Soil biodiversity enhances the persistence of legumes under climate change

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Summary

- Global environmental change poses threats to plant and soil biodiversity. Yet, whether soil biodiversity loss can further influence plant community's response to global change is still poorly understood.
- We created a gradient of soil biodiversity using the dilution-to-extinction approach, and investigated the effects of soil biodiversity loss on plant communities during and following manipulations simulating global change disturbances in experimental grassland microcosms.
- Grass and herb biomass was decreased by drought and promoted by nitrogen deposition, and a fast recovery was observed following disturbances, independently of soil biodiversity loss. Warming promoted herb biomass during and following disturbance only when soil biodiversity was not reduced. However, legumes biomass was suppressed by these disturbances, and there were more detrimental effects with reduced soil biodiversity. Moreover, soil biodiversity loss suppressed the recovery of legumes following these disturbances. Similar patterns were found for the response of plant diversity. The changes in legumes might be partly attributed to the loss of mycorrhizal soil mutualists.
- Our study shows that soil biodiversity is crucial for legume persistence and plant diversity maintenance when faced with environmental change, highlighting the importance of soil biodiversity as a potential buffering mechanism for plant diversity and community composition in grasslands.

Introduction

Drivers of global environmental change, such as drought, nitrogen (N) deposition and warming, have been shown to dramatically shift plant community composition and primary productivity, and reduce plant diversity (Stevens *et al.*, 2004; Clark & Tilman, 2008; Hautier *et al.*, 2015; Buermann *et al.*, 2018; Liu *et al.*, 2018; Stevens *et al.*, 2018; Ploughe *et al.*, 2019). Global change also threatens soil biodiversity, leading to increasing concerns about the consequences of soil biodiversity loss (Veresoglou *et al.*, 2015; Geisen *et al.*, 2019; Tibbett *et al.*, 2020; Zhou *et al.*, 2020). The majority of existing studies suggest that biodiversity underpins the stable provision of ecosystem functions (Hautier *et al.*, 2014; Tilman *et al.*, 2014; Hautier *et al.*, 2015; Isbell *et al.*, 2015). However, we know little about whether a diverse soil community can help to maintain plant diversity and stabilize community composition under global environmental change.

Soil biodiversity, including numerous soil organisms, plays fundamental roles in the dynamics of plant community composition, and the maintenance of plant diversity and multiple ecosystem functions (Philippot *et al.*, 2013; Wagg *et al.*, 2014; Jing *et al.*, 2015; Delgado-Baquerizo *et al.*, 2020; Guerra *et al.*, 2020; Thakur *et al.*, 2020). A recent study shows that the yield of a legume crop (pea) following drought was enhanced by soil microbial diversity (Prudent *et al.*, 2020). Furthermore, a conceptual study shows high soil biodiversity may help stabilize plant community composition and maintain plant diversity when faced with global change (Yang *et al.*, 2018). A better understanding of how soil biodiversity can contribute to stabilizing plant communities will advance our ability to predict the consequence of global change factors on vegetation dynamics.

Drought, with increases in the frequency and intensity in many regions, can cause the loss of plant diversity and large changes in plant community composition (Hoover *et al.*, 2014; Liu *et al.*, 2018; Ploughe *et al.*, 2019). Drought has more detrimental impacts on herbs than grasses, leading to plant communities dominated by grasses (Hoover *et al.*, 2014; Liu *et al.*, 2018). Past studies have reported that soil microbial communities can promote the resistance of legumes and deciduous trees to drought (Xi *et al.*, 2018; Allsup & Lankau, 2019; de Vries *et al.*, 2020; Prudent *et al.*, 2020). Besides, soil mutualists, such as arbuscular mycorrhizal (AM) fungi and plant growth-promoting bacteria, can promote the resistance of plant growth to drought (Mariotte *et al.*, 2017; Rubin *et al.*, 2017; Wu, 2017; Armada *et al.*, 2018; Z. Zhang *et al.*, 2019). In this case, a diverse soil community, especially the presence of soil mutualists, could reduce the negative effect of drought on plant communities by increasing the performance of legumes and herbs.

Warming has been shown to reduce the temporal stability of primary productivity, but to increase the abundance of grasses in an alpine grassland in the Tibetan Plateau (Ma *et al.*, 2017; Liu *et al.*, 2018), and in North American prairie without changes in plant diversity (Whittington *et al.*, 2013; Cowles *et al.*, 2016). However, a recent study reported that there will be a decline in plant diversity as species losses induced by warming generally exceed species gains (Harrison, 2020). In experimental grasslands, the growth of legumes and herbs was dramatically suppressed by soil biodiversity loss, while grasses increased and dominated at low soil biodiversity (Wagg *et al.*, 2014). These results indicate that higher soil biodiversity can enhance the performance of legumes and herbs. However, whether soil biodiversity can benefit legumes and herbs under and following warming is still poorly understood.

It is well known that N deposition is a major driver of the loss of plant species (Stevens *et al.*, 2004; Suding *et al.*, 2005; Clark & Tilman, 2008; Bai *et al.*, 2010; Zhang *et al.*, 2014). Usually, N deposition changes plant community composition by favouring grasses over other species, and therefore, contributes to the extinction of legumes and herbs. Legumes and herbs are more dependent on the presence of AM fungi and N-fixing bacteria (van der Heijden *et al.*, 2008a; Hoeksema *et al.*, 2010; van der Heijden *et al.*, 2016). A previous study shows the presence of AM fungi can decrease the negative effect of N deposition on plant communities by promoting the performance of legumes (van der Heijden *et al.*, 2008b). Therefore, high soil biodiversity could be crucial for the performance of legumes and herbs during and following N deposition, because a diverse soil community is more important for legumes and herbs than grasses (Wagg *et al.*, 2014).

High dominance of some species in plant communities can reduce available resources, favour competitive exclusion, and therefore, lower plant diversity (McNaughton & Wolf, 1970; Koerner *et al.*, 2018). Soil biodiversity loss can decrease soil nutrient availability, and therefore, increase competition among plant species, giving dominant species (e.g. grasses) an advantage (De Deyn *et al.*, 2004; Philippot *et al.*, 2013; Wagg *et al.*, 2014; Delgado-Baquerizo *et al.*, 2020). The loss of soil biodiversity increased the dominance of grasses, leading to a decrease in plant diversity (De Deyn *et al.*, 2004; Wagg *et al.*, 2014). The presence of AM fungi can improve plant diversity by promoting the growth of legumes under N deposition (van der Heijden *et al.*, 2008b). Similarly, because soil biodiversity is important for the growth of herbs and legumes, we expect that soil biodiversity would maintain plant diversity under global change disturbances and increase the recovery of plant diversity by promoting the growth of these species following disturbances.

