), the goodness-of-fit index (GFI, >0.9), and the root mean square error of approximation (RMSEA, <0.05).

To better review empirical studies about the plant-soil microbial alpha diversity relationship, we conducted a literature search on Web of Science. Papers were also surveyed based on the reference lists in the relevant articles, and 25 pieces of evidence were obtained in total. We divided these independent studies into two categories—observational and experimental—and the ecosystems were further identified by forest or grassland. We then classified the reported relationships as significant for bacteria, fungi, both, or nonsignificant, based on the results of original publications (see Table S10 in the supplemental material).

To test for the relationship between plant beta diversity and soil microbial beta diversity, we performed the Mantel test to examine the correlation between the plant community dissimilarity matrix (Bray-Curtis distance of plant species composition table) and the microbial community dissimilarity matrix (Bray-Curtis distance of microbial OTU abundance table). The pairwise Bray-Curtis distance between samples was calculated using the R package *vegan*. A partial Mantel test was used to test the relationship between plant beta diversity and soil microbial beta diversity after controlling for other factors. To identify the effect of plant and edaphic properties on soil microbial community composition, canonical correspondence analysis (CCA) and permutational multivariate analysis of variance (PERMANOVA) were performed in the R package *vegan*. The percentages of variations in community composition explained by variables were tested through distance-based multivariate analysis for a linear model (DistLM), which was conducted in DISTLM_forward3 software (15). Spearman's rank correlations were used to examine the relationships between the relative abundance of microbial phyla/classes and plant and edaphic properties.

To test the plant diversity-soil microbial resistance stability relationship, the general linear regression model was used to examine the correlation between plant richness and soil microbial community dissimilarities (Bray-Curtis distance between noninvasion and invasion treatment at the same plant richness level). If greater plant richness induced greater soil microbial resistance, the microbial community dissimilarities resulting from plant invasion would be smaller. To better understand the ecological processes, community dissimilarities were further partitioned into turnover (referring to species substitution processes) and nestedness (referring to species loss processes) components (using Sørensen dissimilarity indices of the Baselga family) using the R package *adespatial* (42, 62). The two components were also associated with plant richness by general linear regression models. The response ratio (RR) was used to quantify significant responses of microbial taxonomic/functional groups to plant invasion. If the 95% confidence interval (CI) of a response variable overlaps with zero, the RR is not significantly different; otherwise, the RR is significant (63).

Data availability. The sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number [PRJNA601082](https://www.ncbi.nlm.nih.gov/sra/PRJNA601082).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.8 MB.

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We declare no conflicts of interest.

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