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RESEARCH ARTICLE

Small-sized soil organisms drive the legacy of soil addition in a degraded grassland

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Abstract

1. Plants can alter the abiotic and biotic components of the soil ecosystem, and this can result in soil legacies that facilitate or inhibit the growth of succeeding plants. To what extent soil legacy effects are due to soil biotic or abiotic characteristics is still poorly understood.
2. In a microcosm experiment, we grew one plant species (*Leymus Chinensis*) in soil containing legacy effects of a prior soil inoculation event where soil from donor sites was inoculated at a receiver site. We tested whether soil legacies originating from the field plots differentially shaped the assemblage of soil microbial communities and plant performance, and how these effects are changed by the removal of specific size groups of soil biota.
3. Our microcosm experiment showed that soil legacies from a one-time soil addition experiment in a degraded grassland can affect the soil microbial composition 4 years later. This legacy effect was positively influenced by the amount of soil originally added to the field plot. By testing four types of biotic fractions differing in size, we found that small-sized organisms (<20 μm), in particular fungi, were the most important for legacy formation and plant growth.
4. Our results highlight the importance of inoculum density and smaller-sized microbes in the formation of soil legacies. We conclude that the interaction between plant and soil microbes is a driver in the restoration of degraded grassland after soil addition. Disentangling the contributions of different biotic groups in the soil to plant growth can provide a theoretical basis for the preparation of bio-inoculants that can be used for the sustainable restoration of degraded ecosystems.

KEYWORDS
biotic fractions, plant–soil interactions, soil inoculation, soil legacy effect, soil microbiome

Yuhui Li and Yixin Sun contributed equally to this work.

1 | INTRODUCTION

Abiotic and biotic characteristics of the soil can be shaped by the plants growing in the soil (Bardgett & van der Putten, 2014). This can result in soil legacies that can affect the performance of other plants growing in the soil later (Teste et al., 2017; van der Putten et al., 2013). Recent work has shown that these plant–soil legacies can persist in the soil for several years or longer, and therefore they can have long-term consequences for plant community assembly and productivity (Heinen et al., 2020). By adding soil, for example via soil inoculation, the legacy of the soil can be transferred to another location and this can change the development of the plant and soil biome, and even influence ecosystem functions at the recipient site (Wubs et al., 2016). It was also found that the effects of these legacies on plant growth and soil characteristics at the recipient site are influenced by the amount of soil that is inoculated (Han et al., 2022). However, the amount of soil that is inoculated also changes the availability of resources such as soil-available nitrogen, either directly or indirectly via changes in soil organic matter quality or quantity (Cong et al., 2014; Jing et al., 2022). To what extent soil legacy effects are due to biotic or abiotic characteristics of the inoculated soil, and whether single introductions of soil from different ecosystems can generate distinct soil legacies is still poorly understood.

The soil biome is a critical driver of soil processes such as nutrient cycling, thereby supporting primary productivity and plant diversity (Wagg et al., 2019). Most studies focus on the net effects of the whole soil biome on plant performance. However, plant growth can be differently affected by soil organisms of varying body sizes and functions, and soil biotic groups of different sizes can be complementary in promoting plant growth (Wang et al., 2019). These different size groups of organisms can affect other groups in the soil food web. For example, macrofauna and mesofauna can alter microbial and nematode communities via direct trophic interactions, but also via their impact on the availability of resources (David, 2014). One way to study the impact of different size fractions of soil fauna on the performance of plants is to re-inoculate separately sieved fractions to sterilized soil (Wagg et al., 2014; Wang et al., 2019). Although the biotic component of soil legacies was found to have long-term effects on community assembly (Wubs et al., 2019), the quantitative contributions of differently sized organisms on the biotic legacies are largely unknown as many field studies were just based on sequencing, especially in long-term legacies formation in degraded ecosystems.

Soil legacy effects change over time. This can happen due to feedbacks between plants and soil communities and can lead to stronger effects over time, where initial differences in the soil cause changes in plants that subsequently lead to larger differences in the soil (Wubs et al., 2016). However, soil legacy effects that are initially present may also disappear, resulting in convergence in soil communities returning to their original state within a short period of time, for example, due to overriding effects of the environment (Kardol et al., 2009). Understanding the relationship between the persistence of soil legacy effects and plant growth is critical for a more in-depth understanding of plant–soil feedbacks and interactions.

In this study, we examined how long-term soil legacies generated in a field experiment by a single soil addition event affect a newly formed soil biome and plant performance in a microcosm experiment. We also tested whether these legacies are affected by how much soil was inoculated originally in the field, and what the relative contribution is of different biotic groups to the legacy effect. We tested the following hypotheses: (1) After 3 months of growth by a common plant species, soil communities in microcosms inoculated with soil organism groups from donor sites and from experimental plots will differ and (2) communities arising from inocula made from soil collected from experimental field plots that originally received the higher amount of soil inoculum will resemble the donor community more closely than those that received the lower amount of soil. (3) Soil communities will be more dissimilar to the donor community when they are inoculated with both larger and smaller-sized soil fauna from field plots than when inoculated with small-sized biota only, because original differences in larger soil organisms in the inoculum will influence micro- and meso-community composition due to food-web effects.

