Soil legacies and drought regulate plant diversity-productivity relationships

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Abstract

How historical and concurrent drought regulate plant diversity-productivity relationships through altering soil microbial communities remains a key knowledge gap. We addressed this gap with plant diversity-productivity relationship experiments under drought and ambient conditions over two phases (Phase I: soil conditioning, and Phase II: plant response). Our results reveal that plant diversity and drought interacted and caused divergent soil microbial communities in Phase I, leading to soil microbial legacies. These soil legacies interacted and caused more pronounced plant diversity-productivity relationships in Phase II, reflecting increased net biodiversity effects over time. Complementarity effects were most positive in plant communities with highest plant richness and in the Drought-Ambient (Phase I-II) treatment, and selection effects were most negative in these communities. Our results highlight the importance of soil microbial communities in driving positive plant diversity effects, and future rainfall changes can cause complicated patterns in the biodiversity-ecosystem functioning relationships through soil microbial legacy.

Introduction

Soil microbes have been highlighted as a candidate factor driving plant diversity-productivity relationships (Chen *et al.* 2019; Liang *et al.* 2019; Jia *et al.* 2020; van Ruijven *et al.* 2020). The majority of experimental studies testing this idea have compared plant diversity-productivity relationships in live versus sterilized soils (Maron *et al.* 2011; Luo *et al.* 2016), or through inoculating sterile substrates with different microbial guilds (Klironomos *et al.* 2000; Schnitzer *et al.* 2011). In natural ecosystems, however, plant diversity can influence intact soil biota and abiotic properties through biochemically diverse litter production and root exudates (Stephan *et al.* 2000; Kowalchuk *et al.* 2002; Millard & Singh 2010; Milcu *et al.* 2013; Mommer *et al.* 2018), and this soil legacy can feed back to influence subsequent plant communities, leading to so-called "plant-soil feedbacks" (PSFs) (Bever 1994; van der Putten *et al.* 2013). To date, experimental evidence illuminating the influence of soil microbes on plant diversity-productivity relationships through PSFs remains limited (but see Jing *et al.* 2015; Wang *et al.* 2019; Jia *et al.* 2020).

Soil pathogens can contribute to plant diversity-productivity relationships by enhancing the appearance of a complementarity effect (niche differentiation and facilitation among species, CE) via negative density dependence. Here, if plant performance is reduced in monocultures due to accumulation of species-specific soil pathogens, then plants are likely to perform better in multiple-species mixtures due to dilution of pathogenic effects (Maron *et al.* 2011; Schnitzer *et al.* 2011; Mommer *et al.* 2018; van Ruijven *et al.* 2020). Soil mutualists could contribute to diversity effects as well, through enhancing resource partitioning in mixtures, as plant communities with high richness might host diverse mutualist communities and different guilds have different host effects (Maherali & Klironomos 2007; Wagg *et al.* 2011; Jing *et al.* 2015; Wang *et al.* 2019; Jia *et al.* 2020). Soil microbes are likely to also influence the selection effect (the likelihood of the presence of a particular productive plant species increases in a species-rich community, SE), as particular microbial

1

guilds (e.g., species-specific pathogens and mutualists) will enhance or reduce the dominance of a particular plant species in mixtures (Vogelsang *et al.* 2006; Wagg *et al.* 2011; Walder *et al.* 2012). It is the prerequisite of soil microbe-mediated biodiversity effects that soil microbial assemblages and the strength of PSFs differ among plant species richness levels (Mommer *et al.* 2018), but the experimental test for this assumption is lacking.

There is currently growing evidence that drought can alter soil abiotic and biotic properties, mainly through two non-exclusive mechanisms: 1) specialized soil pathogens become dormant under drought stress, and they are subsequently reactivated by rewetting (Kaisermann *et al.* 2017; Meisner *et al.* 2018), meaning plant communities under drought receive a reprieve from pathogens; 2) plants select for mycorrhizal fungi guilds which promote plant efficiency of soil water capture under drought stress (Querejeta *et al.* 2009; Mariotte*et al.* 2017). Consequently, drought can generate a soil microbial legacy that has long-lasting effects on subsequent plant communities (de Vries *et al.* 2012; Kaisermann *et al.* 2017; De Long*et al.* 2019). However, little is known about how drought and plant diversity interact to cause combined soil legacy effects on plant performance and biodiversity-productivity relationships.

Plant diversity and drought-induced soil legacy could interact with subsequent drought events to regulate plant performance. For example, drought could simply weaken soil legacy effects/feedbacks on future plants and shift PSFs from positive and negative to neutral (Fry*et al.* 2018; Snyder & Harmon-Threatt 2019). Previous studies also suggest that plant drought resistance is enhanced when growing in conspecific soil with legacy of historical drought events (de Vries*et al.* 2012; Lau & Lennon 2012; Allsup & Lankau 2019). However, these studies tested single plant species, but how soil legacy of plant diversity and drought interact with subsequent soil moistures to influence diversity effects remains unexplored.