In this study, the dilution-to-extinction approach using serial dilutions of soil was employed to create a gradient of soil biodiversity (Salonius, 1981; Hol *et al.*, 2015b; Yan *et al.*, 2015; Roger *et al.*, 2016; Kurm *et al.*, 2018; Domeignoz-Horta *et al.*, 2020). We established microcosms, simulating a local grassland, along soil biodiversity gradients under controlled conditions, and then we investigated how soil biodiversity loss affected plant diversity and community composition during and following N deposition, warming and drought (Fig. 1). We hypothesize that (1) less variance in plant community responses could be observed at high soil biodiversity during global change disturbances; and that (2) high soil biodiversity can enhance the recovery of plant community responses following global change disturbances.

Materials and Methods

Experimental design

Warming, N deposition and drought disturbances were included in the present study, as they are major global change factors determining the functions of soil microbial communities (Delgado-Baquerizo *et al.*, 2017; Zhou *et al.*, 2020). There were three parallel experiments. Each global change factor was combined with high, moderate and low soil biodiversity treatments (Fig. 1), resulting in three treatments for each experiment. Besides, a high soil biodiversity treatment without global change disturbance was regarded as the control for all three experiments. There were three treatments for each experiment plus one control and each is replicated six times, for a total of 60 grassland microcosms. In reality, with global change factors influencing plant diversity and community composition, soil biodiversity loss can occur as a result of several global anthropogenic changes, such as extreme climate changes, land-use change, agricultural intensification, and nutrient eutrophication (Tsiafouli *et al.*, 2015; Geisen *et al.*, 2019; Rillig *et al.*, 2019; Tibbett *et al.*, 2020; Zhou *et al.*, 2020). Therefore, the control in the present study represented an initial status, which was used to evaluate the effect of global change factor and soil biodiversity by comparison.

Soil biodiversity manipulation

The soil was collected from the top 20 cm of a local grassland in Brandenburg (52.466°N, 13.303°E). The soil is an Albic Luvisol and has the following properties: 73.6% sand, 18.8% silt and 7.6% clay; pH 7.1 (calcium chloride), 6.9 mg of phosphorus per 100 g of soil (calcium acetate-lactate), 0.12% N and 1.87% carbon (Rillig *et al.*, 2010). Field soil was passed through a 0.5 cm mesh to remove large roots and stones. About 6 kg of fresh soil was stored at 4°C for 3 days until soil dilution. The rest of the soil (40 kg) was sterilized by autoclaving for 90 min at 121°C. Although autoclaving can alter physical and chemical soil properties (Salonius *et al.*, 1967; Dietrich *et al.*, 2020), this side effect should have a minor impact on our results, because all soil used in the experiment had been sterilized with the same autoclaving procedure.

The dilution-to-extinction approach has been widely used to investigate soil biodiversity–ecosystem functions relationships (Salonius, 1981; Hol *et al.*, 2015b; Yan *et al.*, 2015; Roger *et al.*, 2016; Kurm *et al.*, 2018; Domeignoz-Horta *et al.*, 2020). Previous studies using this approach suggest that rare soil microbial species are lost first during the dilution (Salonius, 1981; Hol *et al.*, 2015b; Yan *et al.*, 2015; Roger *et al.*, 2016; Kurm *et al.*, 2018; Maron *et al.*, 2018). In the same manner, rare soil microbial species are likely more sensitive to global change factors than

