An invasive plant rapidly increased the similarity of soil fungal pathogen communities

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- **Background and Aims:** Plant invasions can change soil microbial communities and affect subsequent invasions directly or indirectly via foliar herbivory. It has been proposed that invaders promote uniform biotic communities that displace diverse, spatially variable communities (the biotic homogenization hypothesis), but this has not been experimentally tested for soil microbial communities, so the underlying mechanisms and dynamics are unclear. Here, we compared density-dependent impacts of the invasive plant *Alternanthera philoxeroides* and its native congener *A. sessilis* on soil fungal communities, and their feedback effects on plants and a foliar beetle.
- **Methods:** We conducted a plant–soil feedback (PSF) experiment and a laboratory bioassay to examine PSFs associated with the native and invasive plants and a beetle feeding on them. We also characterized the soil fungal community using high-throughput sequencing.
- **Key Results:** We found locally differentiated soil fungal pathogen assemblages associated with high densities of the native plant *A. sessilis* but little variation in those associated with the invasive congener *A. philoxeroides*, regardless of plant density. In contrast, arbuscular mycorrhizal fungal assemblages associated with high densities of the invasive plant were more variable. Soil biota decreased plant shoot mass but their effect was weak for the invasive plant growing in native plant-conditioned soils. PSFs increased the larval biomass of a beetle reared on leaves of the native plant only. Moreover, PSFs on plant shoot and root mass and beetle mass were predicted by different pathogen taxa in a plant species-specific manner.
- **Conclusion:** Our results suggest that plant invasions can rapidly increase the similarity of soil pathogen assemblages even at low plant densities, leading to taxonomically and functionally homogeneous soil communities that may limit negative soil effects on invasive plants.

Key words: Above- and below-ground interactions, biotic homogenization, community divergence, density-dependent impacts, fungal pathogens, plant invasions, plant–soil feedbacks, rapid responses.

INTRODUCTION

Non-native plant invasion is recognized as an important driver of species loss in terrestrial ecosystems (van Hengstum *et al.*, 2014). Of particular importance, some invasive species increased the taxonomic similarity among invaded communities, known as biotic homogenization, at local and regional scales, owing to increases in their abundance and decreases in native species abundance (Winter *et al.*, 2009). Biotic homogenization could further increase the susceptibility of ecosystems to environmental change, representing a severe threat to biodiversity, and may trigger unpredictable but important ecological and evolutionary consequences (Olden *et al.*, 2004). To date, however, the majority of existing studies evaluating the ecological consequences of plant invasions have focused on above-ground biotic communities, and the extent to which soil biotic communities are affected by non-native plant invasions has been less explored (Vilà *et al.*, 2011; Simberloff *et al.*, 2013). Even less

is known about whether plant invasions can rapidly increase the taxonomic similarity among soil biotic communities (but see Dickens *et al.*, 2013; Piper *et al.*, 2015; Lu *et al.*, 2018; Le Roux *et al.*, 2018).

Soil biota, a critical determinant of species diversity and ecosystem stability, are particularly sensitive to environmental changes, such as non-native plant invasions (Veresoglou *et al.*, 2015). Soil biotic communities can be modified by plants in a species-specific and density-dependent manner as a function of root exudates and volatiles or changes in soil properties (Sasse *et al.*, 2018; Schulz-Bohm *et al.*, 2018). For example, some non-native plant invasions have decreased or increased the richness and α -diversity of soil microbial communities (Broz *et al.*, 2007; Lekberg *et al.*, 2013), and restructured interaction networks among soil microbes (Mamet *et al.*, 2017, 2019). However, only a few studies so far have reported that non-native plant invasions increased the similarity among soil microbial

communities in the field (Dickens *et al.*, 2013; Piper *et al.*, 2015; Lu *et al.*, 2018; Le Roux *et al.*, 2018), potentially leading to biotic homogeneity. However, the results of these studies may be confounded by the diverse abiotic and biotic environments, invasion histories and soil microbial communities in the field. Moreover, the impacts of plants on soil microbial communities might be density-dependent (Petermann *et al.*, 2008). For instance, high-density populations of some plants accumulate species-specific pathogens, thus decreasing the per capita fitness of these plants (the Janzen–Connell effect) (Petermann *et al.*, 2008; Chen *et al.*, 2019). Therefore, manipulative studies across plant densities are essential for disentangling the mechanisms, dynamics and consequences of changes in soil microbial composition due to plant invasions.