Here, we performed plant diversity-productivity relationship experiments under drought and ambient conditions over two phases (Fig. 1). In the conditioning phase (Phase I), plant communities with a range of species richness levels grew in homogeneous soils, and soil microbial communities were trained by these plants. In the response phase (Phase II), newly-established plant communities grew with soil inoculums which the same plant communities conditioned. Drought was manipulated in both phases to stimulate drought legacy effects and to assess responses to drought from these legacy effects. We addressed two key research questions: 1) Does plant diversity and drought cause soil legacy effects through influencing soil microbial communities? (2) How does this soil microbial legacy regulate plant diversity-productivity relationships?

Materials and Methods

Plant species and soil

We selected twelve herbaceous species that co-occur in natural old fields in southern China for setting up model plant communities, including three grasses (*Dactyloctenium aegyptium* ,*Digitaria radicosa* and *Isachne repens*), eight non-legume forbs (*Celosia argentea* , *Amaranthus viridis* ,*Achyranthes aspera* , *Emilia sonchifolia* , *Ageratum conyzoides* , *Eclipta prostrata* , *Ludwigia hyssopifolia* and*Capsella bursa-pastoris*), and a legume (*Mimosa pudica*).*Isachne repens* and *Achyranthes aspera* are perennial species, *Mimosa pudica* can be either an annual or perennial and the rest are annual species. Seeds were collected from January to February 2019 in old fields in Fengkai County, China. Soil was collected in an old field in Fengkai County, China (23.51°N, 111.82°E). The climate is subtropical monsoon with a mean annual temperature of 19.6°C and a mean annual precipitation of 1532.8 mm. On March 22nd 2019, topsoil (15cm depth) was collected, sieved at 1cm mesh to remove large particles and organic debris, and homogenized thoroughly prior to the experiment.

Phase I: Conditioning soil

We performed a soil conditioning phase of the experiment to obtain specified soil communities of different plant communities at the Heishiding nature reserve in Fengkai (23.46° N, 111.90° E). Plant communities with different species richness levels were established in the collected field soil and subjected to ambient or drought treatments. Five plant diversity treatments were established, including monoculture, two, four, six and twelve

2

species mixtures. Each of the twelve species used in this experiment were planted as monocultures, and each monoculture was replicated two times. For the two, four, or six species mixtures, species compositions were determined using separate random draws from the twelve species pool (Table S1), and each drawn species composition was replicated twice. In total, this experiment consisted of 5 plant diversity levels × 2 moisture treatments × 12 replicates = 120 plant communities. Each community consisted of twelve individual plants, and all plant species had equal density in mixtures.

Seeds were first surface sterilized by rinsing in 75% ethanol for 2 minutes and then germinated in sterilized vermiculite. On April 28th 2019, twelve two-week-old seedlings were transplanted into each mesocosm (24 cm diameter, 19 cm height), and plants that failed during the first week were replanted. All mesocosms were regularly watered with 600 ml every four days for the first six weeks to avoid drought stress. On June 15th, half of the mesocosms continued to be watered regularly (i.e., ambient treatment), and the remaining ones received one third of water amount of the ambient treatment (the drought treatment). Mesocosm location was randomized monthly to avoid position effects.

Volumetric soil moisture content at 5 cm depth was monitored by GS-3 soil moisture probe (Decagon Devices, Inc. WA) monthly to make sure soil moisture treatments were effective. Over the period of Phase I, the soil moisture treatments caused significantly different soil moisture contents among mesocosms (Table S2, Fig. S1a). Plants were grown for 18 weeks. In the beginning of September 2019 when all species were in their reproductive stages, plants were removed in all mesocosms. Soil was thoroughly homogenized for each mesocosm, and a subsample was collected and stored at -80 °C for soil microbial DNA sequencing. For the twelve species treatment, we randomly selected five mesocosms for microbial analyses in each moisture treatment. A subsample of 30% (by volume) mesocosm soil was stored at -20 as soil inoculum prior to Phase II.

Phase II: Community response to soil legacy and drought

Bulk soil was collected at the Heishiding nature reserve and sterilized by gamma-irradiation at 25kGy. Gamma irradiation has been shown to effectively sterilize soil with minimal impacts on other soil properties (Berns *et al.* 2008). Mesocosms (21cm diameter, 17cm height) were filled with 70% (by volume) sterilized bulk soil, 10% conditioned soil and 20% sterilized bulk soil. This approach allowed us to reduce the impacts of differences in soil abiotic properties on plant growth, and explore the role of soil microorganisms (Smith-Ramesh & Reynolds 2017). Two-week-old seedlings were transplanted into mesocosms on May 13th 2020, and the plant community compositions were consistent with those that provided soil inoculum in Phase I. Plants that failed during the first week were replaced. During the first three weeks, all mesocosms again received 600 ml water every four days.

On June 2nd 2020, soil moisture treatments were established. The water amounts used in ambient and drought treatments was consistent with those in Phase I. There were four soil moisture regimes over Phase I and II, namely, ambient treatments in both phases (A-A), drought treatments in both phases (D-D), ambient in Phase I + drought in Phase II (A-D) and drought in Phase I + ambient in Phase II (D-A) (Fig. 1). In total, there were 5 plant richness levels x 2 soil moistures in Phase I x 2 soil moistures in Phase II x 12 replicates = 240 mesocosms. During the period of experiment, volumetric soil moisture content at 5 cm depth was monitored regularly every two weeks by GS-3 soil moisture probe (Decagon Devices, Inc. WA). Over the period of Phase II, the soil moisture treatments caused significantly different soil moisture contents among mesocosms (Table S2, Fig. S1b). We randomized the mesocosms every two weeks. Plants were harvested from August 5th to 11th 2020 when all species were in their reproductive stages. Aboveground biomass was sorted by species. Belowground biomass was harvested at the mesocosm level, because root systems cannot be clearly sorted out to individual species. Plant materials were oven-dried at 60 for 48 h and weighted to determine biomass.

