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Asymmetric succession in soil microbial communities enhances the competitive advantage of invasive alien plants

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Abstract

Background Biological invasions pose an escalating threat to native ecosystems. The accumulation of invasive alien plants worldwide is not saturated yet, underscoring the persistent and growing impact of invasions. Soil microorganisms play a key role in the process of alien plant invasion. However, the temporal dynamics of microbial communities has rarely been determined during the invasion owing to the dearth of long-term, in situ experimental systems.

Results Here, we examined the temporal succession of soil microbial communities 8 years after experiment setup in a common garden. Bacterial communities displayed divergent temporal succession, with invasive plants exhibiting higher turnover rates. Invasive alien plants reduced stochasticity in bacterial communities, likely acting as an environmental filter on community assembly. Plant growth-promoting microbes underwent higher succession rates in invasive alien plants compared to native plants, suggesting that invasive alien plants may possess a distinct advantage in fostering a favorable microbiota for their own growth and establishment. In sharp contrast, native plants selectively increased succession rates of specific plant pathogens. Furthermore, the microbial co-occurrence network was more complex in invasive plants, suggesting that invasive plants foster intricate relationships among microbial communities.

Conclusions Therefore, the asymmetric succession in soil microbial communities enables invasive plants recruit beneficial microbiota from the surrounding soil. These results deepen our understanding of the mechanism underlying plant invasion and provide novel insights into predicting the ecological consequences resulting from widespread plant invasion. This knowledge can be incorporated into management strategies to address the evolving challenges posed by invasive plants.

Keywords Soil microbe, Microbial succession, Plant invasion, Assembly process, Mercenary strategy

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Introduction

The incidence of biological invasion intensifies complex global environmental challenges, including biodiversity loss, biota homogenization, and habitat fragmentation [1], which has become a pervasive aspect of global change. Invasive alien plants, characterized by their rapid growth and substantial biomass accumulation, often colonize and expand swiftly, leading to reduced plant diversity and the formation of monocultural communities in new habitats [2]. Invasive alien plants can disrupt ecosystem dynamics by altering soil biogeochemical pools and energy fluxes. These disruptions can have long-term consequences, potentially leading to the degradation of ecosystems and the loss of valuable ecosystem services [3]. Previous studies have primarily focused on deciphering the mechanisms that enable invasive alien plants to establish and spread more successfully than their native counterparts [4–7].

In recent years, there has been growing recognition of the pivotal role that soil microorganisms associated with plants play in enhancing plant reproduction, fitness, nutrient acquisition, and resistance to environmental stresses [8]. Invasive plants like *Mikania micrantha*, *Ageratina adenophora*, and *Flaveria bidentis* can increase soil carbon and nitrogen availability by enriching microbes involved in these processes [9, 10, 2, 11]. Increased access to limiting nutrients (e.g., C, N, P) can thus stimulate the rapid growth and reproduction of exotic invasive plants [12]. Plant growth-promoting microbes, such as arbuscular mycorrhizal fungi (AMF), have been found to colonize the roots of exotic *F. bidentis* more extensively than those of co-occurring native plants [13], and AMF inoculation has facilitated the competition of *F. bidentis* [14]. Furthermore, many plant-soil feedback (PSF) experiments have demonstrated positive effects on invasive plant species in soils conditioned by the invasive plants themselves [15]. For example, *Lespedeza cuneata* and *Prosopis juliflora* benefit from a history of invasion, with these positive effects attributed to modifications in soil microbial communities[17, 18]. In contrast, many native plant species exhibit less growth on soil conditioned by invasive plants, which is largely due to allelochemical effects by invasive plants or accumulation of soil-borne pathogens. However, most of these studies are based on single-time observations due to the dearth of long-term, in situ experimental systems, so we cannot determine whether these phenomena are temporary or vary over time. In addition, without longitudinal data, predicting the future ecological consequences of invasion remains challenging. Therefore, studies on chronosequence-based microbial successions after plant invasion are necessary to address this uncertainty.

Alien plant invasions are temporally dynamic; however, the role of soil microorganisms in driving invasions is often overlooked, especially in terms of temporal components [15]. Owing to the uncertainty in succession trajectories, which can exhibit patterns of convergent, divergent, cyclical or linear, etc. [18], one of the major challenges in understanding biological invasion lies in the difficulty of predicting its ecological consequences. The litter input from invasive alien plants typically supports larger microbial populations [19], triggering a cascade of microbial succession. Additionally, specific root exudates, such as allelochemicals, can attract specific microbes and potentially accelerate the temporal succession of microbial communities. These dynamics introduce a level of unpredictability in forecasting whether microbial communities will exhibit divergent or convergent behaviors, making it challenging to know the ecological outcomes of plant invasions.

Previous studies have shown that alien plant invasion can trigger an increase in relative abundances of soil microbes involved in nutrient cycling [2, 9] or a decrease in pathogenic microbes[21]. However, it remains unclear whether all functional groups within soil microbial communities respond uniformly to plant invasion. In this context, we hypothesize that (1) alien plant invasion may result in nonuniform succession dynamics among various microbial groups, specifically activating those that promote plant growth over time, thereby enhancing the temporal successions of these microorganisms, and (2) as increased nutrient availability triggers microbial activities in soils alien plants, invasive plants may increase the complexity of microbial networks, resulting in more nodes and keystone taxa.