Changes in soil biotic community composition as a result of plant invasions could negatively, neutrally or positively affect subsequent plants (referred as plant–soil feedback, PSF) (van der Putten *et al.*, 2013), depending on the relative contributions of soil pathogens, soil mutualists and plant functional traits (e.g. defence) (van der Putten *et al.*, 2016; Lu *et al.*, 2018). Soil biota are expected to promote plant invasions because of the non-native plants' evolutionary novelty (Callaway and Ridenour, 2004). However, empirical evidence is inconclusive. A body of studies reported that invasive plants perform better than native plants in their 'home soil' (i.e. soil conditioned by plants of the same species), apparently as a result of release from their specialist enemies or the accumulation of generalist mutualists (Inderjit and van der Putten, 2010). However, others have found neutral or even strong negative PSFs on invasive plants, possibly due to accumulation of pathogens or a lack of necessary mutualists (Nuñez *et al.*, 2009; Callaway *et al.*, 2013). Moreover, soil microbes can also influence foliar herbivores via host plants and in turn indirectly affect plant invasions. The role of herbivores in plant invasions has been well recognized (Maron and Vila, 2001; Mitchell *et al.*, 2006). For instance, non-native plants experiencing relatively weaker herbivory from resident herbivores than native plants is considered an important reason underlying plant invasions (the enemy release hypothesis) (Keane and Crawley, 2002). But how soil biota affects foliar herbivores and in turn affects plant invasions has been little explored (Lu *et al.*, 2018). Therefore, the role of soil biota, indirect impacts via foliar herbivory in particular, in non-native plant invasions remains an open debate.

Understanding the mechanisms of PSF is critical for predicting the role of soil biota in non-native plant invasions. However, our knowledge is limited, leaving the direction and strength of PSF largely unpredictable (De Long *et al.*, 2019). Recent studies with native plants have found linkages between the strength of PSFs and soil biota community diversity, the abundance of a specific soil biota, and plant root traits (e.g. the root-to-shoot mass ratio) (Semchenko *et al.*, 2018; Carrillo *et al.*, 2019; Wilschut *et al.*, 2019). For example, in a study with eight intracontinentally range-expanding and native *Geranium* species, Wilschut *et al.* (2019) found that variations in PSFs among these species could be predicted by the abundance of the root nematode *Meloidogyne hapla* in their rhizosphere. Thus, variations in root traits or interactions with differing groups of soil biota might contribute to the observed different PSFs between native and non-native plants. However, no study so far has explored and compared the biotic mechanisms underpinning

PSFs between phylogenetically related native and non-native invasive species.

To address these gaps in knowledge, we studied the interactions of soil biota with the invasive plant *Alternanthera philoxeroides* and its native congener *A. sessilis* in China. In a previous study with field-collected rhizosphere soil spanning 15 latitudinal degrees, we found that bacterial and pathogenic fungal communities were more taxonomically similar for pairs of rhizosphere soils associated with the invasive plant than for pairs of rhizosphere soils associated with the native plant, suggesting that *A. philoxeroides* invasion might have increased the taxonomic similarity of these soil biotas at large scales (Lu *et al.*, 2018). Moreover, soil biota had a greater negative impact on the native versus invasive plants and on the introduced beetle (*Agasicles hygrophila*) feeding on the native versus invasive plants (Lu *et al.*, 2018), suggesting that the soil biota can promote *A. philoxeroides* invasions directly or indirectly via foliar herbivores. However, the soil fungal and bacterial communities as well as climate and soil properties changed with latitude (Lu *et al.*, 2018), so the observed changes in the soil biotic community plus the PSFs on plants and the foliar beetle might be due to the interactions of these factors and plant invasions. Moreover, the mechanisms underlying plant–soil interactions for the native and invasive species remain unknown.

To test the direct and indirect (via foliar herbivory) reciprocal plant–soil interactions and explore the underlying mechanisms, we conducted a PSF experiment and a laboratory bioassay to examine PSFs associated with the native and invasive plants and the introduced beetle. We also characterized the soil fungal community using high-throughput sequencing to evaluate the effects of native and invasive plants at varying densities on the richness and taxonomic composition of total, saprotrophic, pathogenic and arbuscular mycorrhizal (AM) fungi. To explore the potential mechanisms of the PSFs, we explored the linkages between PSFs and the richness and abundance of these functional guilds of fungi. Specifically, we predicted that (1) the invasive plant could quickly increase the taxonomic similarity of soil pathogen assemblages; (2) the invasive plant would perform better than the native plant when grown in invasive plant-conditioned soil (i.e. home soil advantage); (3) soil biota would increase the performance (e.g. larval mass) of insects feeding on detached leaves of the native plant only, regardless of the identity of the conditioning plant, as we found before (Lu *et al.*, 2018); and (4) PSFs on the native plant and the beetle feeding on it would be predicted by the abundance of soil pathogens, but this pattern would be absent for the invasive plant given its greater defence against root infection (Lu *et al.*, 2015b).