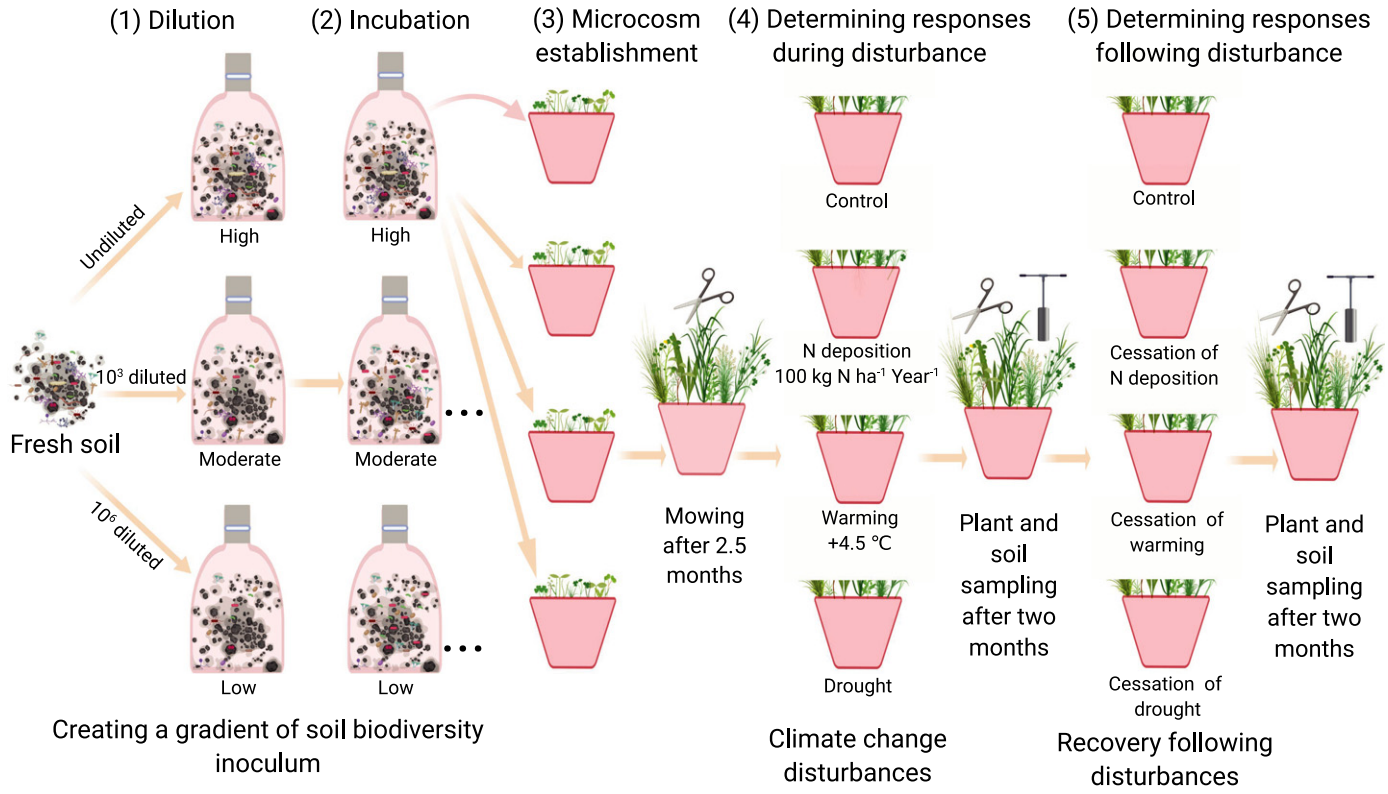

Fig. 1 Overview of the experiment. (1) Dilution. High, moderate and low soil biodiversity inocula were created by not diluting or diluting fresh soil 1×10^3 and 1×10^6 times with sterilized soil. Soil inocula were stored in plastic bags. Sterile water was added to each plastic bag to reach the initial moisture content of the local grassland. (2) Incubation. Plastic bags were sealed with sterilized cotton plugs and rubber bands to avoid microbial contamination but allow gas exchange. All bags were incubated in a dark room at 20°C for 2 months when similar microbial abundance was observed among different dilutions of soil inocula. (3) Microcosm establishment. Each microcosm was filled with a mixture of 200 g DW of soil inoculum and 6.8 kg DW of a sterilized substrate, planted with 24 seedlings of 12 plant species (two individuals of each species), and then maintained in a glasshouse for 2.5 months. (4) Determining responses during disturbance. All plant shoots were removed 5 cm above the soil surface before the implementation of global change disturbances. After 2 months of global change treatments, plant shoots were harvested by species and soil was collected by mixing three cylindrical soil cores into one composite sample for each microcosm. (5) Determining responses following disturbance. After 2 months of recovery, plants and soil were sampled as in the previous harvest.

common species (Gaston, 2008; Zhou *et al.*, 2020), indicating rare species could be lost first during global change disturbances. Therefore, this approach can be used to simulate a realistic loss of soil biodiversity.

The undiluted fresh field soil (10^0) was used as the 'high' soil biodiversity treatment. The 10^{-1} dilution treatment was created by mixing 200 g dry weight (DW) of fresh soil with 1800 g DW sterilized soil. And then 200 g of the 10^{-1} dilution were mixed with 1800 g DW sterilized soil to obtain the 10^{-2} dilution treatment. We repeated this procedure several times to reach 10^{-3} and 10^{-6} dilutions. A plastic bag was filled with 2000 g DW of the 10^0, 10^{-3} or 10^{-6} soil dilution to create high, moderate or low soil biodiversity treatments, respectively (Fig. 1). There were nine bags (three bags for each soil biodiversity treatments) in total.

After dilution, there was an incubation phase allowing the regrowth of soil microbes to reach similar microbial abundance among different dilutions of soil inoculum. Sterile water was added to each plastic bag to reach the initial moisture content of the local grassland. All bags were closed with a sterilized cotton plug and a rubber band to avoid microbial contamination but allow gas exchange (Hol *et al.*, 2015a), and then were stored in a dark room at 20°C. The incubated soil was homogenized by shaking and turning the bags every 2 wk.

Our previous study showed that a gradient of soil microbial diversity has been successfully created using the dilution-to-extinction approach after 2 months of incubation (G. Yang, M. Ryo, J. Roy, S. Hempel, C. M. Rillig, unpublished). Compared with the undiluted soil, microbial diversity of soil inoculum was reduced by 56.7% in the 10^{-3} dilution and by 77.1% in the 10^{-6} dilution, and less abundant soil taxa were first removed during dilution. Besides, the soil microbial biomass was fully recovered. These results have been reported in a previous study using the same soil inocula (G. Yang, M. Ryo, J. Roy, S. Hempel, C. M. Rillig, unpublished). The magnitude of biodiversity loss by soil dilution is larger than that induced by single or few global change factors (Zhou *et al.*, 2020), but soils typically face multiple factors simultaneously, which can reduce soil fungal diversity to similar levels as obtained at low soil biodiversity treatment (Rillig *et al.*, 2019).

Microcosm establishment and sampling

Grassland microcosms were established using 60 pots (22.5 cm diameter and 16.5 cm height). Each pot received 200 g DW of soil inoculum, which was carefully homogenized with 6.8 kg DW of a sterilized (autoclaved for 90 min at 121°C) 1 : 1 sand : field soil mixture. Twelve typical species from the local grassland were used in this study, including four species of grasses (*Holcus lanatus*, *Anthoxanthum odoratum*, *Lolium perenne* and *Festuca rubra*), four species of herbs (*Daucus carota*, *Achillea millefolium*, *Hieracium pilosella* and *Plantago lanceolata*) and four species of legumes (*Trifolium repens*, *Vicia cracca*, *Medicago lupulina* and *Lotus corniculatus*). All seeds were obtained from Rieger-Hofmann GmbH (Blaufelden-Raboldshausen, Germany), surface sterilized with 70% alcohol for 2 min, germinated at room temperature in sterilized sand, and watered with sterile water. Simultaneous germination of different species was ensured by varying the start time (as done in a preliminary study). Each pot was planted with 24 post-germination seedlings of 12 plant species (two individuals for each species). All microcosms were maintained in a glasshouse with 22°C for 16 h during the day and 18°C for 8 h at night. High-pressure sodium lamps (400 W) were used to subsidize light when the light intensity was below 50 klx. Each microcosm was watered twice weekly to maintain gravimetric soil moisture of 12 to 18%. All microcosms were weighed biweekly to balance water content and their locations were randomly re-assigned at this same time throughout the experiment.

Two and a half months after planting, plant shoots were removed at 5 cm above the soil surface (Fig. 1). The experimental procedures here, with the 2.5 months of an establishing stage, aimed at bringing the majority of microbial taxa at densities close to their carrying capacity in their new environment. Global change disturbances were conducted after the first harvest. Microcosms with the control, N deposition and warming treatments were watered twice weekly to maintain the same gravimetric soil moisture of 12 to 18% by weight. The microcosms with the drought treatment received constant amount of water (300 ml) only when most legumes and herbs started to wilt (Weißhuhn *et al.*, 2011), and water content was balanced biweekly to 12% of gravimetric soil moisture. Each microcosm with the N deposition treatment received 1.29 g of ammonium nitrate (NH_4NO_3, based on the soil surface in microcosm). This amount of N added in each microcosm is equivalent to 100 kg N ha^{-1} yr^{-1}, which reflects a high N deposition level (van der Heijden *et al.*, 2008b; Rillig *et al.*, 2019). Microcosms with warming were maintained under increased temperature (+4.5°C) (Delgado-Baquerizo *et al.*, 2017; Rillig *et al.*, 2019). To increase the temperature of microcosms, a heating cable (Exo Terra PT-2012; Hagen Deutschland GmbH & Co. KG, Holm, Germany) was wrapped around the outside of each pot, which was independently controlled by a temperature controller (Voltcraft ETC-902; Conrad Electronic SE, Hirschau, Germany) for each pot (Supporting Information Fig. S1). The temperature controller has a sensor buried in the pot and can maintain a set temperature with ± 1°C dynamics in the pot by switching off and on the heating cable (Rillig *et al.*, 2019).