Partitioning of biodiversity effects

The net biodiversity effect (BE) of a polyculture mesocosm was measured by the difference between the observed yield and its expected yield, which was generated by a species proportional abundance multiplied

3

by biomass estimated in monoculture. CE was the average performance of species above or below their expected performance. SE was measured by the covariance between the monoculture yield of species and their change in relative yield in the mixture. These various effects can be related by an additive partition (Loreau & Hector 2001):

$$BE = CE + SE = N \ RY_i \ M_i + N \ cov(RY_i, M_i)$$

where i was the component species in the mixture, N was the number of component species, $\Delta P\Psi_i$ was the deviation from expected relative abundance of species i in the mixture, and M_i was the yield of species i in its monoculture. Positive CE indicates that species yields in a mixture are on average higher than expected values of the component species (the weighted average monoculture yields), and positive SE indicates that species with higher-than-average monoculture yields dominate the mixture.

Soil microbial DNA sequencing

DNA was extracted from soil samples using the MOBIO PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA). DNA quality, concentration and purification were checked on 1% agarose gel electrophoresis and NanoDrop One UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, USA). The V4 hypervariable region of the 16S rRNA for bacteria and second internal transcribed spacer (ITS2) region of the rRNA operon for fungi were amplified using primer combinations 515F/806R and ITS3F/ITS4R respectively. Polymerase chain reaction (PCR) were conducted using BioRad S1000 (Bio-Rad Laboratory, CA), with a 50 μl mixture containing 25 μl of 2 × Premix Taq, 10 mM of each primer, 60 ng DNA and nuclease-free water. The PCR amplification had an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C (30 s), annealing at 52 °C (30 s), extension at 72 °C (30 s), and a final extension at 72 °C (10 min). PCR products were extracted and purified using E.Z.N.A.® Gel Extraction Kit (Omega, USA). Amplicon libraries were prepared using NEBNext® Ultra DNA Library Prep Kit for Illumina[r] (New England Biolabs, USA) followed the manufacturer's protocol, and PE250 sequencing was performed on NovaSeq 6000 Sequencing System (Illumina, San Diego, USA).

Operational taxonomic units (OTUs) were categorized at 97% sequence similarity using the UPARSE package, and taxonomic assignment for bacteria and fungi was performed using Silva and Unite database respectively. Singleton sequences, chloroplast or mitochondria (16S amplicon) sequences and sequences that could not assign to the kingdom level were removed from the final OTU table. Each sample was rarefied to the same number of reads as the smallest sample (52190 and 29940 reads for bacteria and fungi respectively) for further analyses. Alpha diversity for soil bacteria and fungi was measured using the exponential Shannon-Weiner index based on the rarefied OTU tables. Fungal OTUs were assigned to arbuscular mycorrhizal fungi (AMF) and putative plant pathogens using FUNGuild (Nguyen *et al.* 2016) and further checked based on literature (Roncero *et al.* 2003; Wu *et al.* 2008; Mendes *et al.* 2013).

Statistical analyses

Effects of plant richness and soil moisture content on soil bacterial and fungal community compositions were examined using the distance-based redundancy analysis (db-RDA). Microbial OTU composition data were Hellinger transformed, and Bray-Curtis dissimilarity was used to measure community distance. Plant richness and log-transformed soil moisture content (the average values of mesocosms over the phase I) were used as explanatory variables. The db-RDAs were performed in R using "capscale" function in package "vegan" (Oksanen *et al.* 2019).

To evaluate effects of plant species richness and soil moisture treatments on plant and soil variables in Phase I (i.e., soil bacterial and fungal diversity, the OTU richness and relative abundance of AMF and fungal pathogens, community biomass, BE, CE and SE), we performed general linear mixed-effect models using the "lme" function in R package "nlme". Plant species richness (log-transformed) and soil moisture treatments in Phase I were included as fixed factors. Separate regression analyses were then performed to test whether there were linear relationships between plant species richness and these response variables in

4

each soil moisture treatment. Plant community compositions were used as random factor in the models mentioned above.

To test effects of plant species richness, soil moisture treatments from Phases I and II on plant variables of Phase II (i.e., plant community biomass, BE, CE and SE), we performed general linear mixed-effect models. Plant species richness, soil moisture treatments from Phases I and II were included as fixed factors, and plant community compositions and mesocosms that provided soil inoculums were used as the random factors. Plant species richness was log-transformed. The between-group comparisons were performed using the Tukey's HSD tests. All analyses were conducted in R (R development core team 2020).