MATERIALS AND METHODS

Study species

Alternanthera philoxeroides (alligatorweed, Amaranthaceae) has invaded an area up to 36.8° N in China (Lu *et al.*, 2015a). *Alternanthera philoxeroides* frequently co-occurs with its native congener *A. sessilis* in terrestrial habitats as well as in aquatic habitats. In China, *A. philoxeroides* is a noxious weed in land, farmland in particular, where it is hard to manage because of its high tolerance of abiotic and biotic

stresses in terrestrial habitats (Lu and Ding, 2010). In comparison with the native plant, the invasive plant is more resistant to root infection, potentially due in part to its higher coarse-to-total root mass ratio (Lu *et al.*, 2015b). Both species are also damaged by the specialist beetle *Agasicles hygrophila* (Chrysomelidae), which was introduced to control *A. philoxeroides* (Lu *et al.*, 2015a).

Plant–soil feedback experiment: conditioning phase

We conducted the PSF experiment in Wuhan (Hubei province, China; 30.48° N 114.35° E), where the native plant propagates solely via seeds and the invasive plant propagates solely via clonal stems. The PSF experiment consisted of conditioning and feedback phases. For the conditioning phase, we collected top soil (10 cm) from a nearby abandoned agricultural field (both species are commonly found in this type of habitat) where neither the native nor the invasive plants had occurred, to avoid potential legacy effects of either species on the results. In the laboratory we sieved and homogenized the soil and used it to fill 72 5-L experimental pots (25 cm diameter and 20 cm depth). We planted similar-sized (~10 cm length) native or invasive plants at densities of one, three and five individuals per pot, hereafter referred to as low, middle and high densities, respectively. The native and invasive plants were propagated from Wuhan field-collected seeds and cut stems (~5 cm length), respectively, in trays in a naturally lit greenhouse at Huazhong Agricultural University in Wuhan. We filled the trays with a mixture of peat and sand (2:1 in volume) that had been sterilized by γ -irradiation (dose >25 Gy).

We then positioned all pots randomly in a naturally lit greenhouse. We replicated each treatment combination (2 species $\times$ 3 densities) 12 times, but mortality during the trial caused the final replicates for the treatment combinations to range from eight to ten, resulting in 53 pots in total. Odds of a plant surviving did not depend on species ($F_{1,66} = 0.1$, $P = 0.7561$), density ($F_{2,66} = 0.5$, $P = 0.6017$) or their interaction ($F_{2,66} = 0.1$, $P = 0.9141$). During the experiment we watered the plants when necessary. Five months later, we removed all plant materials (including roots and shoots). We collected soils from each pot and kept them separately for the feedback experiment, and stored a subsample at -20°C for DNA extraction. We bleached and rinsed the soil collection tools (with gloved hands) between pots to reduce cross-contamination among sets of conditioned soils.

Plant–soil feedback experiment: feedback phase

We conducted the second phase of the feedback experiment in April 2018 in the same greenhouse. For this phase, we divided the soil from each of the first-phase pots into 12 parts that were each assigned to one of the following treatments (each replicated three times): (1) sterilized soil (the same source as in the conditioning phase) + native plant; (2) sterilized soil + invasive plant; (3) non-sterilized soil + native plant; and (4) non-sterilized soil + invasive plant. In total there were 636 pots (53 conditioned soils $\times$ 2 test plant species $\times$ 2 sterilization treatments $\times$ 3 replicates). For each second-phase pot, we mixed 300 mL of conditioned soil with 700 mL of a sterilized peat

and sand mixture (3:1 by volume). For sterilized soils pots, we treated the soil and mixture of peat and sand with γ -radiation (dose >25 Gy). We planted a single invasive or native plant (~10 cm length) grown in sterilized peat and sand mixture (see above for details) in each pot. Four months later, we collected 20 fully opened leaves (mass of these leaves was <1 % of individual plant mass; thus, their mass was not included in plant mass) from the top of each plant in each pot and stored them (for a maximum of 9 d) at 4°C for the beetle bioassay. Because there were not enough leaves in some pots, we pooled the plant leaves collected from pots of the same treatment combinations. After that, we collected plant shoots then rinsed the roots to remove soil, dried shoots and roots at 80°C for 48 h and weighed them.

