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Plant landscape abundance and soil fungi modulate drought effects on plant–soil feedbacks

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Plant–soil feedbacks (PSF) play an important role in determining plant community structure and dynamics. However, previous studies have provided mixed results for the relationship between PSF and plant landscape abundance (i.e. abundance across local communities). This may reflect the mediation of climate factors on PSF. Here, we tested how PSF of tree species varied with local abundances by growing seedlings in conspecific versus heterospecific soil and how simulated drought altered PSF–plant abundance relationships. Six tree species were selected and half of the seedlings were grown under ambient moisture conditions, while the others experienced a 2-month period of drought following 3-months of growth under ambient moisture conditions. Fungal communities in the rhizosphere soil were analysed using DNA amplicon sequencing to link shifts in soil fungi to the observed PSF. We found that drought reduced negative PSF for all plant species except one species (*Lithocarpus lohangwu*). In the drought treatments, PSF were positively correlated with the relative abundance of total putative pathogens, but negatively correlated with the proportion of unique pathogens (those pathogens that were present in conspecific soil rather than heterospecific soil, thereby potentially species-specific). In addition, we found that PSF only significantly predicted plant relative abundance in the drought treatment, indicating that abiotic stress made PSF a stronger predictor of plant landscape abundance. This finding also implies that future extreme drought events could promote the dominance of the abundant plant species, thereby leading to the loss of biodiversity. Collectively, our results provide evidence for microbial mechanisms of PSF and suggest that accounting for abiotic stress can make PSF a stronger predictor of plant landscape abundance due to the omnipresence of stress under natural conditions.

Keywords: drought, plant diversity maintenance, plant landscape abundance, plant–soil feedbacks, plant–soil interactions, specific pathogens

Introduction

Plant species change soil abiotic and biotic conditions in ways that either promote or harm plants via positive or negative plant–soil feedbacks (PSF). These PSF can

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be measured by comparing plant performance in conspecific versus heterospecific soils (van der Putten et al. 2013). Negative PSF can contribute to plant species coexistence via negative density-dependence (Bever et al. 1997). Species average PSF has been linked to plant landscape abundance (i.e. abundance across local communities), and connections between PSF and plant landscape abundance provide an important test of the potential role of PSF in regulating local community dynamics. However, mixed results for the relationships are illustrated, such as positive (Klironomos 2002, Mangan et al. 2010, Kulmatiski et al. 2017, Heinze et al. 2020), negative (Maron et al. 2016) and neutral relationships (Reinhart 2012). A recent meta-analysis illustrates that globally, plant landscape abundance has a weakly positive relationship with PSF (Reinhart et al. 2021). These inconsistent findings may be the result of variation in environmental conditions across ecosystems (Smith-Ramesh and Reynolds 2017, De Long et al. 2019). Understanding which environmental conditions change the direction of PSF can largely enhance our understanding of how PSF contribute to plant community dynamics.

Outcomes of PSF often depend on interactions between plants and soil microbes (Ke et al. 2015, van der Putten et al. 2016, Smith-Ramesh and Reynolds 2017). Soil microbial communities consist of common species that are associated with a range of plant species and unique/specific species that affect only a few plant species. When plant species are negatively affected by host-specific pathogens, they may be able to escape those pathogens when grown in heterospecific soil, negative PSF occur. However, when plant species share common pathogens, neutral PSF are expected because plants cannot escape from pathogens in heterospecific soils. Because of this, unique pathogens likely have more pronounced effects in driving population dynamics of host plants than common pathogens (Bever et al. 2015, Ricklefs 2015, 2016, but see Hersh et al. 2012). Accumulations of unique symbiotrophs (e.g. mycorrhizal fungi) and saprotrophs (i.e. decomposers) generally produce positive PSF via increasing plant nutrient acquisition and releasing nutrients from organic matter, respectively (van der Putten et al. 2016, Bennett and Klironomos 2019, Chen et al. 2019, Liang et al. 2019). Overall, the relationship between PSF and plant abundance will be positive if abundant plant species suffer from more common pathogens and less host-specific pathogens than less-abundant species, and it will be negative if abundant plants suffer from more host-specific pathogens. Therefore, understanding how common and unique microbes drive PSF would be of particular importance for predicting the relationships between PSF and plant abundances.

Plant–soil feedbacks can be altered by environmental context, such as drought (Fry et al. 2018, Xi et al. 2018, Snyder and Harmon-Threatt 2019, Crawford and Hawkes 2020), which is a key climate change factor in terrestrial ecosystems and influences plant ecophysiology and soil microorganisms (Kaisermann et al. 2017, Kannenberg and Phillips 2017). Chronic drought (drought persists in a low or moderate strength) may select for increases in mycorrhizal fungi, potentially increasing positive feedback effects (Kivlin et al. 2013, Revillini et al. 2016). Under wetter conditions, soil pathogens are more diverse and abundant (Augspurger and Kelly 1984, Tedersoo et al. 2014), and thus negative PSF should be more prevalent (Bennett and Klironomos 2018). Extreme drought might contribute to negative PSF as well (Suzuki et al. 2014, Kaisermann et al. 2017), as plants may become more susceptible to soil pathogens due to increased stress and some host-specific pathogens can adapt quickly to drought stress (Chakraborty 2013). To date, however, few studies have explicitly explored how drought effects on PSF vary among plant species with different landscape abundances. It will be of particular importance to fill this gap, as drought events, as a key driver of climate change, are increasing in frequency and exert long-term and vital influences on ecosystem function and community structure (Jentsch et al. 2007).

Here, we investigated how simulated drought influenced seedling performance of tree species with different relative abundances in soil conditioned by conspecific or heterospecific adults. We used six tree species which cooccur in the Heishiding 50-ha forest dynamics plot. The seedling phase is of high importance for plant establishment and regeneration, and seedling performance determines the temporal dynamics of species landscape abundance. Soil fungal communities were measured because soil fungi play crucial roles in driving plant–soil feedbacks (Semchenko et al. 2018). We addressed three key and complementary questions: 1) does drought alter PSF and the PSF–plant landscape abundance relationship? And 2) how do both unique and common soil fungal guilds (pathogens, symbiotrophs and saprotrophs) drive the variation of PSF under different watering conditions?