Results

Soil microbial legacy of plant diversity and drought from Phase I

Amplification of 16S rRNA yielded 18,990 bacterial OTUs. The db-RDA showed that soil bacterial community structure was significantly influenced by soil moisture content ($F_1 = 23.91, P < 0.001$, Fig. 2a) and plant species richness ($F_1 = 4.06$, $P < 0.001$, Fig. 2a), indicating that drought and plant diversity caused significant alterations to the soil bacterial communities and potentially creating legacies for future plant-soil feedbacks. These factors explained 21.86% of variation in bacterial community composition. In general, drought treatments significantly decreased bacterial diversity (Table S3). There was a marginally significant relationship between soil bacterial diversity and plant species richness in ambient treatments ($P = 0.081$) and there was no evidence of a relationship observed in drought treatment ($P = 0.108$, Fig. 2b). However, bacterial diversity was greater in ambient than drought treatments at monoculture and two-species mixtures (Fig. 2b).

Amplification of ITS2 yielded 3444 fungal OTUs. Soil moisture treatment ($F_1 = 12.94$, $P < 0.001$, Fig. 2a) and plant species richness ($F_1 = 3.78$, $P < 0.001$, Fig. 2a) had significant influences on soil fungal community composition, explaining 14.32% of variation in fungal community composition, and again producing soil fungal legacies from plant diversity and drought. Drought treatments significantly decreased soil fungal diversity (Table S3). However, there was a positive relationship between soil fungal diversity and plant species richness under ambient conditions ($r = 0.45$, $P < 0.001$), while no significant relationship was observed under drought ($P = 0.257$, Fig. 2c), resulting in a significant plant species richness x soil moisture treatment interaction in Phase I (Table S3).

Drought significantly reduced the richness and relative abundance of AMF (Table S3). Drought further affected the relationship between AMF and plant species richness, resulting in a significant plant species richness x soil moisture interaction in Phase I for both AMF OTU richness and relative abundance (Table S3). AMF richness increased with plant species richness in both moisture treatments while the plant richness effects were more pronounced in the ambient treatments ($r = 0.85$, $P < 0.001$ for ambient and $r = 0.19, P = 0.002$ for drought treatments, Fig. 2d), and the relative abundance of AMF was positively related to plant species richness only in the ambient treatments ($r = 0.45$, $P < 0.001$, Fig. 2e). There was a negative relationship between OTU richness of fungal pathogens and plant species richness in the ambient treatment ($r = 0.13$, $P = 0.010$, Fig. 2f) but there was no significant relationship observed in the drought treatment ($P = 0.802$, Fig. 2f), resulting in a significant plant species richness x soil moisture interaction (Table S3). The relative abundance of fungal pathogens increased with increasing plant species richness in ambient conditions ($r = 0.92$, $P = 0.008$, Fig. 2g) while there was no significant relationship in drought conditions ($P = 0.111$, Fig. 2g). Drought treatments significantly increased the relative abundance of fungal pathogens in all plant species richness levels (Table S3).

Community biomass and biodiversity effects in Phase I

Drought significantly decreased community biomass (Table 1, Fig. 3a). There was a significant positive relationship between plant species richness and community biomass in the ambient treatments, and no correlation was detected in the drought treatments, reflecting a plant species richness x soil moisture treatment interaction (Table 1, Fig. 3a). Driving the differences in the diversity-productivity relationships between

5

moisture treatments, BE and CE were lower in drought compared to ambient treatments (Table 2, Fig. 4a and b). CE increased with plant species richness, while SE values were more negative with plant species richness (Table 2, Fig. 4b and c).

Community biomass and biodiversity effects in Phase II

Community biomass significantly increased with plant species richness in Phase II (Table 1, Fig. 3b). However, plant richness effects on community biomass significantly varied with soil moisture treatments, resulting in a significant plant species richness x soil moisture in Phase I x soil moisture in Phase II interaction (Table 1).

Over all treatments, the BE increased with plant species richness (Table 2, Fig. 4d). However, the CE and SE showed different directions of responses to plant species richness. Positive CE values were greater in higher plant species richness levels, while negative SE increased with higher plant species richness (Table 2, Fig. 4e and f). Soil moisture treatments in Phase I significantly influenced the BE, CE and SE in Phase II (Table 2). Both the BE and CE were greater in mesocosms with drought treatments than those with ambient treatments from Phase I (Fig. 4d and e). However, drought treatments from Phase I caused more negative SE in Phase II (Fig. 4f). Drought in Phase II generally decreased the magnitude of positive BE and CE values, and it had no general influence on SE (Table 2, Fig. 4).

Plant species richness and soil moisture treatments of Phase I and II interacted to influence both the CE and SE (Table 2). In mesocosms with two species, the CE did not vary among soil moisture treatment combinations, while at higher richness levels, the CE was greater in the Drought-Ambient treatment and lower in the Ambient-Ambient treatment compared to the other treatment combinations (Fig. 4e). There were no significant differences in SE among soil moisture treatment combinations for mesocosms with two or four species. However, in treatments with six or twelve species, the SE was positive in the Ambient-Ambient treatment, and the values were negative for the other treatment combinations. The negative SE was greatest in the Drought-Ambient treatment and with twelve plant species (Fig. 4f). Relationships between species richness and the BE did not vary among treatment combinations of soil moisture in Phase I and II (Fig. 4d), and thus there was no plant species richness x soil moisture in Phase I x soil moisture in Phase II interaction (Table 2).