DNA sequencing

We characterized the fungal community for the soil samples collected from each pot at the end of the conditioning phase as in Lu *et al.* (2018). Briefly, DNA was extracted from the soil samples, and internal transcribed spacer 2 (ITS2) genes were amplified with the primers gITS7 (5'-GTGARTCATCGARTCTTTG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). For each sample, a unique 12-bp barcode was added at the 5'-end of the reverse primer and two PCR reactions were conducted. The PCR products were purified, pooled for each DNA sample, and then prepared with a TruSeq DNA kit (Sangon Biotech, China) and sequenced with the Illumina MiSeq platform at the Environmental Genome Platform of Chengdu Institute of Biology, Chinese Academy of Sciences (for details of sequencing see Li *et al.*, 2016).

Raw sequences were analysed with the QIIME pipeline (<http://qiime.org/>). All reads were trimmed and assigned to each sample based on their unique barcodes, and the two paired-end reads were merged using the FLASH-1.2.8 software. High-quality merged sequences (read length was ~340 bp, without ambiguous base N) were clustered into operational taxonomic units (OTUs) against the UNITE fungal database with 97 % identity as a cutoff, and chimeric sequences were removed with the UCHIME algorithm. All sequences were resampled to 10 000 reads per sample and OTU taxonomy was assigned with the Ribosomal Database Project (RDP) Classifier program (<http://rdp.cme.msu.edu/>) with the confidence cutoff of 80 % (for details of the bioinformatics analysis see Zhang *et al.*, 2018). Classification of the zygomycete fungi was updated according to Naranjo-Ortiz and Gabaldón (2019). This phylogeny differs from that of Spatafora *et al.* (2016), which has the Glomeromycota as the subphylum Glomeromycotina within the Mucoromycota. Fungal OTUs were split into putative saprotrophs and pathogens based on the data available in FUNGuild (Nguyen *et al.*, 2016). Glomeromycota were assigned as AM fungi. Original sequencing reads have been deposited in the NCBI database (SRP283306).

Laboratory bioassay

To test the soil biota impacts on the beetle via native and invasive plants, we conducted a bioassay in the laboratory with

the plant leaves collected in the PSF experiment for each treatment combination. This laboratory bioassay is a powerful way to test how variations in plant palatability or defence impact insect performance (Hoskins and Craig, 1962). We used offspring of Wuhan field-collected *A. hygrophila* that had been raised on *A. philoxeroides* for one generation in the laboratory. We individually transferred newly hatched larvae into 9-cm-diameter Petri dishes (one Petri dish per leaf) lined with moist filter paper. We placed the Petri dishes in a laboratory with the temperature held at 28 °C and a natural light/dark photoperiod (~14:10 h). We kept the filter papers moist, and replaced leaves with intact leaves collected from plants incubated in soils that received the same conditioning treatment every other day. After 10 d we finished the experiment, and weighed the beetles after 24 h of starvation.

Data analysis

We conducted analyses with SAS 9.4 (SAS Institute, Cary, NC, USA). We calculated OTU richness of the total, saprophytic, putative pathogenic and AM fungal communities. We tested their dependence on conditioning species identity, density and their interactions with ANOVA. We calculated the Euclidean dissimilarity, which reflects both the presence/absence of OTUs and changes in OTU abundance (i.e. number of reads), between pairs of fungal communities. We then performed ANOVAs to test the dependence of pairwise taxonomic dissimilarity on combinations of conditioning plant species identity, density and their interactions. We also tested the impact of conditioning plant species identity, density and their interaction on the abundance of the 30 most abundant fungal genera classified as putative pathogens and the total relative abundance of rare putative pathogenic genera (each relative abundance was <0.01 %) with ANOVA. When significant interactive effects occurred in ANOVAs, we examined differences among treatment combinations with adjusted means partial difference tests ($P < 0.05$).

For the PSF experiment, we averaged the data for plant total, shoot and root mass and beetle mass across the three replicates for each of the 212 treatment combinations. Then we quantified PSFs on plant total, shoot and root mass and larval beetle mass as their $\ln$ response ratios between non-sterilized and sterilized soils in the feedback phase, where values higher or lower than zero would be evidence of positive or negative feedback, respectively. We conducted mixed ANOVAs to test the dependence of PSFs on the fixed factors of conditioning species identity and density, tested species identity, their interactions and the random factor of conditioning pot nested within conditioning species identity and density. We conducted a similar mixed ANOVA to test the impacts of these factors and soil sterilization treatments on plant root-to-shoot mass ratio. In addition, we conducted one-sample Student's t -tests to test whether PSFs differed from zero for the two species.