Materials and methods

Site description and sample collection

In 2012, we initiated a common garden experiment at Langfang Experimental Station of the Chinese Academy of Agricultural Sciences in Hebei province, China (39° 30′ 42″ N, 116° 30′ 42″ N). The experiment included four types of annual plants: invasive *Ambrosia artemisiifolia* L. and *Bidens pilosa* L and native *Chenopodium serotinum* L. and *Setaria viridis* L. The two invasive plants were selected due to their severe ecological impact and widespread distribution in China, including the experiment site [21, 22]. Two native plants frequently co-occur in areas invaded by the two invasive plants in China and have been used as control natives [13, 23] (Fig. 1a). This setup resulted in two treatments: the native treatment involved monoculture of *C. serotinum* or *S. viridis*, and the invasive treatment involved intercropping of *A. artemisiifolia* and *C. serotinum*, *A. artemisiifolia* and *S. viridis*, *B. pilosa* and *C. serotinum*, and

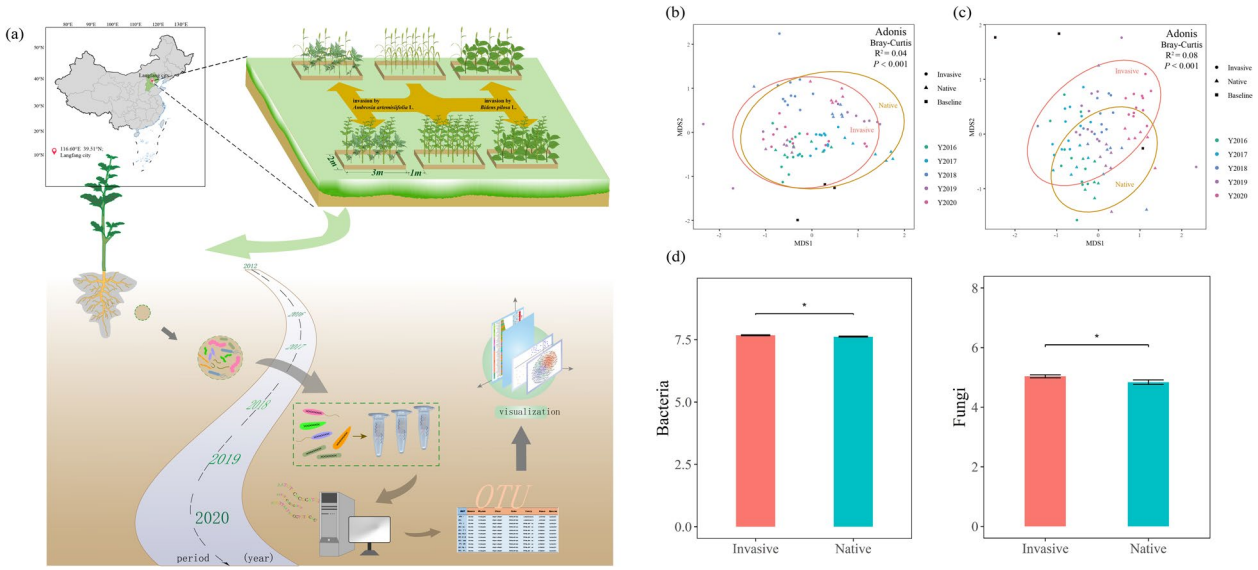

Fig. 1 Effects of alien plant invasion on soil microbial communities. **a** Illustration of the experiment treatments. The native treatment includes all samples from monocultures of *C. serotinum* and *S. viridis*; the invasive treatment includes all intercropping samples from the intercropping of *A. artemisiifolia* and *C. serotinum*, *A. artemisiifolia* and *S. viridis*, *B. pilosa* and *C. serotinum*, and *B. pilosa* and *S. viridis*. "Baseline" refers to soil samples collected at the beginning of the experiment in 2012. Then soil samples were taken yearly from 2016 to 2020. **b** NMDS ordination of bacterial communities based on Bray–Curtis dissimilarity, with the Adonis analysis indicating significant among treatments. **c** NMDS ordination of fungal communities. **d** Shannon diversities of bacterial and fungal communities. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

B. pilosa and *S. viridis*. Additionally, bare fallow soil was used as a control. Each type of plantation had three replicate plots, each measuring 3 m in length, 2 m in width, and spaced 1 m apart. This design resulted in a total of 21 plots [(2 monocultural plantations + 4 intercropping plantations + 1 bare fallow soils) × 3 replicates], arranged in a randomized complete block design. Our aim was to investigate the impact on soil microbial communities in native species when invasive species are introduced, a scenario that frequently occurs in nature. Therefore, we focused on comparing mixtures and monocultures of native species, excluding monocultures of invasive plants from our data analysis. A total of 100 seeds (50 seeds of each plant in intercropped plots) were randomly broadcasted by hand in each plot at the beginning of the experiment, with no additional seeding or fertilizer applied in the subsequent years. Plants were removed as needed to maintain the original experimental design, and aboveground biomass was manually removed before the growth season to reduce the shading effect on plant growth.

Soil samples were collected from three bare fallow plots as baseline samples in October 2012. After allowing 4 years for system stabilization, soil samples were collected from each plot every October from 2016 to 2020, resulting in a total of 108 samples. The 15 samples from bare fallow soils were not involved in downstream analyses. Before collecting soil in each plot, we randomly selected five cores of 20-cm depth and 30-cm width and removed 1 cm of litter from the surface soil. Then we collected soils in cores, avoiding roots. The collected soils were homogenized, sieved through a 20-mesh sieve to remove impurities, and stored in polyethylene (PE) bags at − 80 °C for 16S rRNA and ITS2 gene sequencing. Fresh soils were used to analyze soil properties.

Measurements of environmental variables

For each sampling point, we determined the plant biomass by placing plant tissues per 0.25 m^2 into paper bags and drying them at 80 °C for 72 h until a constant weight was achieved. We determined soil geochemical properties by traditional methods [24]. Briefly, we measured soil organic matter content (SOM) by a potassium dichromate oxidation external heating method[26]. We measured soil pH in a soil suspension (soil:water of 1:2.5) using a pH meter (Mettler-Toledo Instruments, Shanghai, China). We determined total nitrogen (TN) by the Kjeldahl method with sulfuric acid as accelerator[27], total phosphorus (TP) by a molybdenum antimony colorimetry method after extraction by sodium carbonate[28], and total potassium (TK) by flame photometry after extraction using sodium hydroxide[29].