After 2 months of the implementation of global change treatments, plant shoots were cut 5 cm above the soil surface and sorted by species, oven-dried for 72 h at 60°C and weighed (Fig. 1). Three cylindrical soil cores (8-cm depth and 1-cm diameter) were collected from each microcosm and mixed into one composite sample. The holes were filled right after sampling with the same sterilized 1 : 1 sand : field soil mixture used for microcosm establishment. The sampled soil in each microcosm was sieved to 4 mm, homogenized, and stored at −80°C until DNA extraction. All global change treatments were stopped after the second harvest. All microcosms were maintained at the same condition as before the first harvest to enable the microcosms to recover from the experimental treatments. Two months later, plant and soil were sampled using the method described earlier. Based on the macronutrients of the maximum biomass production removed by the first harvest, 400 ml of a modified Hoagland nutrient solution was added to each microcosm after each harvest. The solution consisted of the following nutrients: 7.8 mM KNO_3, 5.2 mM $Ca(NO_3)_2$, 1.3 mM KH_2PO_4, 2.6 mM $MgSO_4$, 5.9 μM Fe(Na)-EDTA, 46 μM H_3BO_3, 9.1 μM $MnCl_2$, 0.32 μM $CuSO_4$, 0.77 μM $ZnSO_4$, 0.1 μM H_2MoO_4. Root biomass was not measured in this study, because the destructive sampling of roots during the second harvest could affect the recovery of plants following disturbances, resulting in a confounding effect of treatment and sampling.

Soil fungal and bacterial diversity

We extracted DNA from each soil inoculum and soil samples during and following global change treatment from 250 mg soil, using DNeasy PowerMax Soil Kit (MoBio Laboratories Inc., Carlsbad, CA, USA), following manufacturer's instructions. The taxonomic composition of soil fungal and bacterial diversity was determined using Illumina MiSeq high-throughput sequencing with fITS7 and ITS4 for fungi and 515f and 806r for bacteria (Fierer *et al.*, 2005; Ihrmark *et al.*, 2012).

DADA2 in *R* was used to obtain denoised, chimera-free, nonsingleton fungal amplicon sequence variants (ASVs) (Callahan *et al.*, 2016), following the standard operating procedure as implemented in Rillig *et al.* (2019). Raw reads were demultiplexed allowing no error in the index sequence for sample assignment. Primers were removed, and, for fungi, this included the removal of the reverse complement sequence of the reverse and forward primer sequence in the forward and reverse reads, respectively, using cutadapt (Martin, 2011). Reads with more than one and two maximum expected number of errors for forward and reverse reads were excluded. Nonsingleton ASVs were inferred on a sample basis. Chimera were identified *de novo* as sequences that corresponded to subsets of two more abundant sequences and removed. Taxonomic annotation of fungal ASVs was performed using the Naive Bayesian Classifier (Wang *et al.*, 2007) against UNITE (Nilsson *et al.*, 2019). RDPII database was used for bacteria taxonomic annotation (Cole *et al.*, 2013). Taxonomic annotations at any rank were considered robust at a 100% bootstrap confidence threshold. Internal transcribed spacer (ITS) ASVs and 16S ASVs not annotated to fungi and bacteria, respectively, were removed. Sample reads were rarefied

to a common sequencing depth to account for varying sequencing depth among samples. There were 1000 reads for bacteria and 100 reads for fungi.

Statistical analysis

For each parallel experiment, one-way ANOVA was employed to investigate how soil biodiversity loss and the implementation or termination of global change treatment influenced all variables. The same method was used to test the effect of soil biodiversity on plant diversity, shoot biomass production of plant community, grasses, herbs and legumes before global change disturbance. We used Duncan's multiple range test to compare the differences among the control and soil biodiversity treatments before, under and following global change disturbance at the 0.05 probability level. Data were log-transformed if needed to ensure normal distributions of residuals and homoscedasticity. All data analyses were performed in the software R v.4.0.0 (R Core Team, 2020). The package VEGAN was used to calculate diversity index. For data visualization, we used the packages GGPLOT2, RESHAPE2 and COWPLOT (Wickham, 2007, 2016; Wilke, 2019). Packages DPLYR, TIDYR, AGRICOLAE, CAR, ENVSTATS and TIBBLE were used for data manipulation and statistical analysis. R script and data are available in the Supporting Information Notes S1 and Dataset S1, respectively.

Results

Shoot biomass production during global change disturbances

Drought strongly decreased the overall shoot biomass of the plant community and for each functional group (Fig. 2a–d). This was particularly pronounced for legumes with reduced soil biodiversity. For instance, drought decreased the shoot biomass of legumes by 89% at high soil biodiversity, and by 95% at moderate soil biodiversity (Fig. 2d). In contrast to drought, N deposition increased the overall shoot biomass of the plant community. Shoot biomass of grasses and herbs increased with N deposition at all soil biodiversity treatments, whereas the shoot biomass of legumes was decreased by N deposition under reduced soil biodiversity (Fig. 2e–h). Warming did not alter the overall shoot biomass of the plant community