Material and methods

Experimental design

The experiment was performed at the Heishiding Ecological Station of Sun Yat-Sen University (23°46'N, 111°90'E). The climate is sub-tropical with a mean annual precipitation of 1532.8 mm and a mean annual temperature of 19.4°C. The experiment consisted of three factors: soil inoculum (conspecific soil, heterospecific soil), soil water (ambient, drought) and plant species identity. We chose six plant species based on seed availability and landscape commonness in a 50-ha permanent forest dynamic plot, including three arbuscular mycorrhizal (AM) plants (*Canarium album*, *Altingia chinensis* and *Litsea lancilimba*) and three ecto-mycorrhizal (ECM) plants (*Castanopsis fissa*, *Castanopsis hystrix* and *Lithocarpus lohangwu*) (Brundrett 2009). The subtropical forest plot is highly diverse, with 250 tree species in total. We selected these plant species, because these species represent a large range of plant landscape abundance (*L. lancilimba*, *A. chinensis*, *L. lohangwu*, *C. album*, *C. hystrix* and *C. fissa* are ranked as the 3rd, 14th, 48th, 57th, 73rd and 119th abundant species, respectively) and showed no apparent spatial segregation across the landscape. Plant abundance was recorded through a census for all individual trees and shrubs in the Heishiding plot in 2017.

In November–December 2018, we collected seeds from 6–8 random individuals for each tree species across the forest plot. All seeds were surfaced-sterilized to eliminate potential confounding effects on PSF by introduction of seed-borne soil organisms (1 min 75% ethanol, 3 min 2% NaOCl, 1 min 75% ethanol and 3 min distilled water) and stored at 4°C prior to experiments. In March 2019, seeds were germinated in pots filled with sterilized sand, and left to grow for two weeks. Each treatment combination was replicated six times, resulting in a total of 144 pots.

Soil inoculum collection

In June 2019, we randomly selected 12 focal adult trees for each species in the 50-ha plot, and each tree was at least 30 m apart from other conspecific trees. We collected four soil samples (15 cm depth) underneath each focal tree (Supporting information), pooled the soil samples and used as one conspecific soil inoculum. Using an approach similar to that described in Bennett et al. (2017), for each focal tree, four heterospecific adult trees (i.e. any heterospecific tree species located in the forest plot, not necessarily the other five tree species used in this experiment), were randomly selected within 10 m distance from the focal tree (Supporting information). One soil sample was collected underneath each heterospecific tree and the four soil samples were pooled as one heterospecific soil inoculum, which was used as a comparison to each individual conspecific soil feedback pairing (Supporting information). One sampling site consisted of one pair of conspecific and heterospecific soil inoculums of one plant species, and there were 6 plant species × 12 sampling sites = 72 sampling sites. This sampling approach allowed us to have real replicates for conspecific and heterospecific soil inoculums considering random spatial variation of soil properties in the forest plot, thus avoiding the potential disadvantages associated with mixing soil samples across experimental units (i.e. individual conspecific adults in this present study) and improving precise estimates of soil microbial effects on plants (Reinhart and Rinella 2016). We measured individual PSF as the difference of plant growth between individual conspecific soil and its heterospecific soil pairing. Soil samples had substantial fine roots (i.e. roots with a diameter of two millimetres or less, Gu et al. 2016). Large organic debris and roots were removed from soil samples, and we kept fine roots because they host diverse rhizosphere microbes. In total, we collected 72 pairs of conspecific and heterospecific soil samples, and stored at 4°C prior to seedling transplanting.

Soil feedback experiment

Background soil was collected in multiple sites in Heishiding forest, mixed and homogenized to remove litter and stones. All background soil was sterilized with gamma radiation (35 KGy). Gamma irradiation has been shown to effectively sterilize soil with minimal impacts on other soil properties (Berns et al. 2008). The soil was characterized by 3.72% C, 0.13% N, 0.015% P and a pH_{H2O} of 4.8. Pots (8 cm diameter, 30 cm height) were filled with sterilized background soil and live soil inoculum (volume ratio 9:1). One two-week old seedling was transplanted into each pot. Pots were randomly arranged in the shade house and re-arranged weekly to minimize possible artefact effects. Seedlings were allowed to grow for 3 months, and each pot received 64 ml of water every 3 days, according to the average monthly precipitation (127.5 mm) of the last 10 years (based on the long-term records in the Heishiding ecological station).

In the beginning of September 2019, we established soil water treatments; in the ambient treatment, pots were watered regularly as previously described, whereas in the drought treatment, watering was reduced by 90% (10% of ambient treatments) during the last two months. We used this ratio because it was the threshold value above plant wilting according to our preliminary test. All plants were destructively harvested in the beginning of November 2019 to determine dry biomass following oven drying (60°C, 48 h). Soil samples were collected and sieved at 2 mm. Sieved soil samples were stored at −20°C and used for soil fungal DNA sequencing. An additional 5 g sieved soil sample from each pot was oven-dried (105°C, 24 h) to determine soil moisture content. Our results showed that the drought treatments caused a 52% reduction of soil moisture compared to the ambient treatments (Supporting information), causing a stress for plant growth.