Discussion

Our study addressed a key knowledge gap, namely, how past environmental conditions and plant communities influence soil microbial diversity which subsequently creates PSFs that can fundamentally alter plant diversity-productivity relationships. We found that 1) plant diversity and drought resulted in different soil microbial communities, leading to soil legacies that had subsequent effects of plant-soil feedbacks; 2) and that these soil legacies of drought and plant diversity influenced the strength of subsequent plant diversity-productivity relationships; 3) plant communities in soil that experienced past drought conditions usually had more positive complementarity effects and more negative selection effects.

Soil microbial communities shaped by plant diversity and drought

We observed pronounced impacts of plant species richness and soil moisture treatments on community composition of soil bacteria and fungi, indicating an effective conditioning process in Phase I. It has been frequently observed that soil microbial diversity increases with plant diversity, and the hypothesized mechanisms are that root exudates, litter heterogeneity and microhabitats provide more niche opportunities for soil microbes in more diverse plant communities (Youssef & Elshahed 2009; Millard & Singh 2010). However, our results demonstrated that the diversity of microbial communities did not always increase with greater plant diversity. We found that fungal overall diversity and AMF diversity increased and fungal pathogen diversity decreased with plant species richness under ambient conditions, while plant species richness increased AMF diversity under drought conditions. We did not observe any relationship between overall bacterial diversity and plant species richness. Our findings were consistent with an increasing realization that the relationship between microbial and plant diversity can exhibit complicated patterns that might differ for different func-

6

tional guilds (Rottstock *et al.* 2014; Prober *et al.* 2015; Schlatter*et al.* 2015; Dassen *et al.* 2017; Mommer *et al.*2018; Jia *et al.* 2020).

The positive relationships between overall fungal diversity or AMF diversity with plant diversity could reflect the strong host specificity of these microbes and thus plant mixtures harboured a mix of fungal guilds associated with different plant species (Rottstock *et al.*2014; Dassen *et al.* 2017). The neutral relationship between bacterial diversity and plant species richness likely reflected functional redundancy of soil bacteria in our model systems. Thus, high plant species richness did not increase bacterial diversity nor resulted in increased functioning of the whole bacterial community (Allison & Martiny 2008; Louca *et al.* 2018). Under ambient conditions, the relative abundance of fungal pathogens increased with plant species richness, likely reflecting that increased root biomass and exudates stimulated the growth of more generalist soil pathogens (Eisenhauer*et al.* 2017) However, high-density root systems likely impeded species-specific soil pathogens in locating their hosts, and thus some specialized pathogen species present in monocultures were absent in plant mixtures, leading to decreased fungal pathogen richness with plant species richness (Mommer *et al.* 2018).

Biodiversity declined under drought stress for soil bacteria, fungi and AMF. AMF relative abundance decreased and fungal pathogen relative abundance increased under drought conditions. These findings reflect that drought likely limited activities of some guilds that were sensitive to drought stress and selected for particular drought-adapted guilds such as dormant pathogens and drought-resistant AMF (Mariotte*et al.* 2017; Meisner *et al.* 2018). Our results provided evidence that plant diversity and drought can condition soil microbial communities, leading to soil legacy effects that can alter subsequent PSFs (De Long *et al.* 2019).

The more pronounced plant diversity -productivity relationships in Phase II

In Phase I, the BE was not correlated with plant species richness in the ambient treatments even though the values were positive, and the BE was absent in the drought treatments, causing a non-significant plant diversity-productivity relationship (Fig. 3a). In Phase II, however, the BE increased with plant species richness, regardless of soil moisture treatments (Fig. 4d). Our results provided evidence that the positive plant diversity-productivity relationships were more pronounced over time, and soil microbes were one mechanism behind this finding (Schmid*et al.* 2008; van Ruijven *et al.* 2020).

These findings were in line with several studies that attributed long-term biodiversity effects to increasing niche complementarity in resource use and facilitation (van Ruijven *et al.* 2005; Cardinale*et al.* 2007; Fargione *et al.* 2007). However, our results show that these temporal patterns in plant diversity-productivity can be primarily driven by PSFs (Maron *et al.* 2011; Schnitzer *et al.* 2011; Kulmatiski *et al.* 2012; Jing *et al.* 2015; Wang*et al.* 2019). We found that the positive CE-plant richness relationships were more pronounced in Phase II compared to Phase I. A possible explanation was that accumulations of species-specific soil pathogens decreased community biomass in the monocultures, while the community biomass of mixtures was promoted due to enhanced diversity of AMF (van Der Heijden *et al.* 1998; Wagg *et al.* 2011; van Ruijven *et al.* 2020). Future studies are necessary to test this hypothesis through identifying species-specific fungal species and recording their shifts in abundance as plant species richness increase.

Interestingly, we observed that the SE became more negative in higher plant richness levels in Phase II (Fig. 4f), and this result was consistent with previous findings that the positive selection effects become negative over time (Fargione *et al.* 2007; Marquard*et al.* 2009). In the present study, unproductive plants, *D. aegyptium* , increased their performance more than productive species in high plant richness levels, leading to a negative selective effect (Fig. S3). In other words, unproductive species might suffer from stronger negative density dependence than productive species, and the possible mechanism was that productive species could produce dense and large root systems in mixtures that declined dilution effects of species-specific soil pathogens (Mommer *et al.* 2018; van Ruijven *et al.*2020). Previous studies have suggested that negative PSFs can maintain species richness by preventing productive species from dominating the plant community (Bever 2003; Adler & Muller-Landau 2005; Petermann*et al.* 2008; Mangan *et al.* 2010). The implication of our results is that soil conditioning can alter biodiversity effects by increasing complementarity

and decreasing selection effects through PSFs.