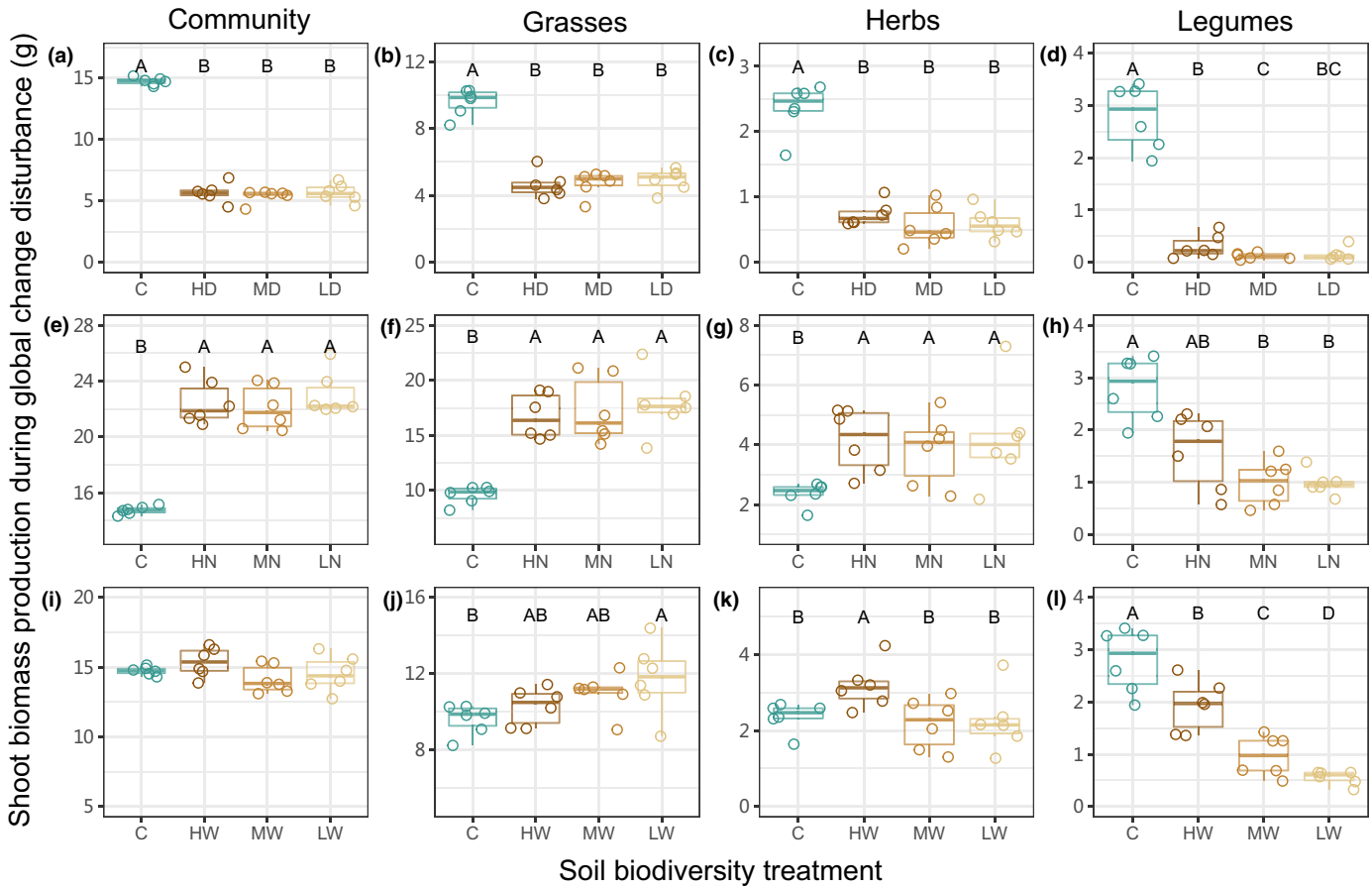

Fig. 2 The effects of soil biodiversity loss and global change factors on the overall shoot biomass of plant community (a, e, i), grasses (b, f, j), herbs (c, g, k) and legumes (d, h, l) during global change disturbances. H, high soil biodiversity; M, moderate soil biodiversity; L, low soil biodiversity; C, control (ambient condition); D, drought; N, nitrogen deposition; W, warming. Boxplots with different capital letters indicate significant differences ($P < 0.05$). The ends of the boxplot are the upper (75th percentile) and lower (25th percentile) quartiles; the vertical line inside the box is the median (middle quartile); the upper and lower whiskers represent responses outside the middle 50%.

(Fig. 2i). Similar to N deposition, biomass was redistributed among species of different functional groups. Compared with the control, warming increased the shoot biomass of grasses at low soil biodiversity, while enhancing the shoot biomass of herbs with high soil biodiversity (Fig. 2j,k). However, warming decreased the shoot biomass of legumes, particularly with reduced soil biodiversity (Fig. 2l). For instance, there were 31%, 65% and 80% of reductions at high, moderate and low soil biodiversity, respectively (Fig. 2l). In general, when faced with these global change disturbances, the growth of legumes experienced less of a decrease when soil biodiversity was maintained at a high level. Furthermore, soil biodiversity loss did not alter shoot biomass of plant communities, grasses, herbs and legumes before global change disturbance (Fig. S2).

Shoot biomass production after the termination of global change disturbances

There were no significant differences among treatments in the overall shoot biomass of the plant community, grasses and herbs following the cessation of the drought and N deposition treatments (Fig. 3a–c,e–g). Compared with the control, the shoot biomass of legumes at high or moderate soil biodiversity had fully recovered from global change disturbances (Fig. 3d,h,l). Nevertheless, there were still significant differences between the control and global change treatments at lower soil biodiversity levels (Fig. 3d,h,l). These results indicate that high soil biodiversity can enhance the recovery of legumes from global change disturbances. Besides, the ability to recover depended also on global change factor. For instance, full recovery required high soil biodiversity following drought, while moderate soil biodiversity was enough for recovery following N deposition and warming (Fig. 3d,h,l). We observed that warming has a legacy effect on the shoot biomass of grasses and herbs (Fig. 3i–k). Similar responses of grasses and herbs was observed during and following warming disturbance.

Plant and soil microbial diversity

Drought reduced plant diversity compared to control conditions in the case of high soil biodiversity and this negative effect was even stronger in moderate and low soil biodiversity (Fig. 4a). For