Fungal DNA sequencing

We randomly selected three conspecific or heterospecific soil samples for each species and water treatment combination to use for analysing the soil fungal community, for a total of 72 soil samples. Soil microbial DNA was extracted using the MOBIO PowerSoil DNA Isolation Kit. DNA quality, concentration and purification were checked using 1% agarose gel electrophoresis and NanoDrop One UV-Vis Spectrophotometer. The second internal transcribed spacer (ITS2) region of the rRNA operon for fungi was amplified using primer ITS3F/ITS4R. Polymerase chain reaction was conducted using BioRad S1000. Amplicon libraries were prepared using the NEBNext UltraDNA Library Prep Kit for Illumina following the manufacturer's protocol, and PE250 sequencing was performed on NovaSeq 6000 Sequencing System. We removed primers, chimera and low-quality reads from sequencing data using CUTADAPT ver. 1.9.1. Operational taxonomic units (OTUs) were clustered at 97% sequence similarity using the UPARSE package, resulting into a total of 3836 OTUs, and taxonomic assignment for fungi was performed using the Unite database (Kõljalg et al. 2005). The resultant OTU abundance tables from these analyses were rarefied to an equal number of sequences per sample to ensure equal sampling depth (35 820 sequences for each sample). Fungal richness for each soil sample was determined using OTU number. For each species, OTUs that were present in conspecific soils rather than heterospecific soils were marked as unique OTUs (Supporting information). FUNGuild was used to assign OTUs to ecological guilds, i.e. symbiotroph, pathogen and saprotroph, with only guild assignments with a confidence of 'probable' or higher being retained (Nguyen et al. 2016). We acknowledge that the ITS2 region does not describe arbuscular mycorrhizal fungi very well, and the classification of this type of mycorrhizal fungi may therefore be biased. OTUs that had multiple function assignments were excluded from the analysis. Total sequence numbers were used to represent the relative abundances of fungal guilds.

Data analyses

The PSF index was calculated using the response ratio (Brinkman et al. 2010):

$$\mathrm{PSF} = \mathrm{Ln}\left(\mathrm{B}_{\mathrm{con}} / \mathrm{B}_{\mathrm{het}}\right)$$

where $\mathrm{B}_{\mathrm{con}}$ and $\mathrm{B}_{\mathrm{het}}$ were total plant biomass in each pair of conspecific and heterospecific soils, respectively. Two-way analysis of variance (ANOVA) was performed to analyse effects of the soil water treatments and plant species identity on PSF. T tests were used to test if values of PSF were different from zero for each treatment combination. Moreover, we performed mixed effect models with mycorrhizae types and soil water treatments as fixed factors and species identity as a random factor in the nlme R package (Pinheiro et al. 2020) to test if PSF differed between AM and ECM trees, and there was no significant difference between these two groups ($p = 0.571$). Plant total biomass was analysed using mixed effect models in the nlme R package, with response species identity, soil watering treatment and soil inoculum as fixed factors and soil sampling site (i.e. site where the paired conspecific and heterospecific soil inoculums were collected, Supporting information) as a random factor because pairings of conspecific and heterospecific soil inoculums were potentially interdependent.

We tested if plant landscape abundance can be predicted by PSF coefficients considered cumulatively across all con- and heterospecific species and if soil water conditions can alter PSF–plant abundance relationships using mixed effect models. Species-level PSF and soil watering treatment were considered fixed factors (with interactions also specified) and species identity was included as a random factor in order to control for species-specific effects that may have confounded the landscape-level patterns we sought to explore. Linear regression for all treatment combinations was performed to test the general relationship between PSF and plant landscape abundance (i.e. $y = $ plant landscape abundance, $x = $ species-average PSF) and we also tested the relationships between PSF and plant landscape abundance under ambient or drought conditions using separate linear regressions. Plant landscape abundance data were natural log-transformed prior to analyses. Due to the relatively low sample size ($n = 6$ for ambient and drought watering conditions each), we performed power analyses (Cohen 1988) in order to determine whether or not the sample sizes were sufficient to draw robust conclusions (power coefficient ≥ 0.8 considered robust) using the pwr() package in R (Champely et al. 2018).

Fungal community composition in conspecific and heterospecific soils for the six plant species were analysed with principal coordinate analysis (PCoA) based on Bray–Curtis distances, using vegan (Oksanen et al. 2020) and ape (Paradis and Schliep 2019) packages in R. This was done to show that conspecific and heterospecific soil fungal communities showed differences that could be (partially) responsible for the observed PSF. OTU data were Hellinger-transformed prior to PCoA. Comparisons between treatments were made via permutation-based multivariate analysis of variance (PERMANOVA, permutations = 999). The proportion of unique pathogenic, symbiotrophic or saprotrophic OTUs was calculated as the ratio of unique OTUs and all putative pathogenic, symbiotrophic or saprotrophic OTUs. In natural ecosystems, although plant species may be dominantly associated with AM or ECM fungi, they can host both AM and ECM fungi (Brundrett 2009), and thus it is more reasonable to group all symbiotrophs together in analysis.

Three-way ANOVAs were performed to test effects of species identity, soil water treatments (ambient versus drought) and soil inoculums (conspecific versus heterospecific soil) on the relative abundances of soil fungal guilds (putative pathogens, symbiotrophs and saprotrophs) and fungal richness. Two-way ANOVAs were performed to test effects of species identity and soil water treatments on the proportion of unique OTUs in each guild. Data natural log-transformations were performed to meet the requirements of ANOVA if needed. When significant differences were detected, Tukey's honest significant difference post hoc tests were used to compare mean values across treatment combinations using emmeans package in R (Lenth 2020). Separate regression analyses were performed to test if PSF were associated with the proportions of unique OTUs in conspecific soil microbial communities under ambient or drought conditions. We tested if PSF can be predicted by the relative abundance of fungal guilds in conspecific soil microbial communities, because previous studies have shown that the outcome of PSF is more closely associated with soil microbes in conspecific soil than heterospecific soil (McCarthy-Neumann and Kobe 2010, Semchenko et al. 2018, Xi et al. 2020). All analyses were performed in R (<www.r-project.org>).

Results

PSF and plant landscape abundance

Outcomes of plant–soil feedbacks varied depending on soil water treatments (Table 1, Fig. 1). Under ambient conditions, PSF were negative (mean value ± 95% confidence interval $= -0.43 \pm 0.06$), while PSF were neutral under drought conditions (mean value ± 95% confidence

Table 1. Interactive effects of soil water treatments (ambient versus drought) and species identity (*Altingia chinensis*, *Canarium album*, *Castanopsis fissa*, *Castanopsis hystrix*, *Lithocarpus lohangwu* and *Litsea lancilimba*) on plant–soil feedbacks. F- and p-values derived from analysis of variance are shown. Bold type indicates significant effects ($p < 0.05$).