Plant diversity legacy interacted with drought legacy and recurrent drought

Our results showed that both the BE and CE were greater in plant communities with a drought legacy (Fig. 4d and e), and the CE was the most pronounced in the Drought-Ambient treatments and in the highest plant richness level, while there was an opposite pattern for the SE, reflecting the interaction between drought and plant diversity legacy and subsequent drought. There were two potential, and not mutually exclusive, mechanisms for these drought legacy effects on the CE. Firstly, drought stress can favor specific soil pathogens and lead to a breakout after rewetting (van der Putten *et al.* 2016; Barnes*et al.* 2018). Increasing susceptibility of plants to soil pathogens during drought stress allows the pathogen population to accumulate in monocultures rather than high-richness mixtures (Preece*et al.* 2019). Some soil pathogens might go dormant when facing unfavorable conditions and such a strategy allows them to quickly recover and occupy empty soil niches after rewetting (Schimel 2018; Crawford & Hawkes 2020). Secondly, post-drought soil has higher nutrient concentrations than soils without historical drought due to a pulse of mineralization following rewetting through the reactivation of microbial activity and enhanced decomposition rates of dead materials (Bloor & Bardgett 2012; Leitner *et al.* 2017), thereby promoting plant growth. Our results likely support the first hypothesis, because we added soil inoculums into background soils (1: 10 in volume ratio) that reduced the effects of nutrient differences, and plant monoculture yields were lower in the Drought-Ambient than Ambient-Ambient treatments (Fig. 3b). The negative SE occurred in the Drought-Ambient treatments and the highest plant richness level, and this was due to increasing dominance of a small-size species (i.e., *D. aegyptium*) in mixtures (Fig. S3). Previous work suggest that grass species have more negative PSFs than forbs (Kulmatiski *et al.* 2008). In our system, grass species might exhibit negative PSFs in monocultures but largely escape it in mixtures due to efficient dilution effects of species-specific soil pathogens.

We found that drought soil legacy was diminished by subsequent drought. For example, the CE and SE were less pronounced in the Drought-Drought than Drought-Ambient treatments, and the CE and SE were consistent in the strength and direction between the Ambient-Drought and Drought-Drought treatments (Fig. 4e and f). Subsequent drought simply slowed down soil processes and limited microbial activities (Bever 2002; Schimel 2018), thereby diminishing the soil legacy effect. Our findings illustrated that historical and subsequent drought events had different directional effects on plant diversity-productivity relationships, and they can interact to regulate net diversity effects and its components (i.e., complementarity and selection effects). Future rainfall changes are predicted to generate complicated patterns in biodiversity-ecosystem functioning (i.e., community productivity, ecosystem carbon cycling, soil mineralization and others) relationships through shaping soil microbial community structure and activity.

Conclusions

Our results illustrated that plant-soil feedbacks critically influenced plant diversity-productivity relationships, likely through the dilution effects of species-specific pathogens in more diverse communities. We also found that past drought can result in a soil microbial legacy that can have subsequent impacts on plant diversity-productivity relationships. Drying-rewetting scenarios can strengthen complementary effects while weakening selection effects. Our results highlight the importance of soil microbial communities in driving plant diversity effects on ecosystem functioning, and that changes to future rainfall patterns can have direct and long-lasting effects on biodiversity-ecosystem functioning relationships through soil legacies created by past rainfall patterns and which interacts with plant diversity and subsequent rainfall.

Acknowledgements

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Table 1 Results from general linear mixed effects model testing how plant richness and soil moisture treatments influenced community biomass in Phases I and II.*F* and *P* values are given. Bold types indicate significant effects ($P < 0.05$).

Variables	*df*	*F* value	*P* value
Phase I			
Species richness (SR)	1, 46	10.26	**0.003**
Soil moisture in Phase I (M1)	1, 69	291.37	**<0.001**
SR × M1	1, 69	10.47	**0.002**
Phase II			
Species richness (SR)	1, 112	46.34	**<0.001**
Soil moisture in Phase I (M1)	1, 112	8.67	**0.004**
Soil moisture in Phase II (M2)	1, 228	331.84	**<0.001**
SR × M1	1, 112	0.21	0.641
SR × M2	1, 228	18.35	**<0.001**
M1 × M2	1, 228	0.38	0.538
SR × M1 × M2	1, 228	5.46	**0.020**

Table 2 Results from general linear mixed effects model testing how plant richness and soil moisture treatments influenced net biodiversity effects (BE), complementarity effects (CE) and selection effects (SE) in

Phases I and II. F and P values are given. Bold types are significant effects ($P < 0.05$). Bold types indicate significant effects ($P < 0.05$).