To explore the potential linkages between PSFs and the community of soil functional guilds, we ran multiple regressions against the abundance (i.e. number of OTU reads) of the pathogen genera that were present in >60 % of samples (ten

genera) and the richness of AM fungi for the native and invasive species or beetle feeding on them separately. The best models were then selected with the stepwise method. We also regressed PSFs with plant root-to-shoot mass ratio for the native and invasive plants separately, and compared the slopes with ANCOVAs. Data were transformed (e.g. square-root transformed) when necessary to improve normality or variance homogeneity.

RESULTS

Ascomycota was the most abundant phylum, followed by Mucoromycota and Basidiomycota, and their abundances were not affected by the conditioning treatment (Table S1, Fig. S1A). Overall, 83.3 % of OTUs could be assigned to a genus (520 in total; the remaining ~17 % could not be assigned because a large number of fungal taxa in the soil remain unknown), with *Mortierella* the most abundant, followed by *Platyglaea* (Fig. S1B). Of the ten most abundant genera, *Humicola* and *Fusarium* had relative abundances that were interactively affected by conditioning species identity and density (Table S2, Figs S2 and S3A).

Conditioning species identity and density interactively affected the overall fungal species richness along with that of putative pathogenic and saprophytic fungi, but had no impact on the richness of AM fungi (Table S3, Fig. 1). The overall richness (Fig. 1A) and the richnesses of putative pathogenic (Fig. 1B) and saprophytic (Fig. 1C) fungal communities were highest when the soil was conditioned by the invasive plant at low density, and the richnesses of putative pathogenic (Fig. 1B) and saprophytic (Fig. 1C) fungal communities were lowest when the soil was conditioned by the invasive plant at middle density.

The taxonomic compositions of the putative pathogenic and AM fungal communities depended on the interaction of conditioning species identity and density (Table S4, Fig. 2). Soils conditioned by the native plant at a high density developed more distinct pathogen communities than soils conditioned by the native plant at lower densities and by the invasive plant, especially at high density (Fig. 2B). Native plant-conditioned soils at high density shared 55.0 and 56.1 % pathogen OTUs with soils conditioned at middle and low densities, respectively. High-density-conditioned soils with the native plant shared 49.3 % pathogen OTUs with invasive plant-conditioned ones (Fig. S3) and had more *Fusarium* and *Plectosphaerella* fungi (Fig. S4A, D). Moreover, assemblages of pathogens varied greatly among the experimental pots that were conditioned by the native plant at high density (Fig. 2B). In contrast, assemblages of AM fungi were more dissimilar among experimental pots that were conditioned by the invasive plant at high density than among experimental pots that received other conditioning treatments, particularly soils that were conditioned by the native plant at high density (Fig. 2D).

Plant–soil feedbacks on plant total and root mass and the beetle were not affected by conditioning species identity or density or by tested species identity or density (Table S5). Plant–soil feedback generally decreased the total, root and shoot mass for the invasive plant and the total and shoot mass for the native plant, and increased beetle mass when it fed on the native plant (Fig. 3A). The

FIG. 1. Richness of (A) total, (B) pathogenic, (C) saprophytic and (D) AM fungal (AMF) OTUs after conditioning by the invasive (Invader) and native (Native) plants at low (L), middle (M) and high (H) densities for one growing season. Mean + 1 s.e. Means with the same letters did not differ in *post hoc* tests ($P \leq 0.05$).

FIG. 2. Taxonomic distance (Euclidean distance) of (A) total, (B) pathogenic, (C) saprophytic and (D) AM fungal (AMF) communities between pairs of soils conditioned by the invasive plant only (Invader = both are invader), the native plant only (Native = both are native) or different species (Different = one is invader and one is native) at varying plant density (L, low; M, middle; H, high). Mean + 1 s.e. Means with the same letters did not differ in *post hoc* tests ($P \leq 0.05$).

FIG. 3. Plant-soil feedback (PSF) effects on total, shoot and root mass of the invasive (Invader) and native (Native) plants and on beetle larvae feeding on detached leaves of the invasive and native plants (A), and PSFs on plant shoot mass when plants were cultivated in soils conditioned by conspecific (home soil) or heterospecific (foreign soil) individuals for one growing season (B). Mean $\pm$ 1 s.e. In (A) n.s. indicates no significant difference from zero ($P > 0.05$) and asterisks indicate a significant difference from zero. **, $P \leq 0.01$; ***, $P \leq 0.001$. In (B) means with the same letters did not differ in *post hoc* tests ($P \leq 0.05$).

negative PSF on shoot mass was weaker for the invasive plant than for the native plant when the plants were grown in native plant-conditioned soils, but this difference did not occur when the plants were grown in invasive plant-conditioned soils (Fig. 3B).