Effect	df	F-value	p-value
Species	5,60	**8.47**	**< 0.001**
Water	1,60	**70.44**	**< 0.001**
Species × Water	5,60	**2.44**	**0.045**

interval = −0.05 ± 0.06). However, the strength of drought effects on PSF varied among tree species, resulting in a significant interaction between water treatments and species identity (Table 1). Drought reduced negative PSF for all species except *L. lohangwu*. ANOVA results showed that plant biomass responses to soil inoculum and its interactions with species identity or water treatments were consistent with that of the PSF values (Supporting information).

Plant landscape abundance was not correlated with PSF coefficients considered across all species cumulatively when data for ambient and drought conditions were pooled ($p = 0.095$, Fig. 2). However, there was a significant interaction between soil watering treatment and PSF coefficients in predicting plant landscape abundance ($p = 0.039$). In the drought treatments, there was a significant positive relationship between PSF and plant landscape abundances ($p = 0.002$, $R^2 = 0.92$), while there was no significant correlation in the ambient treatments ($p = 0.100$, $R^2 = 0.28$, Fig. 2). The power analyses yielded power coefficient of 0.830 and 0.085 for the drought and ambient correlation models, respectively. This means that the results of the drought model are more robust than those of the ambient model.

Soil fungal community

The first two axes of the PCoA accounted for 11.9% and 10.6% of the variation of fungal community composition over all the treatment combinations in both conspecific and heterospecific soils (Supporting information). Results of the PERMANOVA showed that soil water treatments, plant species identity and soil inoculum significantly altered fungal community composition individually ($p < 0.001$ and $R^2 = 0.06$, $p < 0.001$ and $R^2 = 0.12$, $p < 0.001$ and $R^2 = 0.09$, respectively, Supporting information). However, there was an interaction between species identity and soil inoculum, with the soil fungal guild composition of certain plant species differing between conspecific versus heterospecific soils, while others showed no differences between conspecific versus heterospecific soils across a species ($p = 0.036$, $R^2 = 0.07$, Supporting information). Soil fungal richness did not differ among any treatment combinations (Table 2, Fig. 3a). However, drought increased and decreased the relative abundances of pathogens and symbiotrophs, respectively ($p = 0.012$ and 0.001, respectively, Table 2, Fig. 3b–c). Symbiotroph relative abundance was lower in conspecific soil than heterospecific soil ($p < 0.001$, Table 2, Fig. 3c). However, there was an interaction between soil inoculum and

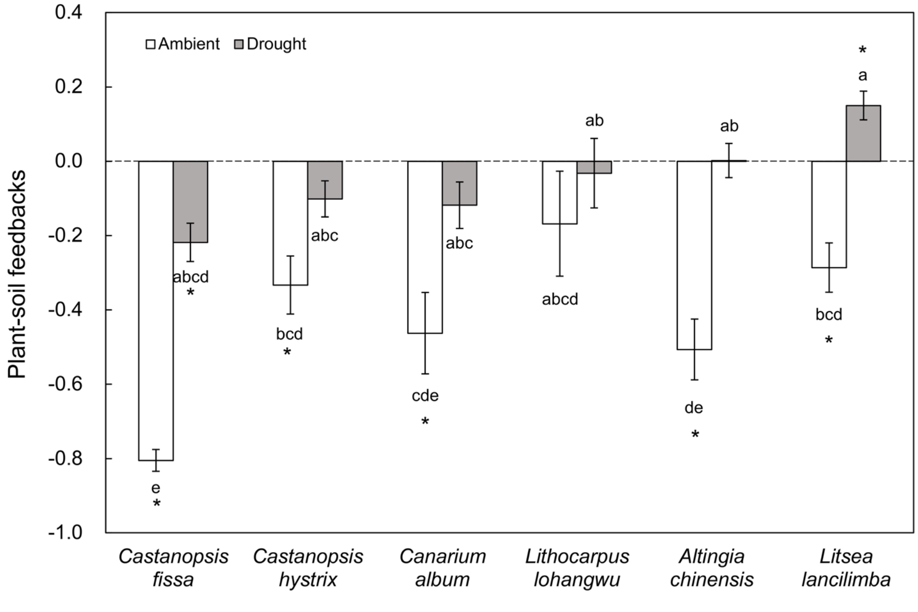

Figure 1. Plant–soil feedbacks under ambient and drought conditions for seedlings of six tree species (*Altingia chinensis*, *Canarium album*, *Castanopsis fissa*, *Castanopsis hystrix*, *Lithocarpus lohangwu* and *Litsea lancilimba*). Means and SE are given ($n = 6$). Bars accompanied by the same lower-case letter do not differ at $p < 0.05$ (Tukey's HSD test), and comparisons were made among all species and watering treatment combinations. Stars indicate PSF that are significantly different from zero at $p < 0.05$ (T test). Species were ordered according to rising plant landscape abundance (from right to left).