Experiments of microbial communities

We extracted soil genome DNA using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad,

CA, USA) and purified the DNA using the MagaBio Soil DNA Purification Kit (Bioer Technology, Hangzhou, Zhejiang province, China). We then assessed DNA quality and concentration using Thermo NanoDrop One (Thermo Fisher Scientific, Waltham, MA, USA) by the absorbance of DNA at 230 nm, 260 nm, and 280 nm.

We amplified the V4 hypervariable region of the 16S rRNA gene for bacteria and archaea using the primer set 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) and the fungal ITS2 gene using the primer set ITS2F (5′-GCATCGATG AAGAACGCAGC-3′) and ITS2R (5′-TCCTCCGCT TATTGATATGC-3′). The 50-μl PCR reaction system included 2×Premix Taq (Takara Biotechnology, Dalian Co. Ltd., Liaoning Province, China), 1 μl of each primer (10 μM), and 3 μl of DNA template (20 ng/μl) with a thermal cycling condition of an initial denaturation at 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. We then purified PCR products using an E.Z.N.A. Gel Extraction Kit (Omega, Bio-tek, Norcross, GA, USA) and sequenced the PCR products on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA).

Data analyses

We processed the sequencing data on a Galaxy pipeline as previously described (http://zhoulab5.rccc.ou.edu) [28]. Briefly, we trimmed sequences to 245 ~ 260 bp for the 16S rRNA gene and 250 ~ 350 bp for the ITS gene and conducted a chimera check with the UCHIME method [29]. The remaining sequences were classified into operational taxonomic units (OTUs) with 97% similarity using the UPARSE algorithm [30]. After singleton removal, sequences were rarefied to 67,259 reads for the 16S rRNA V4 region and to 28,850 reads per sample for the fungal ITS gene. We assigned taxonomic annotations of OTUs to representative sequences by a 16S rRNA training set for the 16S rRNA V4 region amplicon [31] and a UNITE database 8.2 version for the fungal ITS[34] using RDP Classifier. Then we determined the relative abundance (*RA%*) of sequences, which were used in downstream analyses as the following:$RAij\% = \frac{Sij}{\sum_{j=1}^{N} Sij} \times 100$, where *Sij* was the sequence number of the *j*th OTU in the *i*th sample.

Microbial time-decay relationships (TDRs) were determined by similar linear regressions between logarithmic β similarities and logarithmic temporal (year) distance as previously described [33] using the ieggr (V1.6) package in R software (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria) [34, 35]. The equation to evaluate TDRs was as follow:

$$\ln(S_s) = c - \omega \ln(T) + \varepsilon$$

where S_s was the pairwise similarity between pairwise microbial communities within the same treatment, T was the time interval (year), the slope ω measuring the temporal succession rate of microbial communities along time was the TDR value, c was the intercept, and ε was the residual. A bootstrapping of 999 times was used to determine the significance of the TDR value. The significance of differences between the native treatment and the invasive treatment was calculated by comparing observed TDR differences and those in permuted datasets. These analyses were conducted for selected function groups or genera with relative abundance > 0.05%. Functional groups for bacterial communities were predicted using the FAPROTAX method [36], and those for fungi communities were predicted using the FUNGuild method[39].

To construct the co-occurrence network, Spearman's correlation coefficients between OTUs with relative abundance > 0.10% were first calculated using the "microeco" package (version 1.3.0) in R. The Spearman correlation threshold was set using random matrix theory (RMT) with a significance level of $P < 0.01$. We used the igraph package (version 1.4.2) to perform various network analyses. We then performed a one-way analysis of variance (ANOVA) to determine the significance for differences in subnetwork properties between treatments. Networks were then visualized using Gephi software (version 0.10, https://gephi.org/). The value z_i describes how well the node i is connected to other nodes in the same module, and P_i reflects how node i connects to different modules. Nodes with $z_i > 2.5$ and $P_i \leq 0.62$ were considered as module hubs that were highly connected to many nodes in their own modules and $z_i \leq 2.5$ and $P_i > 0.62$ as connectors that were highly linked to several modules [38].

Other statistical analyses were carried out using different packages in R software with details as follows. The β diversities were determined using the Bray–Curtis index [39]. We used a null model analysis to evaluate the relative importance of stochastic and deterministic processes in assembling microbial communities as described by Chase and Myers [40]. Microbial stochastic ratios were determined using the iCAMP (V1.5.3), the ape (V5.3), and the NST (V3.1.8) packages [41]. Simple Mantel tests were conducted with the vegan (V2.3–5) package [42], and the multiple regression on distance matrices (MRM) was conducted with the ecodist (V2.0.9) package. Random forest analysis was performed to identify the individual effects of each variable on soil microbial communities using the randomForest (V4.7–1.1) package[45]. The importance of each factor was estimated by the percentage increase in the mean squared error of variables using the rfPermute (V2.5.2) package by performing 500 permutations on the response variable for evaluation.

Results

Impacts of alien plant invasion on the structure and diversity of microbial communities

Alien plant invasion significantly (Adonis $R^2 = 0.04–0.08$, $P < 0.001$) altered both bacterial (Fig. 1b) and fungal (Fig. 1c) community structures, as revealed by the non-metric multidimensional scaling (NMDS) ordination. Microbial communities in both invasive and native plants were also significantly ($P < 0.05$) different from the baseline treatment in 2012 (Table S1). To test the species effect on microbial community structure, we conducted DMDS for *A. artemisiifolia* and *B. pilosa* separately and found different community structures between invasive and native species for both bacterial and fungal communities (Adonis $R^2 = 0.02–0.07$, $P < 0.02$, Fig. S1).