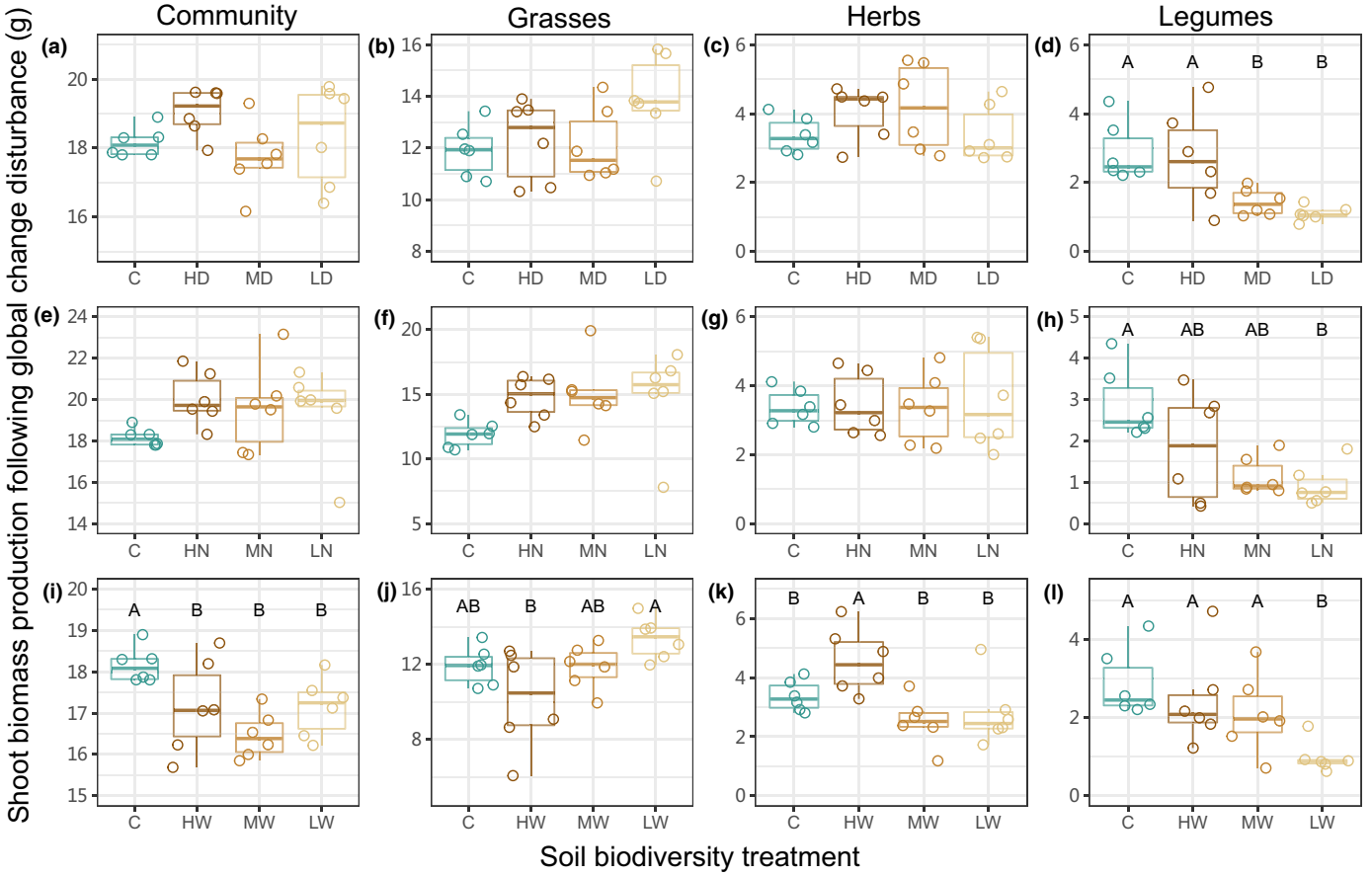

Fig. 3 The effects of soil biodiversity loss and global change factors on the overall shoot biomass of the plant community (a, e, i), grasses (b, f, j), herbs (c, g, k) and legumes (d, h, l) following global change disturbances. H, high soil biodiversity; M, moderate soil biodiversity; L, low soil biodiversity; C, control (ambient condition); D, drought; N, nitrogen deposition; W, warming. Boxplots with different capital letters indicate significant differences ($P < 0.05$). The ends of the boxplot are the upper (75th percentile) and lower (25th percentile) quartiles; the vertical line inside the box is the median (middle quartile); the upper and lower whiskers represent responses outside the middle 50%.

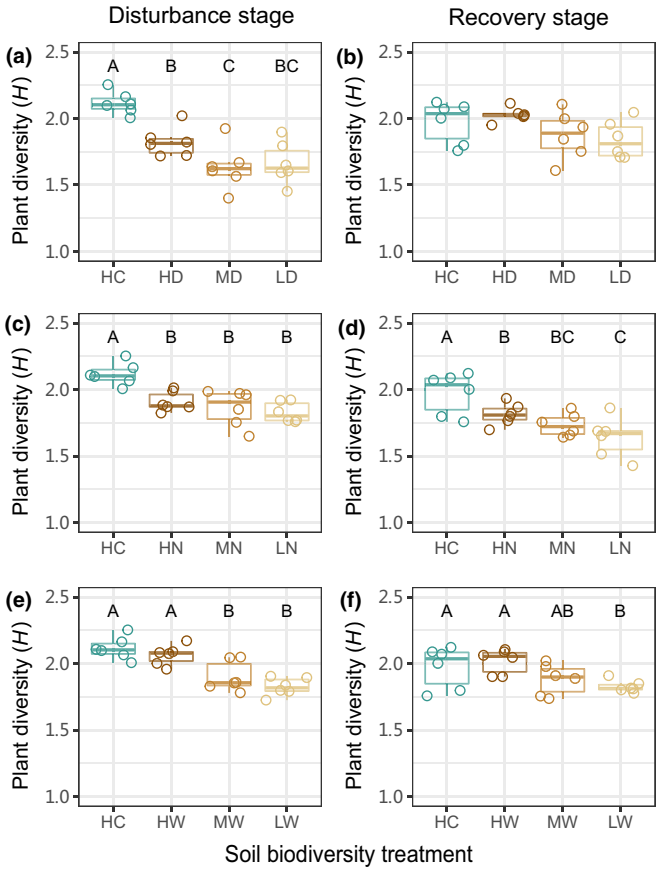

Fig. 4 The effects of soil biodiversity loss and global change factors on plant diversity (*H*) during (a, c, e) and following (b, d, f) global change disturbances. H, high soil biodiversity; M, moderate soil biodiversity; L, low soil biodiversity; C, control (ambient condition); D, drought; N, nitrogen deposition; W, warming. Boxplots with different capital letters indicate significant differences ($P < 0.05$). The ends of the boxplot are the upper (75th percentile) and lower (25th percentile) quartiles; the vertical line inside the box is the median (middle quartile); the upper and lower whiskers represent responses outside the middle 50%.

instance, drought decreased plant diversity by 14% at high soil biodiversity and by 21% at moderate soil biodiversity. On average, N deposition decreased plant diversity by 12% independent of soil biodiversity loss (Fig. 4c). Warming also led to a 12% of reduction in plant diversity with reduced soil biodiversity (Fig. 4e). Plant diversity fully recovered from drought independently of the soil biodiversity level, while soil biodiversity loss suppressed the recovery of plant diversity from N deposition and warming (Fig. 4b,d,f). Besides, soil biodiversity loss did not alter plant diversity before global change disturbance (Fig. S3).

Samples of N deposition in the moderate soil biodiversity treatment were lost during storage (Fig. 5e,f). Compared with the control, bacterial and fungal diversity did not change at high soil biodiversity during global change treatments with the exception of warming, which increased bacterial diversity (Fig. 5a,b,e,f,i,j). The 10^{-6} dilution still resulted in a significant decrease in bacterial and fungal diversity except for bacterial diversity during N deposition (Fig. 5a,b,e,f,i,j). Bacterial diversity fully recovered from global change disturbances (Fig. 5c,g,k), while N deposition and warming had a legacy effect on fungal diversity (Fig. 5h,i). Regarding the changes in the composition of soil microbial communities, fungi in the phylum *Glomeromycota* were eliminated or reduced at moderate and low soil biodiversity during and following global change disturbances, in contrast to high soil biodiversity. However, there is no evidence that soil dilution led to the loss of rhizobia (Fig. S4).