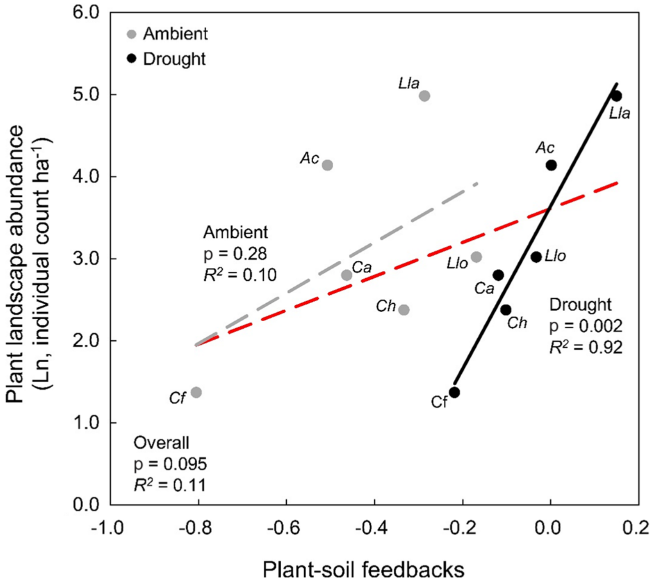

Figure 2. The relationship between plant–soil feedbacks and plant landscape abundance. The solid and dashed lines indicate significant and non-significant correlations for the drought and ambient watering treatments at the level $p < 0.05$, respectively. The red dashed line shows the non-significant correlation when all data points are pooled across ambient and drought treatments. p values and R^2 of fitted lines are given. Plant species are given: Ac = *Altingia chinensis*, Ca = *Canarium album*, Cf = *Castanopsis fissa*, Ch = *Castanopsis hystrix*, Llo = *Lithocarpus lohangwu* and Lla = *Litsea lancilimba*.

water treatments on symbiotroph relative abundance differed among plant species (significant species × water treatment × soil inoculum interaction p = 0.047, Table 2): under ambient conditions, there was greater symbiotroph relative abundance in heterospecific soil than conspecific soil collected from *C. hystrix* compared to drought, but there were no differences between ambient versus drought for the other species (Fig. 3c). Saprotroph relative abundance significantly differed across species, with lower values in *C. fissa* compared to *A. chinensis* and *C. album* (p = 0.012, Table 2, Fig. 3d). Water treatments and soil inoculum interacted and altered saprotroph relative abundance (p = 0.035, Table 2). Specifically, conspecific ambient water treatment soils had a higher saprotroph abundance compared to conspecific drought treatment soils, but heterospecific soils did not differ between watering treatments (Fig. 3e).

There were significant effects of plant species identity on the proportion of unique pathogenic OTUs (p = 0.045, Table 3), with a tendency for lower values in the most abundant species considered here: *L. lancilimba* and *A. chinensis* (significantly lower for *A. chinensis* compared to the 3rd most abundance and the least abundant of the other species and trending lower for *L. lancilimba*; Fig. 4a). The proportions of unique soil fungal OTUs of symbiotrophs and saprotrophs differed among species. The species *C. fissa*, *A. chinensis* and *L. lancilimba* had a higher number of unique symbiotrophs compared to *C. album* and *C. fissa* had a higher number of saprotrophs compared to *C. hystrix* (Table 3, Fig. 4b–c). The proportions of unique OTUs of any guild did not show responses to soil water treatments (Table 3). There were no interactive effects of species identity and water treatments for any fungal variable (Table 3).

Relationships between PSF and soil fungi

PSF were less negative (i.e. more positive) as putative pathogen relative abundance in conspecific soil increased under drought and ambient conditions (Ambient p = 0.035, $R^2 = 0.25$, Drought p = 0.009, $R^2 = 0.32$, Fig. 5a–b). No significant relationships were detected between PSF and symbiotroph or saprotroph relative abundances in conspecific soil at the level of p = 0.05 (Supporting information).

There was no significant relationship between PSF and the proportion of unique putative pathogens in the ambient treatments (P = 0.087, $R^2 = 0.12$, Fig. 5c), but in the drought treatments, PSF became more negative as the proportion of unique putative pathogens increased in conspecific soil (P = 0.002, $R^2 = 0.42$, Fig. 5d). In other words, more negative PSF were associated with a higher amount of unique OTUs and lower common OTUs under drought conditions. There were no significant relationships between PSF and the proportions of unique symbiotroph or saprotroph OTUs under ambient or drought conditions (Supporting information).

Table 2. Interactive effects of species identity (*Altingia chinensis*, *Canarium album*, *Castanopsis fissa*, *Castanopsis hystrix*, *Lithocarpus lohangwu* and *Litsea lancilimba*), water treatments (ambient versus drought) and soil inoculums (conspecific versus heterospecific soil) on fungal richness, and the relative abundances (OTU sequence numbers) of pathogens, symbiotroph and saprotroph. F- and p-values derived from analysis of variance are shown. Significant effects ($p < 0.05$) in bold.

		Fungal richness		Pathogen relative abundance		Symbiotroph relative abundance		Saprotroph relative abundance	
Effect	df	F	p	F	p	F	p	F	p
Species (S)	5,24	1.43	0.251	1.52	0.222	0.91	0.492	**3.75**	**0.012**
Water (W)	1,24	1.83	0.189	**7.34**	**0.012**	**12.87**	**0.001**	2.21	0.150
Soil inoculum (I)	1,24	1.37	0.253	3.76	0.065	**18.00**	**< 0.001**	0.54	0.469
S × W	5,24	0.68	0.642	0.19	0.965	0.57	0.721	1.68	0.179
S × I	5,24	0.39	0.852	0.63	0.680	1.91	0.130	0.77	0.583
W × I	1,24	3.00	0.096	0.03	0.870	0.16	0.696	**5.01**	**0.035**
S × W × I	5,24	0.23	0.947	0.54	0.741	**2.67**	**0.047**	0.13	0.983