Bacterial Shannon diversity was 7.67 ± 0.01 in the invasive treatment, which was significantly ($P < 0.05$) higher than those in the native treatment (7.62 ± 0.02, Fig. 1d, Table S2). Fungal Shannon diversity was also significantly higher in the invasive treatment (5.03 ± 0.05) than in the native treatment (4.84 ± 0.08). Similarly, other diversity indices, including richness and Simpson indices, all increased due to plant invasion (Table S2).

Impacts of alien invasion on microbial temporal succession

To understand the impacts of alien plant invasion on the temporal succession of soil microbial communities, we examined the slopes (ω) of the bacterial or fungal time-decay relationships (TDRs). Significant bacterial TDRs ($P < 0.05$) were observed 8 years after experiment setup, with a ω value of 0.05 for the invasion treatment, which was twice that of the native treatment (Fig. 2a). For fungal communities, alien plant invasion also resulted in a significant successive ω value of 0.07, whereas such succession was insignificant in the native treatment (Fig. 2b). To further dissect microbial temporal succession in different time periods, we estimated the annual TDRs from the 5th to the 8th year. Microbial succession in both the native and the invasive treatments was significant ($P < 0.05$) from the 5th to the 8th year (Fig. S2). Remarkably, bacterial ω values were smaller in the invasive treatment than those in the native treatment until the 7th year but were larger in the invasive treatment in the 8th year, underscoring the importance of temporal dynamics.

We further investigated TDRs in 12 major functional groups, including carbon and nitrogen cycling groups, arbuscular mycorrhizal fungi (AMF), and pathogenic fungi. Half of these functional groups exhibited significant ($P < 0.05$) TDRs in the native treatment, such as nitrogen fixation, nitrification, nitrate respiration, and plant pathogen (Fig. 2c). Alien plant invasion induced significant succession in carbon cycling groups and AMF and amplified 2–3 times ($P < 0.05$) higher TRDs in nitrogen fixation and denitrification groups.

We then examined microbial TDRs at the genus level in response to the invasion of *A. artemisiifolia* and *B. pilosa*, respectively. Only seven genera exhibited significant ($P < 0.05$) temporal succession across all treatments, including *Azotobacter* performing nitrogen fixation, *Virgisporangium* and *Gp7* within Acidobacteria, and two pathogenic fungi, *Aspergillus* and *Metarhizium* (Table S3). Almost all genera in cluster 1, revealed by a heatmap of TDRs' slopes, showed significantly higher TDRs in *A. artemisiifolia* or *B. pilosa* than in the native treatment (Fig. 2d). By contrast, most genera in cluster 3 had lower turnover rates in the invasive treatment compared to the native treatment, including *Cyphellophora*, *Penicillium*, *Nocardia*, and *Paraphoma*. Some microbial genera had significantly higher turnover rates only in the *A. artemisiifolia* invasive treatment, such as *Ilumatobacter*, *Modestobacter*, *Actinoplanes*, and *Caulobacter*, while some other genera, such as *Anaeromyxobacter* and *Rhizobium*, exhibited significantly higher turnover rates only in the *B. pilosa* invasive treatment. These results indicated that the two invasive plants differed in their abilities to modify certain microbial genera.

Community assembly processes underlying microbial successional trajectories

To determine whether microbial communities converge or diverge during succession, we calculated the distances between the native and invasive microbial communities yearly. The variations in bacterial communities increased linearly over time ($r = 0.16$, $P = 0.004$, Fig. 3a), showing a pattern of divergence. In contrast, this divergent pattern was not significant for fungal communities. For bacterial community, stochastic processes accounted for 42.53–49.78% of the community variations, with alien plant invasion significantly decreasing the stochasticity by about 5.00% (Fig. 3b). Furthermore, the relative contribution of the stochastic process in shaping bacteria communities increased over time in the native treatment but remained consistent in the invasion treatment (Fig. S3a). In contrast, stochastic ratios were similar between the native and the invasion treatments in shaping fungal communities, with stochasticity ranging from 70.12 to 79.28% (Fig. 3b, Fig. S3b).

Impacts of alien plant invasion on microbial co-occurrence networks

Recognizing that bacteria and fungi communicate with each other in soil, we constructed co-occurrence networks that encompassed both bacterial and fungal communities. The networks differed profoundly, with the invasive network being more complex (Fig. 4). Using the