Soil dilution decreased Shannon diversity of soil bacterial communities under drought and following drought (Fig. S5a,b). Shannon diversity of soil bacterial communities was not altered during N deposition, but was decreased by soil dilution following N deposition (Fig. S5c,d). Warming reduced Shannon diversity of soil bacterial communities, but this value fully recovered from warming. Shannon diversity of soil fungal communities was not sensitive to treatments during disturbance and recovery stages, with the exception of warming, which decreased in the 10^{-6} dilution treatment during the disturbance stage (Fig. S6). Soil dilution increased Pielou index of evenness of soil bacterial communities during drought and warming (Fig. S7a,c). The Pielou index for the soil bacterial communities was not affected during the recovery stage (Fig. S7a,c). There was an increase in the Pielou index for soil fungal communities during the disturbance stage (Fig. S8a,c,e). The Pielou index for soil fungal communities was not altered following drought and N deposition, and lower evenness was observed at moderate soil biodiversity following warming.

Discussion

Our results suggest that soil biodiversity is of great importance for the persistence of legumes when faced with global change disturbances. We find that global change disturbances decreased the performance of legumes, particularly under reduced soil biodiversity and the loss of soil biodiversity suppressed the recovery of legumes following disturbances. Legumes, associating with rhizobia to fix atmospheric N_2, have a profound effect on multiple ecosystem functions (Spehn *et al.*, 2002; Hector *et al.*, 2007; Zhao *et al.*, 2014; van der Heijden *et al.*, 2016; Xu *et al.*, 2019). The reduction of legumes can decrease N input, which could potentially alter multiple ecosystem functions. Moreover, given that most native grasslands in the world are dominated by grasses or grass-like plants, our study emphasizes the importance of soil biodiversity for the maintenance of plant diversity.

Previous studies show that N deposition reduces the abundance of legumes (Suding *et al.*, 2005; van der Heijden *et al.*, 2008b; Yang *et al.*, 2011), while their abundance was either reduced or promoted by drought (Tilman, 1996; Grant *et al.*, 2014; Ploughe *et al.*, 2019) and could be promoted by warming (Whittington *et al.*, 2013; Cowles *et al.*, 2016). In the present study, drought, N deposition and warming decreased the growth of legumes in experimental grassland. Global environmental change can pose major threats to both plant and soil biodiversity (Stevens *et al.*, 2004; Clark & Tilman, 2008; Stevens *et al.*, 2018; Geisen *et al.*, 2019; Zhou *et al.*, 2020). Here, we show that the loss of soil biodiversity can further decrease the growth of legumes during global change disturbances, and can suppress the

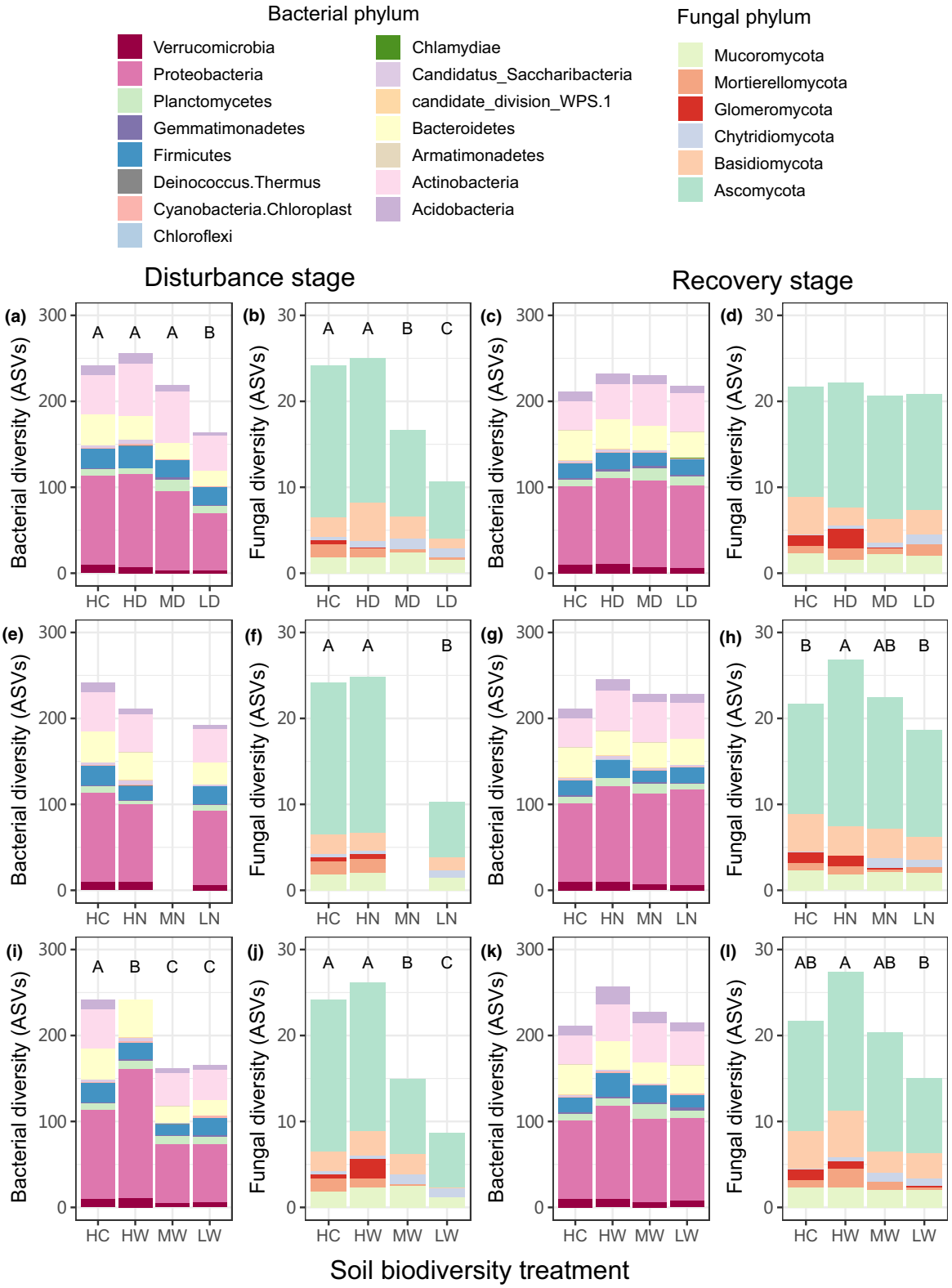

Fig. 5 The effects of the dilution-to-extinction approach and global change factors on soil bacterial (a, e, i, c, g, k) and fungal (b, f, j, d, h, l) diversity during and following global change disturbances. H, high soil biodiversity; M, moderate soil biodiversity; L, low soil biodiversity; C, control (ambient condition); D, drought; N, nitrogen deposition; W, warming. Boxplots with different capital letters indicate significant differences ($P < 0.05$).

recovery following disturbances. Consequently, reduction in legumes can exert a detrimental effect on plant diversity. In addition, because soil biodiversity loss did not affect legumes before global change disturbance, changes in legumes mainly came from soil biodiversity loss with the implementation or termination of global change disturbance. These results indicate the significance

of soil biodiversity in maintaining the performance of legumes during and following global change disturbances.