Plant-soil feedbacks on plant root and shoot mass and on the beetle when it fed on the detached leaves were predicted by the abundance of differing groups of pathogens for both plants and in a plant species-specific manner (Table 1, Fig. 4). Plant-soil feedback on shoot mass decreased with rising abundance of *Powellomyces* for the native but not for the invasive plant (Table 1, Fig. 4A). Plant-soil feedback on beetles feeding on the invasive plant increased with rising abundance of *Gibellulopsis* (Table 1, Fig. 4B), whereas PSF on beetles feeding on the native plant increased with rising AM fungi richness ($R^2 = 0.1104$, $P = 0.05$; Fig. 4C). Moreover, PSFs on shoot mass (invasive plant, $R^2 = 0.248$, $P = 0.0003$; native plant, $R^2 = 0.230$, $P = 0.0008$; Fig. 4D) and root mass (invasive plant, $R^2 = 0.133$, $P = 0.0129$; native plant, $R^2 = 0.095$, $P = 0.0334$; Fig. 4E) decreased with increasing plant root-to-shoot mass ratio for both plants, and there were no differences in the regression slopes between the two species ($P > 0.05$ for both). Invasive plant

root-to-shoot mass ratio was higher than that of the native plant (mean $\pm$ 1 s.e.: 0.187 ± 0.012 versus 0.138 ± 0.014 , $F_{1,175} = 17.56$, $P < 0.001$) regardless of other treatments (interactions with other treatments: $P > 0.05$).

DISCUSSION

Identifying the effects of plant invasions on the soil microbial community and the linkages between soil microbial diversity or composition and the direction or magnitude of PSFs will improve our understanding of reciprocal interactions between non-native plant invasions and soil biota. Our results indicate that rhizospheres of the native plant developed distinct and more divergent assemblages of fungal pathogens at a high plant density than at lower densities or the invasive plant at any density. These results suggest that the invasive plant may rapidly increase the taxonomic similarity of soil-borne fungal pathogens at local and regional scales, because its invasion could decrease the abundance of native plants in the field (Lu *et al.*, 2015a). In addition, the magnitudes of the PSFs on plant roots and shoots and the beetle fed on the plants were predicted by different groups of soil biota (i.e. pathogens and AM fungi) in a plant species-specific manner. This finding suggests that interactions with different groups of soil biota, as well as functional trait variation (e.g. root-to-shoot mass ratio), may underlie varying PSFs between native and non-native invasive plants.

Homogenization of soil fungal pathogens

Native plants can modify soil pathogen communities in a density-dependent manner, and plants growing at high density tend to recruit more diverse and abundant soil-borne pathogens, which has been acknowledged as an important mechanism promoting plant coexistence (i.e. the Janzen-Connell hypothesis) (Janzen, 1970). Our study extends the Janzen-Connell hypothesis by showing that native plants can develop taxonomically distinct and more variable assemblages of soil-borne fungal pathogens at high densities than at low densities, which can also promote species coexistence (Eck *et al.*, 2019). To the best of our knowledge, our study is the first to demonstrate this phenomenon. Although the underlying reason remains unclear, it is possible that the within-species genetic diversity and/or root metabolite variation, both of which can influence soil microbial composition (Sasse *et al.*, 2018; Schulz-Bohm *et al.*, 2018; Eck *et al.*, 2019), of the native plant increased at a high density and in turn promoted more specific and/or rare pathogens than at lower densities, corresponding to a high proportion of unique OTUs found in this study.

However, such a density-dependent impact on the taxonomic composition of fungal pathogens was not observed for the invasive plant. One possible reason may be that root metabolism is more variable among native plant individuals than among invasive plant individuals (Hao Xu & Xinmin Lu, unpubl. data). In addition, the invasive plant developed differing soil pathogen assemblages compared with the native plant, in line with other studies (Mangla and Callaway, 2008; Nelson and Karp, 2013). In combination with our previous study (Lu *et al.*, 2018), this finding suggests that invasion by this non-native plant can rapidly increase taxonomic similarity in the assemblages of soil-borne fungal pathogens at local and regional scales, consistent

TABLE 1. Relationship between the abundance (number of reads) of major putative pathogenic fungal genera and soil feedback effects on plant shoot mass, root mass and beetle mass for the native and invasive species. The best models were selected with the stepwise method. Coefficients are from regressions. NA means not available.