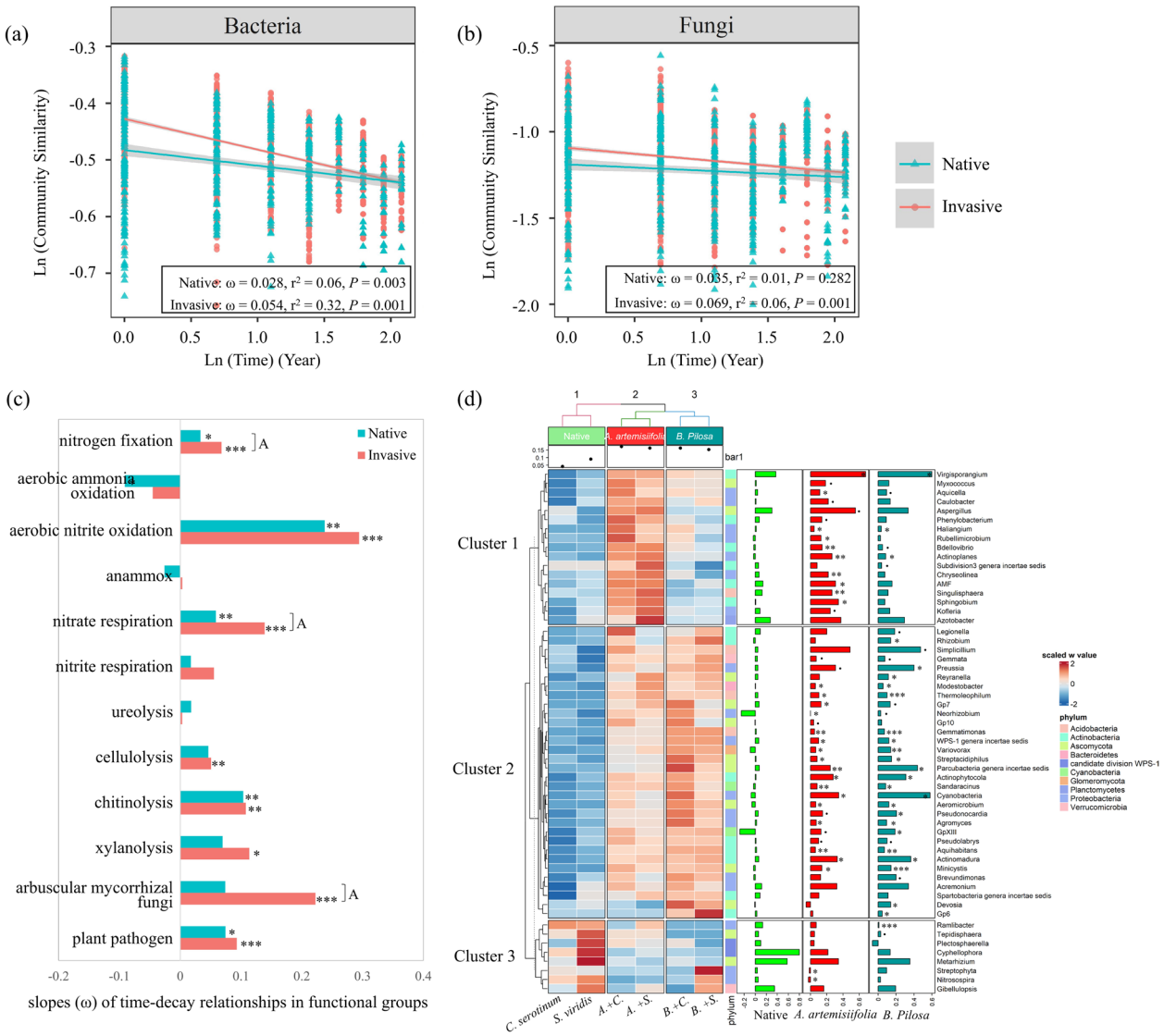

Fig. 2 Time-decay relationships (TDRs) at various levels of microbial communities, including (**a**) the entire bacterial community, **b** the entire fungal community, **c** functional groups, and (**d**) selected genera. The significance (asterisk) of the *ω*-value is determined by bootstrapping 999 times. The significance of differences between treatments was then calculated by comparing the observed slope differences with those in permuted datasets. The letter "A" in diagram (**c**) indicates a significant difference ($P < 0.05$) between the native treatment and the invasion treatment. In diagram **d**, the rates are scaled between − 2 and 2 in the heatmap; the unscaled mean rates are represented by green bars for native plants, red bars for invasive *A. artemisiifolia*, and blue bars for invasive *B. Pilosa*; asterisks over red or blue bars indicate significantly different rates in the invasion treatment compared to the native treatment; the scatter plot refers to the mean rates in each plant. Abbreviations: *A.* + *C.* — intercropping of *A. artemisiifolia* and *C. serotinum*; *A.* + *S.* — intercropping of *A. artemisiifolia* and *S. viridis*; *B.* + *C.* — intercropping of *B. pilosa* and *C. serotinum*; *B.* + *S.* — intercropping of *B. pilosa* and *S. viridis*. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

same threshold for network construction, we obtained similar nodes of 135–160 in these two networks, in which 38.52–48.75% belonged to Ascomycota phyla and 28.75–36.30% belonged to Acidobacteria, Actinobacteria, and Proteobacteria. However, edges in the invasive treatment were 1256, nearly 3 times the number observed in the native treatment (Table S4). The average degree and network centralization were also about 2–3 times higher than those in the native treatment. Furthermore, the network diameter in the invasive treatment was one-third of that in the native treatment.

According to the z_i-P_i plot, OTU36 belonging to *Ramlibacter* emerged as a module hub in both native and invasive treatments (Fig. 4, Table S5). In the native treatment, there were 10 nodes identified as module hubs or connectors, including members of Gp4 and *Blastocatella*