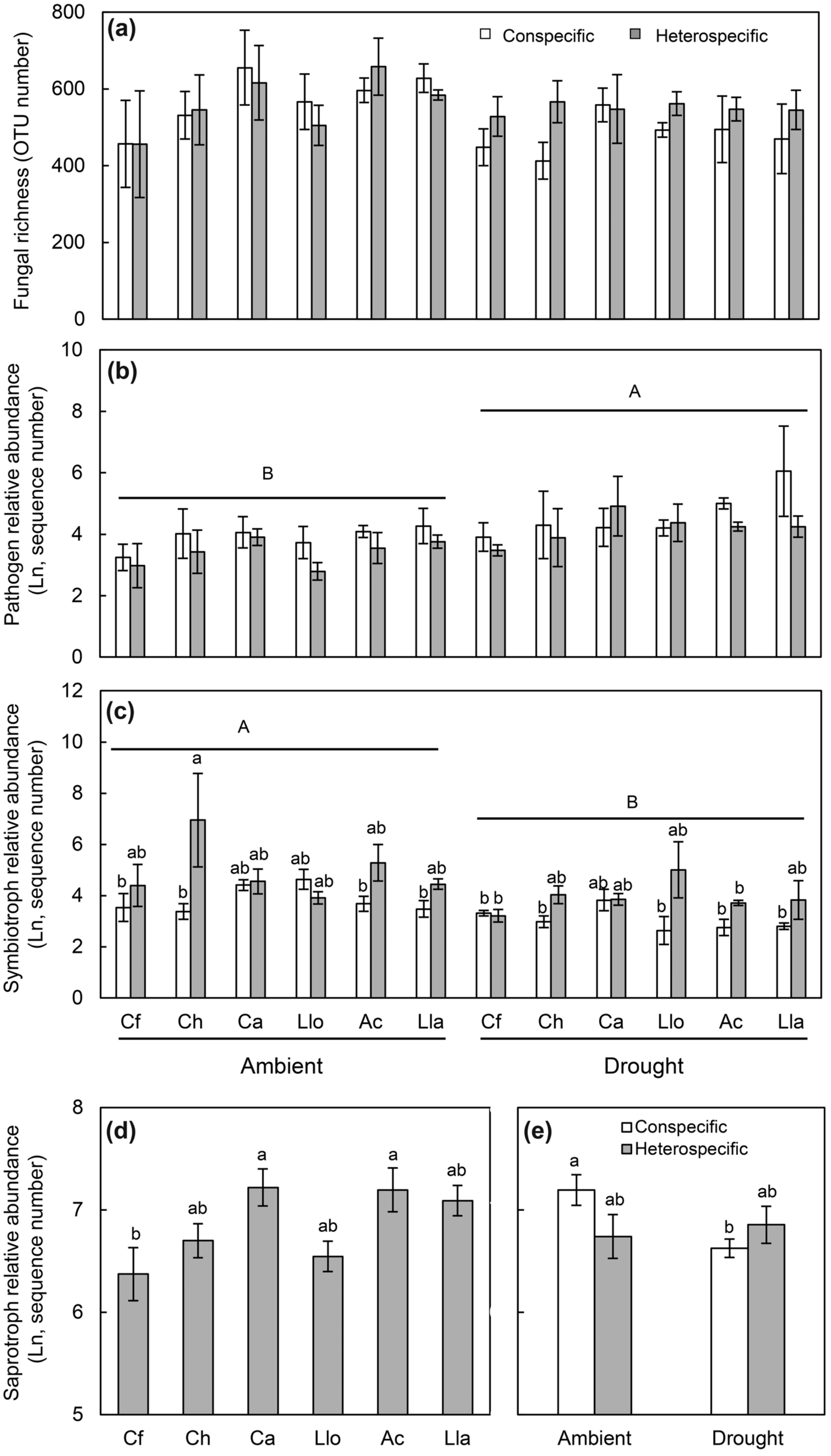

Figure 3. (a) Fungal richness, (b) pathogen relative abundance, (c) symbiotroph relative abundance, and saprotroph relative abundance of microbial communities for (d) all plant species and (e) all water and soil inoculum treatment combinations. Within each panel, groups of bars topped by different uppercase letters or individual bars topped by different lowercase letters differ at $p < 0.05$ (Tukey's HSD). Plant species are given: Ac = *Altingia chinensis*, Ca = *Canarium album*, Cf = *Castanopsis fissa*, Ch = *Castanopsis hystrix*, Llo = *Lithocarpus lohangwu* and Lla = *Litsea lancilimba*.

Table 3. Interactive effects of water treatments (ambient versus drought) and species identity (*Altingia chinensis*, *Canarium album*, *Castanopsis fissa*, *Castanopsis hystrix*, *Lithocarpus lohangwu* and *Litsea lancilimba*) on proportions of unique OTUs in conspecific soil. F- and p-values derived from analysis of variance are shown. Significant effects ($p < 0.05$) in bold.

Effect	df	F-value	p-value
Proportion of unique pathogens			
Species	5,24	**2.71**	**0.045**
Water	1,24	0.85	0.367
Species × Water	5,24	1.19	0.346
Proportion of unique symbiotroph			
Species	5,24	**2.76**	**0.042**
Water	1,24	0.34	0.566
Species × Water	5,24	0.71	0.625
Proportion of unique saprotroph			
Species	5,24	**3.71**	**0.013**
Water	1,24	1.44	0.242
Species × Water	5,24	1.76	0.160