Feedback effect	Fungal genus	Coefficient	P-value	Partial R^2
Native plant	<i>Powellomyces</i>	−0.091	0.03	0.101
Shoot mass	NA			
Root mass	NA			
Beetle mass	NA			
Invasive plant	<i>Spizellomyces</i>	−0.045	0.05	0.078
Shoot mass	<i>Verticillium</i>	0.112	0.02	0.105
Root mass	<i>Gibellulopsis</i>	0.042	0.03	0.028
Beetle mass

FIG. 4. Relationships between PSF effects on plant shoot mass with the abundance of *Powellomyces* (A), on the beetle with the abundance of *Gibellulopsis* (B) and AM fungi richness (C), and on plant shoot (D) and root (E) mass with plant root-to-shoot mass ratio for the native (Native) and invasive (Invader) plants. Sqrt means the data was square-root transformed.

with our first prediction. Further studies with multiple pairs of phylogenetically related native versus non-native plants are needed to test whether the absence of density-dependent impacts on the taxonomic composition of soil pathogens or other groups of soil microbes is widespread among invasive plants in their invaded ranges. If it is common, this may explain why invasive plants generally experienced weaker density-dependent negative conspecific PSFs than native plants (Suding *et al.*, 2013) and their invasion increased taxonomic similarity in soil microbial communities in the invaded range (Dickens *et al.*, 2013; Piper *et al.*, 2015). In contrast, pairs of soils conditioned

by invasive plants at high density developed more divergent AM fungal communities than pairs of soils conditioned by native plants at high density, suggesting that non-native plant invasions imposed varying selections on different groups of soil biota.

PSFs for native and invasive plants

However, we detected no density-dependent PSFs reflecting the sum impacts of soil-borne enemies and mutualists on either the native and invasive plants when grown in soils conditioned

by them. This is likely due to the opposing impacts of pathogens and AM fungi, the short duration of the PSF experiment or the functional redundancy of many soil microbes. In this study, both native and invasive plants were negatively affected by the soil biota regardless of the identity of the conditioning plant, indicating a stronger role of soil-borne enemies than mutualists. Surprisingly, we detected no home soil advantage (i.e. lower negative effects on invasive versus native plants of soil biota from invasive plant-conditioned soils compared with sterilized soils), but rather the foreign soil advantage (i.e. lower negative effects on invasive versus native plants of soil biota from native plant-conditioned soils compared with sterilized soils) identified for the invasive plant was in contrast to our second prediction (i.e. the invasive plant would perform better than the native plant when grown in their home soils). One possible explanation is that the invasive species suppressed some host-specific soil-borne enemies of the native species, which in turn increased native plant relative performance when grown in invasive versus native plant-conditioned soils. For instance, the invasive plant decreased the relative abundance of *Fusarium* and *Plectosphaerella* compared with the native plant at high plant density. It is important to note, however, that we cannot exclude potential effects of other groups of soil biota, such as bacteria and nematodes. The results could also be sensitive to the choice of unconditioned soil to start the experiment.

Our results raise the possibility that the frequency and relative abundance of some specialist enemies of native plants may decrease in invaded communities and thus that the beneficial impacts of soil biota on non-native plant invasions may decrease over time. This may partially contribute to the decreased invasiveness of some non-native plants over time (Speek *et al.*, 2011), given that soil-borne pathogens did not accumulate with residence time (Speek *et al.*, 2015). However, such a foreign soil advantage for the invasive plant in our study may decrease over time or in stressful environments because such soil-borne enemies (e.g. pathogens) only suppressed root growth for the invasive plant. High root mass enabled the invasive plant to tolerate herbivory and abiotic stresses, which facilitate its invasion (Lu and Ding, 2010). This finding highlights the importance of exploring plant–soil interactions from a whole-plant perspective in order to increase our ability to predict long-term impacts of PSF.

Role of pathogens in PSFs

Moreover, the magnitudes of the PSFs on roots and shoots were predicted by differing pathogens for both plants in a plant species-specific manner consistent with our fourth prediction, which at least partially explained the asymmetrical responses of plant roots and shoots and the varying PSFs on the two plants. These findings are surprising but reasonable. First, plant roots associate with diverse soil organisms, some of which may originate from shoots, whereas plant shoots are exposed to foliar microbes and a subset of soil microbes (Bai *et al.*, 2015; Whitaker *et al.*, 2017). Second, plant roots and shoots could respond differently to herbivory or pathogen infections in terms of biomass allocation and defence, as predicted by the optimal defence hypothesis (Kostenko *et al.*, 2013; Mundim *et al.*, 2017). Surprisingly, the potentially varying responses of plant

roots and shoots have rarely been incorporated into above-/below-ground interaction studies. Combined with other studies (Kostenko *et al.*, 2013; Mundim *et al.*, 2017), our results suggest that considering the asymmetrical responses of roots and shoots may provide new insights into above- and below-ground connections. In addition, the higher root-to-shoot mass ratio of the invasive plant compared with the native plant also contributes to the weaker negative PSFs on total and shoot mass for the invasive plant in this study.