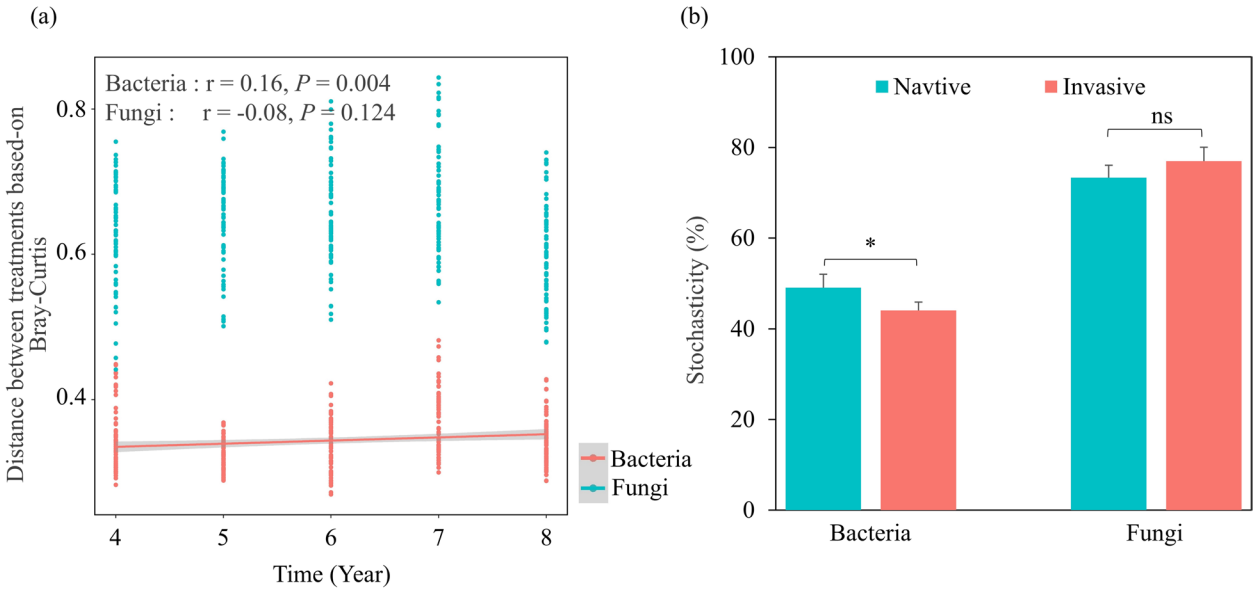

Fig. 3 **a** Community distance between the native and invasive microbial communities based on Bray–Curtis and **b** community stochasticity. Statistical significance in pairwise distance was determined by permutation tests. The Student *t*-test was used to calculate the statistical difference in stochasticity between the native treatment and the invasion treatment. *$P < 0.05$; ns, not significant

belonging to Acidobacteria and plant-pathogenic fungi *Periconia*, *Cladosporium*, and *Penicillium*. In contrast, the invasive network had 21 nodes functioning as connectors, almost all belonging to the phyla Ascomycota, Proteobacteria, and Actinobacteria.

Environmental drivers of microbial communities

Mantel tests were used to determine the relationship between each environmental variable (Table S6) and microbial communities. Soil characteristics, including soil organic matter, soil pH, total nitrogen, total phosphorus, and total potassium, were correlated with bacterial and fungal communities ($P < 0.05$, Table S7). The variation of bacterial community was also significantly explained by plant biomass ($r = 0.15$, $P < 0.05$). The random forest model further confirmed these observations (Fig. S4). These variables collectively contributed to 42.40% of bacterial community variation and 23.11% of fungal community variation ($P < 0.05$, Table S7). However, a large portion of fungal community variation could not be explained by measured variables, signifying the importance of stochastic processes (70.12–79.28%, Fig. 3b) in assembling fungal communities.

Discussion

Although numerous studies have demonstrated how soil microbes drive plant invasion through both direct routes (such as pathogens and mutualists) and indirect routes (such as mediation of nutrient availability) [7, 9, 15], it remains unclear whether these soil microbes respond dynamically to invasion over time or if microbial communities respond uniformly. Our results indicate that invasive alien plants significantly impact the composition of soil microbial communities over time. Specifically, alien plant invasion enhances microbial diversity, accelerates microbial succession, and intensifies microbial associations. Furthermore, invasive alien plants often activate beneficial microbiota that increase resource availability and suppress soil-borne pathogens.

Alien plant invasion leads to nonuniform succession dynamics in microbial communities

Alien invasive plants can recruit mutualistic microbes, such as nitrogen-fixation microbes and AMF, from native mutualist communities to support their establishment [44]. Consistently, we found that functional groups and genera with significantly higher succession rates in invasive plants compared to native plants typically play crucial roles in maintaining plant growth. For example, microbial groups involved in carbon and nitrogen cycling, genera in maintaining plant growth such as *Variovorax* [45] and *Pseudolabrys* [46], carbon-decomposing microbes like *Actinomadura* [47] and *Preussia* [48], phototrophic bacteria such as Cyanobacteria [49], and other beneficial taxa like *Gemmatimonas*, *Pseudonocardia*, and *Simplicillium* [50–52] had higher succession rates in the invasive treatment. This evidence suggests that soil microbial communities in alien invasive plants