Variables	df	BE F value	BE P value	CE F value	CE P value	SE F value	SE P value
Phase I							
Species richness (SR)	1, 34	0.06	0.815	5.46	**0.026**	5.16	**0.030**
Soil moisture in Phase I (M1)	1, 58	241.66	**<0.001**	53.17	**<0.001**	0.09	0.770
SR × M1	1, 58	0.29	0.591	1.71	0.197	3.08	0.084
Phase II							
Species richness (SR)	1, 90	38.2	**<0.001**	97.4	**<0.001**	5.98	**0.016**
Soil moisture in Phase I (M1)	1, 90	18.74	**<0.001**	63.15	**<0.001**	6.93	**0.010**
Soil moisture in Phase II (M2)	1, 90	11.19	**0.001**	5.8	**0.018**	0.34	0.564
SR × M1	1, 90	0.06	0.804	15.67	**<0.001**	10.64	**0.002**
SR × M2	1, 90	6.96	**0.010**	3.96	**0.049**	0.12	0.730
M1 × M2	1, 90	25.88	**<0.001**	59.33	**<0.001**	16.23	**<0.001**
SR × M1 × M2	1, 90	0.54	0.463	26.5	**<0.001**	30.08	**<0.001**

Figure legends

Figure 1 Schematic depiction of the experimental design. The study combined a PSF experiment and a plant diversity-productivity relationship experiment. In the soil conditioning phase (Phase I), plant communities with a range of species richness levels grew in homogeneous soils, and soil microbial communities were trained by the plant communities. In the response phase (Phase II), newly established plant communities grew with soil inoculums which the same plant communities conditioned.

Figure 2 (a) RDA analysis showing how plant species richness and soil moisture content influenced species compositions of soil bacterial and fungal communities in Phase I. SR, plant species richness; SMC, soil moisture content. Effects of plant diversity and soil moisture treatments on (b) bacterial diversity, (c) fungal diversity, (d) AMF OTU richness, (e) AMF relative abundance, (f) fungal pathogen OTU richness and (g) fungal pathogen relative abundance. Means and SE are given. Solid lines indicate significant correlations between plant species richness and microbial variables ($P < 0.05$).

Figure 3 Plant diversity-productivity relationships in the (a) Phase I and (b) II under different soil moisture treatments. The solid lines indicate fitted lines for plant diversity-productivity relationships, and the grey areas indicate the 95% confidence intervals. The dashed line indicates an insignificant correlation ($P > 0.05$). Letter codes indicate Phase I-Phase II soil moisture treatments: A-A = Ambient-Ambient; A-D = Ambient-Drought; D-A = Drought-Ambient; D-D = Drought-Drought.