Impacts of PSFs on insect herbivores

Soil biota could affect foliar herbivores by changing the defence and palatability of plants (Wardle *et al.*, 2004), and in turn indirectly affect non-native plant invasions. In this study, the soil biota (regardless of the identity of the conditioning plant) increased beetle performance on the native plant but had no impact on beetle performance on the invasive plant compared with sterilized soils, which might facilitate the non-native plant invasions indirectly but needs further clarification. This is consistent with our third prediction and implies the existence of differing underlying reasons. Indeed, PSFs on the beetle were positively and negatively linked with the abundances of varying pathogens or richness of AM fungi when feeding on the two plants. Consistently, studies have found that root infection by pathogens or associations with AM fungi could increase or decrease plant defence against foliar insects depending on the interacting species and in turn affect foliar herbivores (Kostenko *et al.*, 2013; Mundim *et al.*, 2017). Our results indicate that the mechanisms underlying PSFs on foliar herbivores could also vary among host species, which may explain the observed plant species-specific above- and below-ground interactions.

Conclusions

In summary, our study found that plant invasions can rapidly modify soil fungal communities, potentially increasing taxonomic similarity in assemblages of fungal pathogens and taxonomic divergence in assemblages of AM fungi once they replace native plants and occur at high density. However, such changes in soil biotic composition did not confer a home soil advantage for the invasive species, which benefited when grown in native-conditioned soils. Furthermore, the PSFs on plant roots and shoots and foliar beetles that fed on the detached leaves of plants were predicted by differing groups of soil fungi in a plant species-specific manner. In particular, plant roots and shoots displayed asymmetrical responses to changes in the soil biota. Our results suggest that plant invasions can rapidly increase the similarity of soil pathogen assemblages even at low plant densities, leading to taxonomically and functionally homogeneous soil communities that may limit negative soil effects on invasive plants.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following: Table S1:

impacts of conditioning species identity (Cond_sp), density (Cond_dens) and their interaction on the relative abundance of the fungal phyla. The phylum Blastocladiomycota had too many zero values to conduct this analysis. Table S2: impacts of conditioning species identity (Cond_sp), density (Cond_dens) and their interaction on the relative abundance of the ten most abundant fungal genera. Significant impacts are shown in bold. Table S3: effects of conditioning species identity (Cond_sp), density (Cond_dens) and their interaction on the richness of total, putative pathogenic, putative saprophytic and AM fungal communities. Significant impacts are shown in bold. Table S4: effects of conditioning species identity (Cond_sp), density (Cond_dens) and their interaction on taxonomic distance (Euclidean distance) between pairwise total, putative pathogenic, putative saprophytic and AM fungal communities. Significant impacts are shown in bold. Table S5: effects of conditioning species identity (Cond_sp), density (Cond_dens), tested species identity (Test_sp) and their interactions and the random factor of conditioning pot nested within conditioning species identity and density on PSFs on plant total, shoot and root mass and beetle mass in the PSF experiment. Significant impacts are shown in bold. Figure S1: relative abundance of common fungal phyla (A) and the ten most abundant fungal genera (B) in soils conditioned by the invasive and native plants at low, middle and high densities for one growing season. Figure S2: relative abundance of the fungal genus *Humicola* in soils conditioned by the invasive and native plants at low, middle and high densities for one growing season. Mean + 1 s.e. Means with the same letters did not differ in *post hoc* tests ($P \leq 0.05$). Figure S3: Venn diagram of unique and shared pathogen OTUs between soils that had been conditioned by the native and invasive plants at low, middle and high densities for one growing season. Figure S4: relative abundance of putative pathogenic fungal genera (A) *Fusarium*, (B) *Leptosphaeria*, (C) *Curvularia*, (D) *Plectosphaerella*, (E) *Ilyonectria* and (F) all rare genera (relative abundance of each genus is $<0.01\%$) in soils conditioned by the invasive and native plants at low, middle and high densities for one growing season. Mean + 1 s.e. Means with the same letters did not differ in *post hoc* tests ($P \leq 0.05$).

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