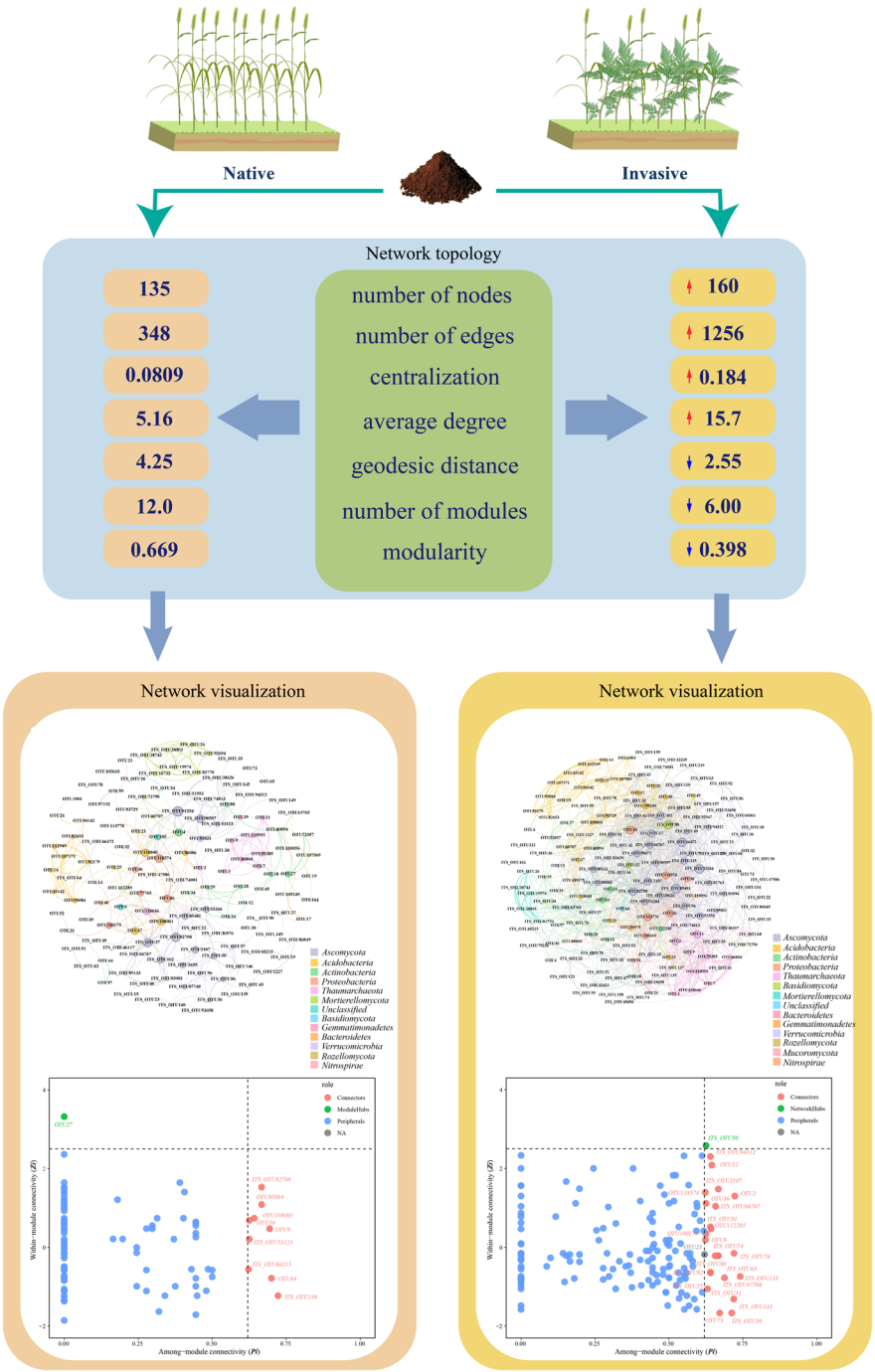

Fig. 4 Co-occurrence networks of microbial communities in the native treatment (left panel) and the invasion treatment (right panel). Connections represent significant (Spearman's $r^2 > 0.24$, $P < 0.01$) correlations. The label of each node is colored according to taxonomic affiliation, and its size is proportional to the number of connections (i.e., degree). Microbial communities include both bacterial and fungal communities. Labels with ITS indicate that these OTUs are derived from fungal communities

underwent succession over time to a unique edge in cultivating beneficial microbiota that increases resource availability.

Conversely, plants typically defend against pathogen attacks by utilizing specific receptors to identify pathogens [53]. It has been demonstrated that invasive alien plants are infected by 84% fewer fungi in invaded

habitats than in their native ranges [54]. This observation supports the "enemy release hypothesis," [5] which proposes that invasive plants are liberated from natural enemies, including pathogens in invasive areas. Similarly, many genera in our study with lower turnover rates in the invasive treatment were pathogenic microbes. Such examples included *Cyphellophora* causing sooty blotch and flyspeck disease in plants [55], soil-borne pathogen *Plectosphaerella* [56], *Acrocalymma* causing crown and root disease [57], *Penicillium* causing a postharvest plant disease named green mold [58], the pathogenic bacteria *Nocardia* [59], and the soil-borne pathogen *Paraphoma* [60].

The asymmetric succession dynamics between beneficial microbiota and pathogenic microbes support our hypothesis that alien plant invasion leads to a nonuniform succession dynamic in various microbial groups, specifically activating those that promote plant growth over time. One possible reason for this nonuniform succession is that plants recruit beneficial microbiota through root exudates and molecules recognized by these microbiota [53]. Certain exudates act as selective recruiters for specific microbial taxa or enrich the rhizosphere with organic carbon, attracting beneficial microbiota. Many invasive alien plants, including *A. artemisiifolia* and *B. pilosa* in our study, secrete allelopathic compounds from their roots [6, 61]. These compounds not only pose a threat to native flora due to their strong phytotoxic activities but also serve as carbon substrates in the soil, attracting specific microbiota. For instance, root residues in *A. artemisiifolia* are rich in sesquiterpenes [61], which increase the abundance of bacterial functional groups involved in carbon and nitrogen metabolism [62]. Additionally, the higher total carbon content in the litter of invasive alien plants compared to native plants may provide more opportunities for decomposer proliferation. For example, the carbon content in the litter of *B. pilosa* is 822.94 g/kg, substantially higher than in *S. viridis* (737.11 g/kg)[65]. To further investigate the reasons behind the asymmetric succession in soil microbial communities, metabonomic analyses and laboratory experiments are needed to identify chemicals that attract beneficial microbiota or suppress pathogenic microbes.

Microbial communities undergo divergent succession trajectories

Long-term experiment systems provide insights into the directionality and convergence of community assembly [33]. We expected a deterministic process to dominant the structuring microbial succession in the invasive treatment due to selective pressure from root exudates [64]. Consistently, alien plant invasion significantly increased the deterministic ratio in assembling bacterial communities. While the stochasticity is higher for fungi than for bacteria in our study, this could be explained by high asexual reproduction, rapid dispersal rates, and resistance to extinction in fungi [65]; furthermore, the much larger size of fungi compared to bacteria may result in lower cell counts and thus less diverse communities [66]. Under deterministic perspective, microbial succession is directional and dissimilar between treatments [67]. Consequently, we observed divergent successional trajectories between native and invasive treatments. Furthermore, our results demonstrate that microbial communities in the invasive treatment evolved towards a particular state that potentially benefits plant growth. This phenomenon highlights the intricate interplay between microbial dynamics and alien plant invasions, contributing to the ecological success of invasive alien species. Network analysis is a valuable tool for identifying and understanding species associations and interactions in complex systems [34, 68]. Higher average degree and average density indicate a more complex network [38], while larger network centralization reflects a more centralized topology. All these topology indices were higher in the invasive treatment than in the native treatment, suggesting that alien plant invasion compacted microbial communities, resulting in nodes being more closely connected. This increased complexity may enhance the adaptability and resilience of invasive alien plants in various environments, underscoring the potential role of microbial interactions in the ecological success of invasive species.