The decrease in legume persistence by soil biodiversity loss could be partly attributed to the absence of soil mutualists, for instance, AM fungi. The present study shows that the dilution-to-extinction approach has eliminated or reduced the diversity of *Glomeromycota*, the phylum containing AM fungal species. For legumes, AM fungi play an important role in the uptake of nutrients (e.g. phosphorus) and water, and therefore, the performance of legumes is often dependent on the presence of AM fungi (van der Heijden *et al.*, 2008b; van der Heijden *et al.*, 2016; Bonfante, 2018; Püschel *et al.*, 2020). Especially, AM fungi can improve plant resistance to stressful disturbances (Delavaux *et al.*, 2017; Wu, 2017; Xie *et al.*, 2018). Besides, soil biodiversity could improve legume performance during and following global environmental change through complex feedbacks between plants and microbes, as described by de Vries *et al.* (2020). For instance, drought resistance could be improved by the interaction between plants and plant growth-promoting rhizobacteria, and by a reduction in heterotrophic microbial activity (de Vries *et al.*, 2020).

Overall soil biodiversity loss could also contribute to a decrease in legume performance. An abrupt decline in the shoot biomass of legumes was observed when soil biodiversity was experimentally reduced (Wagg *et al.*, 2014). A recent study investigated the effects of soil biodiversity loss and drought on the yield production of two pea genotype: the wild type and the nodulation- and mycorrhization-defective mutant (Prudent *et al.*, 2020). It was found that soil biodiversity loss decreased the yield under drought-stressed conditions, independently of pea genotypes, indicating that the detrimental effect of soil biodiversity loss could come from soil microbes in general (Prudent *et al.*, 2020). Furthermore, soil biodiversity loss has been shown to reduce multiple soil functions, such as nutrient provision, decomposition and soil respiration (Philippot *et al.*, 2013; Wagg *et al.*, 2014; Delgado-Baquerizo *et al.*, 2020; Guerra *et al.*, 2020; Thakur *et al.*, 2020). Soil biodiversity loss could affect the performance of legumes, as well as other responses, through mediating soil functions.

In the present study, drought and N deposition decreased plant diversity, consistent with previous studies (Stevens *et al.*, 2004; Clark & Tilman, 2008; Yang *et al.*, 2011; Hautier *et al.*, 2015; Stevens *et al.*, 2018; Y. Zhang *et al.*, 2019). Past studies suggest that warming did not alter plant diversity in grassland ecosystems (Whittington *et al.*, 2013). Our study shows warming decreased plant diversity under reduced soil biodiversity. Moreover, changes in plant diversity mainly came from soil biodiversity loss with the implementation or termination of global change disturbance, because soil biodiversity loss did not affect plant diversity before global change disturbance. These results indicate that high soil biodiversity may help maintain plant diversity during warming. Nitrogen deposition still had a negative effect on plant diversity, and a more detrimental effect was observed at low soil biodiversity. The effects of N deposition could be partly due to the fact that a cessation of N deposition did not entail removing residual N, which could still continue to affect the system. Given that a decrease in N deposition can lead to recovering plant diversity in native grassland (Storkey *et al.*, 2015), our study suggests that the loss of soil biodiversity may suppress the recovery of plant diversity in the short term following N deposition.

In the present study, although warming increased bacterial diversity, N deposition and drought did not affect bacterial and fungal diversity. The duration of global change factor application can determine their effect on soil biodiversity (Yang *et al.*, 2020). As a result, soil bacterial and fungal diversity might be robust to some short-term global change disturbances. Besides, these global change factors did not always induce a loss of soil biodiversity (Zhou *et al.*, 2020). Following global change disturbances, a full recovery of soil bacterial diversity has been observed in the present study, indicating that soil bacteria are highly resilient to global change disturbances, supporting previous work (Bardgett & Caruso, 2020).

In summary, our results underpin the significance of soil biodiversity for maintaining legumes in plant communities experiencing global environmental change. Many studies have shown that global change factors have a strong influence on aboveground and belowground biodiversity (Stevens *et al.*, 2004; Clark & Tilman, 2008; Yang *et al.*, 2011; Hautier *et al.*, 2015; Stevens *et al.*, 2018; Y. Zhang *et al.*, 2019). Our study shows that the loss of biodiversity could result in a negative feedback, which can further decrease plant diversity by decreasing legumes. Numerous studies demonstrate that the maintenance of plant diversity is crucial for ecosystem functioning (Maestre *et al.*, 2012; Hautier *et al.*, 2014; Tilman *et al.*, 2014; Isbell *et al.*, 2015; Weisser *et al.*, 2017; Jochum *et al.*, 2020). Our results contribute to a deeper understanding of the mechanisms that underpin the effects of soil biodiversity under global change, highlighting the key role of soil biodiversity in maintaining plant diversity by promoting the persistence of legumes.

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Author contributions

GY and MCR conceived the ideas and designed the study. GY set up the experiment and collected the data. GY, JR and SDV analysed the data. GY wrote the first draft, and all authors commented on the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 All data supporting the findings of this study.

Fig. S1 Photograph of microcosm warming system.

Fig. S2 The effects of soil biodiversity loss on the overall shoot biomass of plant community, grasses, herbs and legumes before global change disturbances.

Fig. S3 The effects of soil biodiversity loss on plant diversity (*H*) before global change disturbances.

Fig. S4 The effects of soil biodiversity loss and global change factors on rhizobial diversity (ASVs) during and following global change disturbances.

Fig. S5 The effects of soil biodiversity loss and global change factors on Shannon diversity of soil bacterial communities during and following global change disturbances.

Fig. S6 The effects of soil biodiversity loss and global change factors on Shannon diversity of soil fungal communities during and following global change disturbances.

Fig. S7 The effects of soil biodiversity loss and global change factors on Pielou index evenness of soil bacterial communities during and following global change disturbances.

Fig. S8 The effects of soil biodiversity loss and global change factors on Pielou index evenness of soil fungal communities during and following global change disturbances.

Note S1 R script used for data visualization and statistical analysis (access through RSTUDIO and Microsoft NOTEPAD).

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