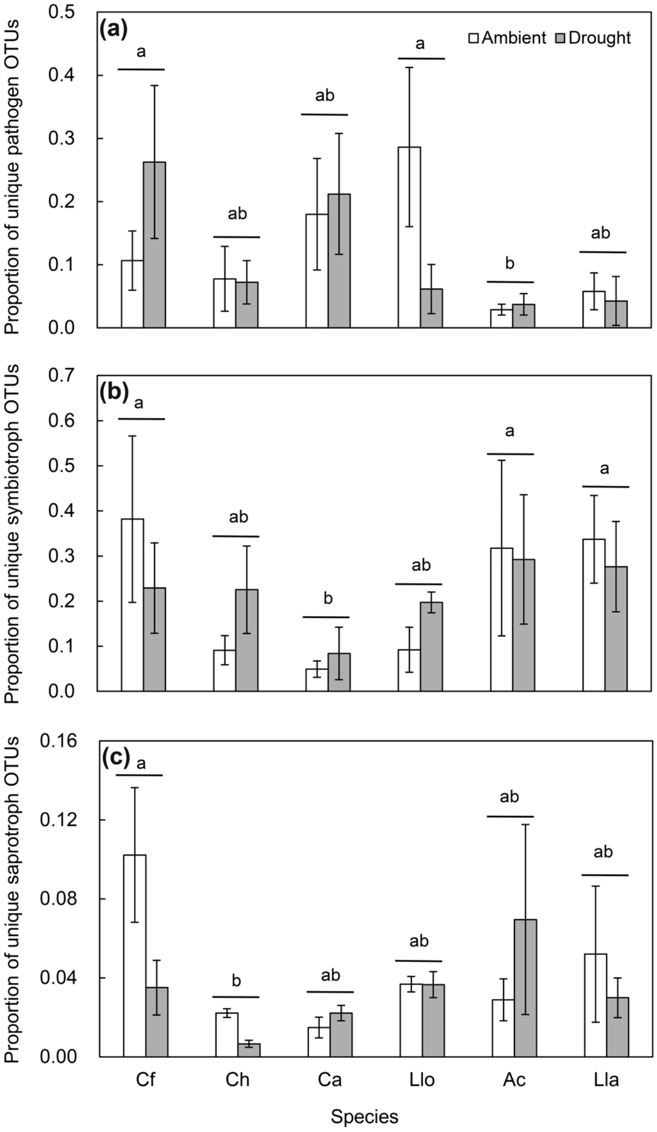

Figure 4. The proportions of (a) unique pathogen (b) unique symbiotroph and (c) unique saprotroph in their fungal guilds of conspecific rhizosphere fungal communities. Means and SE are given (n = 3). Within each panel, groups of bars topped by different lowercase letters differ at $p < 0.05$ (Tukey's HSD). Plant species are given: Ac = *Altingia chinensis*, Ca = *Canarium album*, Cf = *Castanopsis fissa*, Ch = *Castanopsis hystrix*, Llo = *Lithocarpus lohangwu* and Lla = *Litsea lancilimba*.

Discussion

Despite growing recognition that PSF and climate change play an important role in driving plant community dynamics (Reynolds et al. 2003, Bever et al. 2010, Ploughe et al. 2019), very few studies have examined variation of PSF in relation to abundant and less-abundant species under different rainfall patterns. The present study seeks to fill this knowledge gap by examining interspecific PSF for six coexisting tree species under ambient and drought conditions. Our results led to two key findings: 1) drought changed PSF for the two abundant species *L. lancilimba* and *A. chinensis* from negative to positive and neutral, respectively; 2) under drought conditions, PSF were positively related with the relative abundance of all putative fungal pathogens, but negatively related with the proportion of unique putative pathogens (and therefore putatively host-specific).

Drought modulated PSF and the PSF–plant landscape abundance relationship

We found that, in general, PSF were negative under ambient conditions, while drought treatments weakened, neutralised or reversed (i.e. turned positive) negative PSF (Fig. 1). This finding is consistent with previous studies which have shown that concurrent drought neutralized PSF (Fry et al. 2018), or weakened negative PSF (Snyder and Harmon-Threatt 2019). This may have occurred because drought reduced soil microbial biomass, slowed down ecological processes in the rhizosphere, and/or decreased associations between plants and mutualists/antagonists (van der Putten et al. 2016, De Long et al. 2019).

We observed a positive relationship between PSF and plant landscape abundance in the drought treatments for the six plant species, while there was no significant relationship between PSF and plant abundance under ambient conditions (Fig. 2). This suggests that different rainfall change scenarios may have divergent influences on local plant communities. For example, chronic changes in rainfall, such as seasonal dynamics of drying and rewetting, may be important for maintaining species diversity and local abundance patterns (Lasky et al. 2016, Harrison et al. 2018). On the other hand, an extreme drought can increase competitive advantages of abundant species over less-abundant species, thereby exacerbating competitive exclusion and the loss of biodiversity (Grant et al. 2014). Given the generality of PSF effects across systems (Crawford et al. 2019), our results may have implications for many different plant communities. However, power analyses revealed the correlations between plant landscape abundance and drought PSF coefficients were more robust