In ecological network analyses, module hubs and connectors are critical elements. Module hubs, with numerous connections within their modules, and connectors, with high links to multiple modules, are identified as microbial keystone taxa [69]. These keystone taxa typically play important roles in communities. In our study, *Ramlibacter* was a keystone taxon in both native and invasion treatments, which plays a pivotal role in soil N cycling [70]. Additionally, *Periconia*, *Cladosporium*, and *Penicillium*, which are plant-pathogenic fungi, were among the 10 keystone taxa of the native treatment. Therefore, alien plant invasion not only causes divergent microbial compositions but also leads to divergence in soil microbial community interactions.

Conclusion and implications

Our findings provide robust evidence supporting the dynamic aspect of plant microbiome assembly (Fig. 5). Alien plants enhance microbial diversity, accelerate microbial succession, form beneficial associations, and mitigate soil pathogen limitations. Notably, turnover rates vary among microbiota, reflecting their distinct

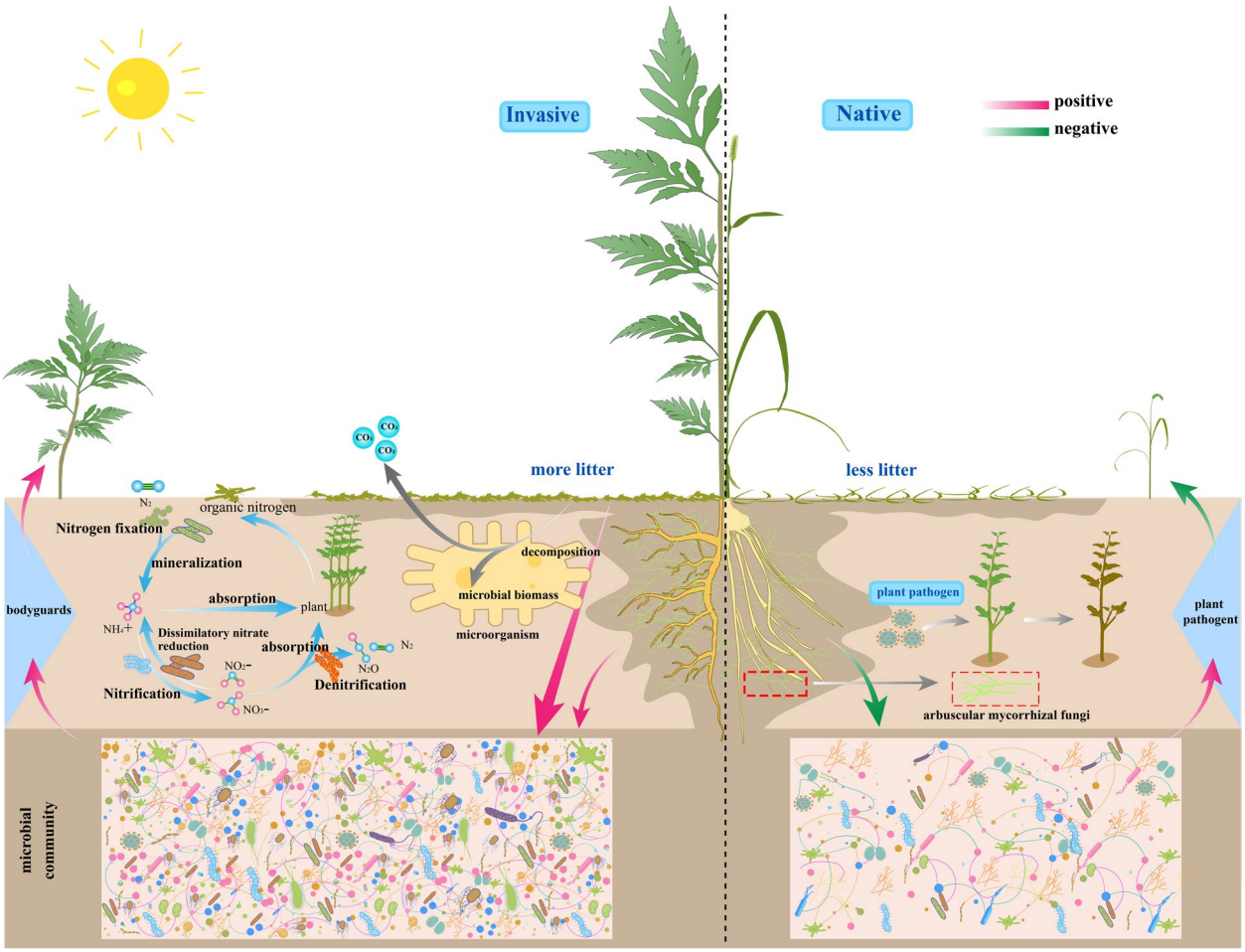

Fig. 5 The asymmetric succession in soil microbial communities due to alien plant invasion. The invasion of alien plants diversifies soil microorganisms and enhances their associations through litter and root effects. It also accelerates the divergent succession of microbial communities by activating beneficial microbiota related to nutrient cycling and mutualistic symbiosis

ecological roles. This comprehensive perspective suggests that the spread of invasive alien plants is facilitated by a strategically assembled microbial support system, offering a comprehensive and multifaceted view of the invasive mechanisms. Understanding the microbial interactions that contribute to alien plant invasion can inform the development of biological control methods, such as introducing natural enemies of specialized pathogens or herbivores. While Asteraceae species are among the most predominant invasive alien plants, the asymmetric succession in soil microbial communities requires verification through additional research involving a broader range of plant categories. With sufficient information, we may be able to predict the ecological consequences of microbial community succession, particularly in scenarios where invasive plants are leading to a global homogenization of plant species.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
FW and MZ developed the study concept and design; QL collected the samples and completed the DNA preparation and environmental genomics experiments; MZ performed data analyses and drafted the manuscript, HZ and XZ performed the data illustration. WL, YY and XG revised the manuscript. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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