Figure 4 Net biodiversity, complementarity and selection effects at different species richness levels in Phases I (a, b, c) and II (d, e, f). Means and SE are given. Letter codes indicate Phase I-Phase II soil moisture treatments: A-A = Ambient-Ambient; D-A = Drought-Ambient; A-D = Ambient-Drought; D-D = Drought-Drought.
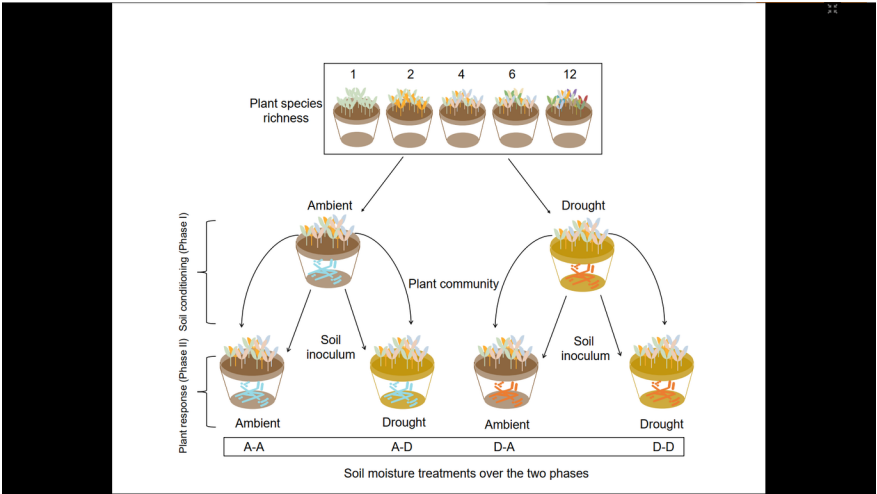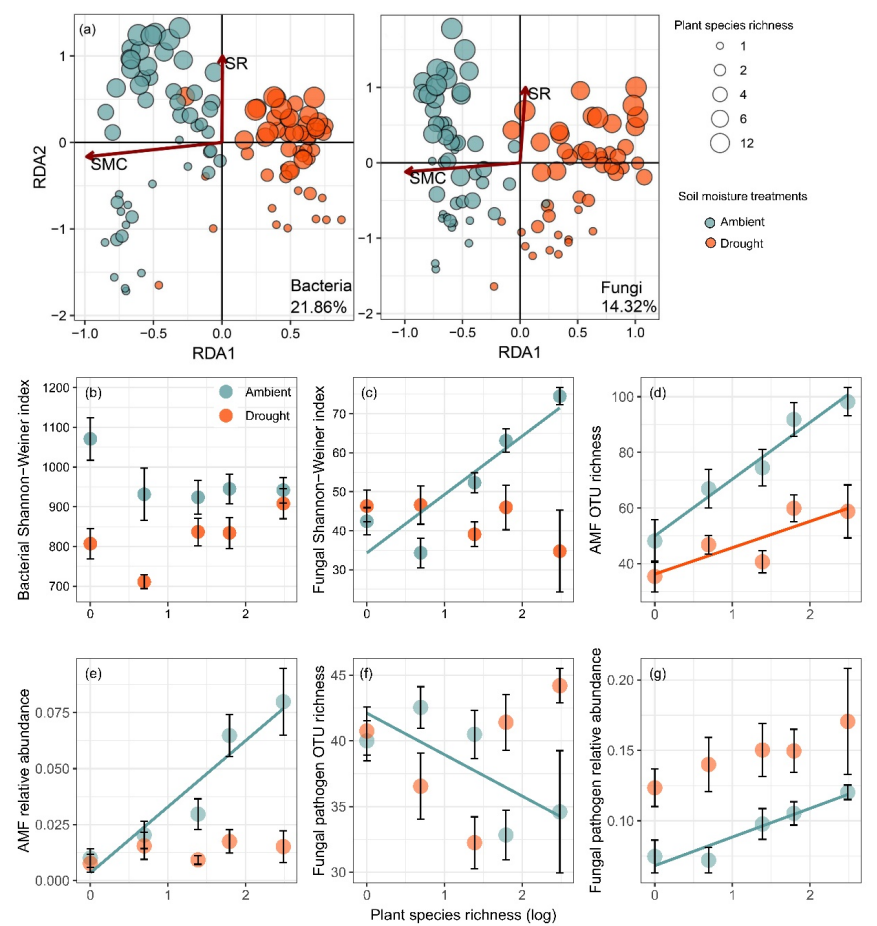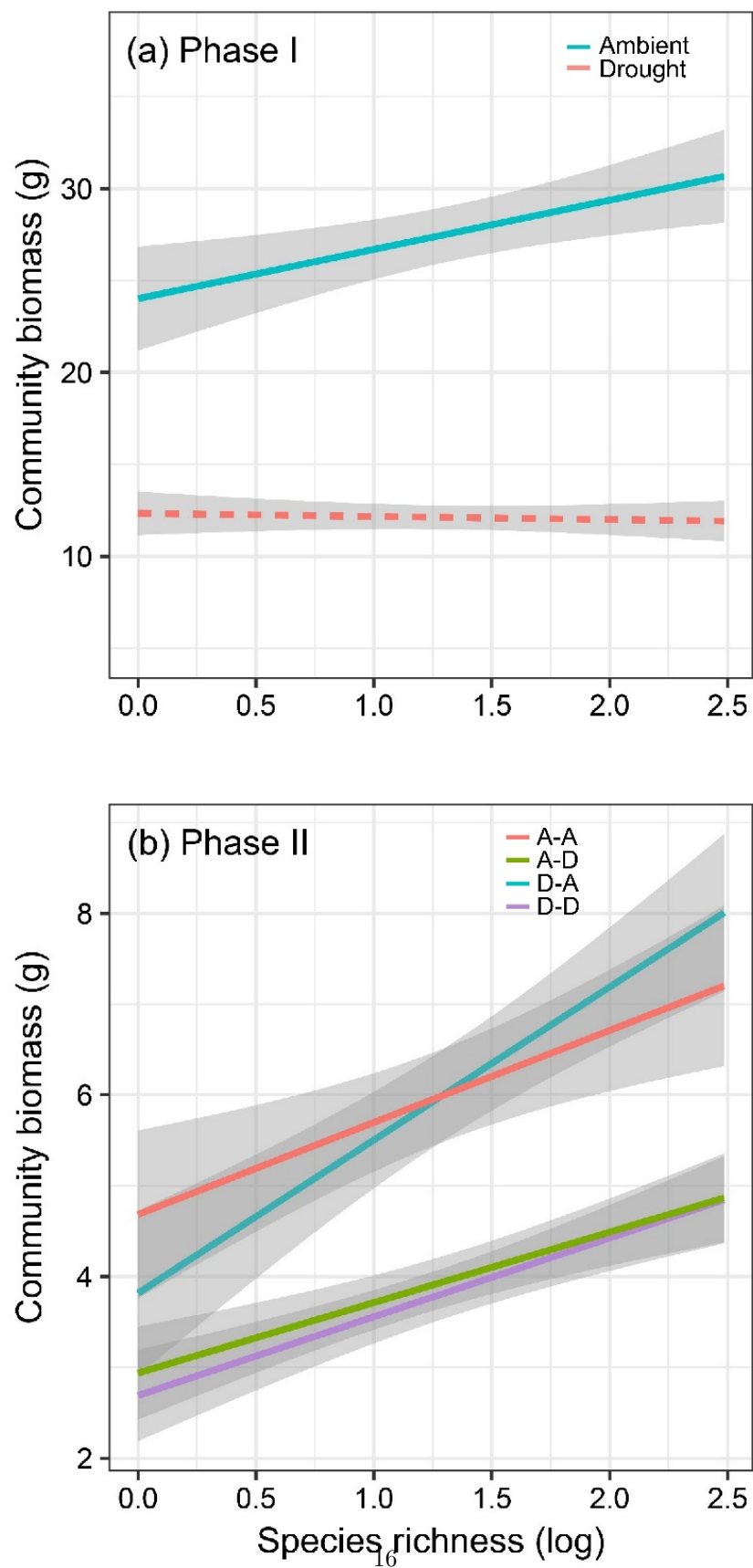
Figure 1

Figure 2

Figure 3

Figure 4