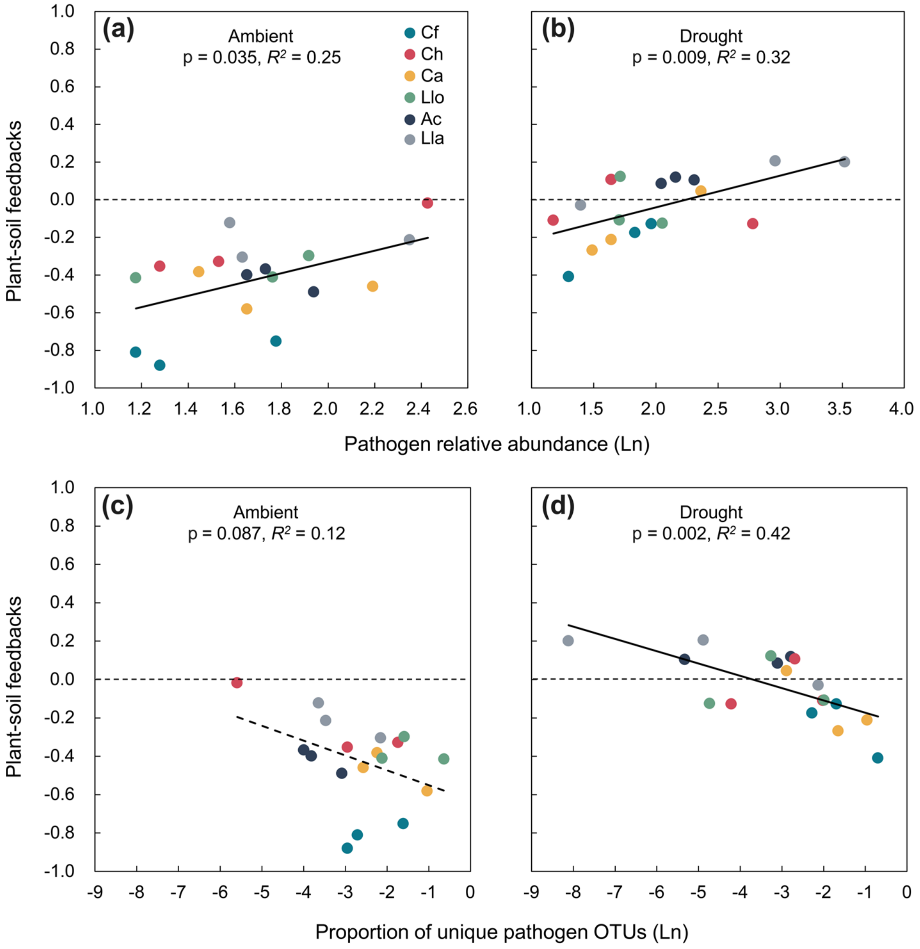

Figure 5. The relationships between pathogen relative abundance (Ln-transformed) and PSF across six plant species in the (a) ambient and (b) drought treatments, and the relationships between proportion of unique pathogens (the ratio of unique, potentially species-specific and total pathogen OTUs) in the (c) ambient and (d) drought treatments. The solid and dashed lines indicate significant and non-significant correlations between drought and ambient watering treatments at the level $p < 0.05$, respectively. The p-values and R^2 of fitted lines are given. Plant species are given: Ac = *Altingia chinensis*, Ca = *Canarium album*, Cf = *Castanopsis fissa*, Ch = *Castanopsis hystrix*, Llo = *Lithocarpus lohangwu* and Lla = *Litsea lancilimba*.

than correlations between plant landscape abundance and ambient PSF coefficients, indicating the necessity for greater samples sizes. Future studies are necessary to validate our findings for more plant species across multiple ecosystems and under different precipitation regimes. Further empirical studies are also required to explicitly test the influences of extreme drought events through comparing PSF under extreme versus chronic drought conditions.

Our results indicate that abiotic stress makes PSF a stronger predictor of plant landscape abundance. In the present study, we simulated drought stress following moist conditions in the drought treatments. The drought conditions imposed in this experiment are likely more realistic to the moisture conditions experienced by plants in natural communities (i.e. intermittent periods of drier versus wetter soil) compared to the constantly moist conditions in the ambient treatments. Combined with the findings of other studies, our results support the idea that accounting for abiotic and biotic stress makes PSF a stronger predictor of plant landscape abundance because such stressors are ubiquitous under natural conditions.

Relationships between PSF and soil fungi

There was a significant positive relationship between PSF and the relative abundance (i.e. sequence number) of putative fungal pathogens in conspecific soil, regardless of soil water treatments (Fig. 5a, b). This positive relationship between PSF and the relative abundance of putative fungal pathogens, combined with the positive relationship between PSF and plant landscape abundance under drought conditions, likely reflects that the abundant plant species host greater abundances of common pathogens because of their abundance in the ecosystem. In other words, the abundant plant species may be more heavily colonised by common pathogens, because host-generalized pathogens may prefer to infect the abundant plant species since they represent a more available resource. Further, common pathogens may not cause fatal effects on host plants compared to specific pathogens (e.g. Janzen–Connell effects) (Janzen 1970, Connell 1971, but see Hersh et al. 2012), and the abundant species may have stronger tolerance to common pathogens than less-abundant species, which may partially explain why the abundant plant species hosted more pathogenic fungi overall, yet experienced more positive PSF.

The relationship between PSF and the proportion of unique (i.e. potentially host-specific) pathogens (i.e. the ratio of unique pathogens and all putative pathogens) was negative under drought conditions (Fig. 5d). The two abundant species (*L. lancilimba* and *A. chinensis*) tended to have a lower proportions of unique pathogen OTUs compared to some of the other species (Fig. 4a). The unique pathogens detected here likely contained species-specialized pathogens and reflect the differences in fungal communities between conspecific and heterospecific soils, and thus in theory may drive PSF. This finding was also supported by the results of the PERMANOVAs that showed significant differences in overall soil fungal community composition driven by con- versus heterospecific soils (and interactions with species identity) (Supporting information). In this sense, the two abundant species may have historically experienced higher pathogen pressure, as well as fungal communities that were overall different in comparison to heterospecific soils. The abundant species may have evolved to be more defensive against pathogens, and thus host less specialized pathogens than less-abundant species that experience greater negative effects of specialized pathogens (Reinhart et al. 2021).

Our results highlight the potential for soil fungal guilds to modulate plant–soil feedbacks, but we realize that there are important knowledge gaps that limit our ability to make more precise inferences on connections between soil fungi and PSF. First, the resolution of fungal taxonomy down to species level is difficult, and many OTUs cannot be identified at even the genus or family level, reflecting that soil fungal communities remain a black box. Despite this weakness, the comparison of soil microbial community composition can be performed based on the OTU tables. Second, although the knowledge of functional guilds of fungi (i.e. pathogen, symbiotroph and saprotroph) is accumulating, to date, a limited number of OTUs have identified functions (in our study, 84% of fungal OTUs could not be assigned to a functional guild). As a consequence, these knowledge gaps prevent us from identifying host-specificity of soil fungi. Therefore, advanced understanding of host-specialized microbes will contribute to work assessing the roles of specialist versus generalist microbes in driving the ecological patterns of PSF.

Conclusions

Our results show that simulated drought stress can weaken the strength of negative PSF and even switch the direction of PSF for the most abundant species. We found that there was a negative relationship between PSF and the proportion of unique OTUs in putative fungal pathogens, likely reflecting that specialized fungal pathogens, rather than generalized pathogens, drive negative PSF. The positive relationship between PSF and plant landscape abundance indicates that drought can increase competitive advantages of the abundant species over less-abundant species through switching PSF from negative to positive or neutral. Our results pull focus on the fact that abiotic stress can increase the capacity of PSF to predict plant landscape abundance.

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Author contributions

Nianxun Xi: Conceptualization (lead); Data curation (lead); Formal analysis (equal); Funding acquisition (lead); Methodology (lead); Writing – original draft (lead); Writing – review and editing (equal). **Kerri M. Crawford**: Conceptualization (equal); Writing – review and editing (equal). **Jonathan R. De Long**: Conceptualization (equal); Formal analysis (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.vx0k6djtw> (Xi et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

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