2 | MATERIALS AND METHODS

2.1 | Soil-addition field experiment

The study site is located in a degraded grassland near the Erguna Forest-Steppe Ecotone Research Station of the Chinese Academy of Sciences (50°10′46″ N, 119°22′56″ E). We have appropriate permits granted by the Erguna Forest-Steppe Ecotone Research Station from the Institute of Applied Ecology, Chinese Academy of Sciences to carry out our field study, and no licence was required. A soil-addition field experiment with three replicated blocks was initiated in 2018 (Figure 1a). Added soil originated from two ecosystem types (meadow steppe (S; hereafter referred to as 'steppe') and upland meadow (M; hereafter referred to as 'meadow')) that were located 20 km apart. For both ecosystems, soil was collected from three different locations 1 km apart as three replicates of donor soil, and the soil was added in two amounts (a layer of 1 cm and a layer of 3 cm of added soil, hereafter called 'low' and 'high', respectively). Therefore, 12 plots with donor soil addition were established in each block; three replicates for four groups (S-low, S-high, M-low and M-high). Each plot (2 × 2 m) was separated by 2 m wide paths. In 2021, we randomly selected 12 soil addition plots from one same block to be used for the microcosm experiment (Figure 1b). We also collected the soil from the recipient site but outside the plots, from an area without soil inoculation, then sterilised the soil and used it as bulk soil for the microcosms experiment.

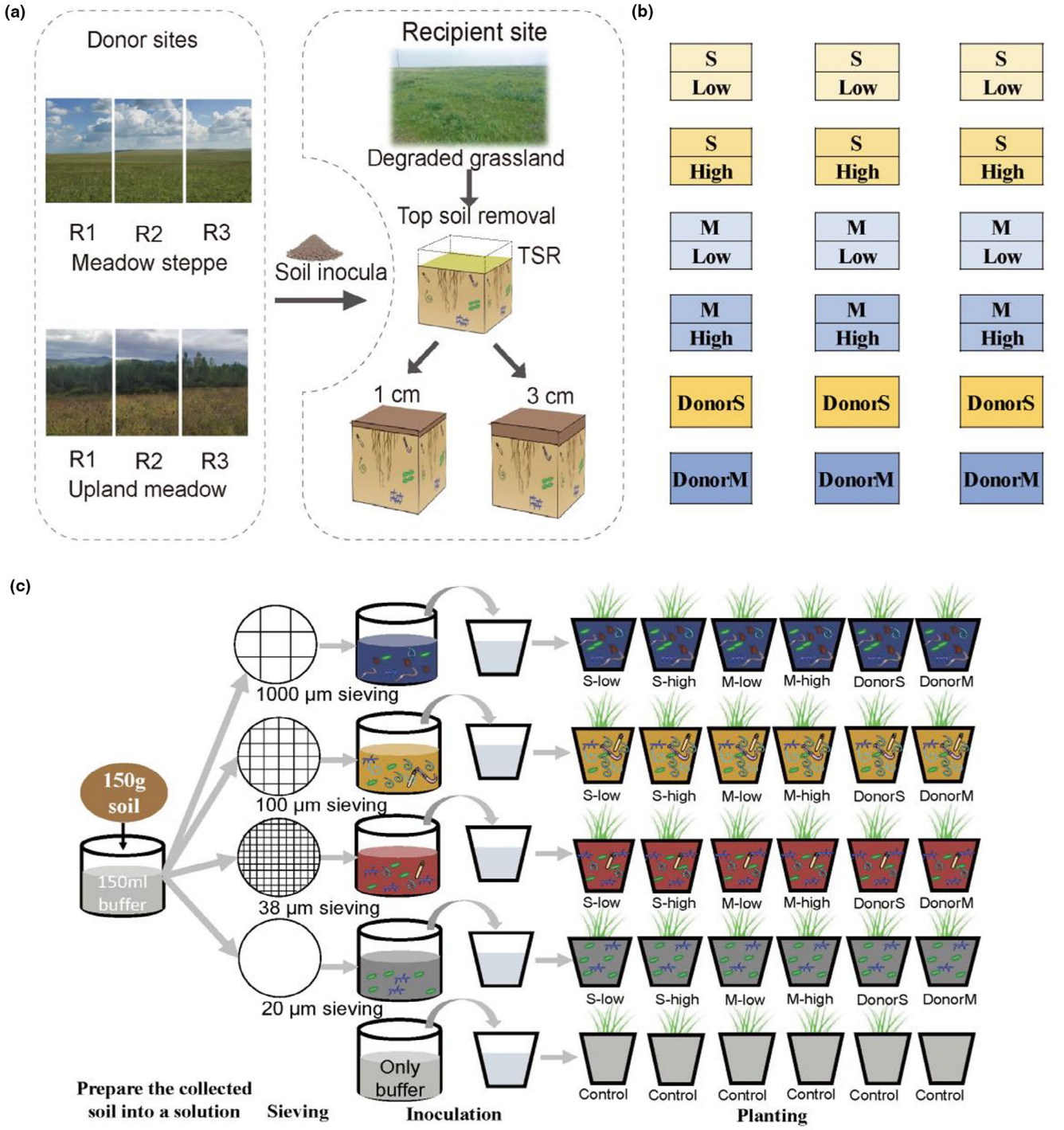

FIGURE 1 Scheme of the experimental design. (a) The experimental design of the soil inoculation field experiment: Two amounts of meadow (M) and steppe (S) soils were inoculated in a degraded grassland. (b) The 18 soils that were selected for the microcosm experiment from the soil inoculation field experiment, containing three replicates of four different soil inoculations and two donor soils. (c) Experimental design of the microcosm experiment: Soils collected from the field plots from the soil addition experiment (S-low, S-high, M-low and M-high) and the donor sites (DonorS and DonorM) were sieved to create soil solutions containing four different sized fractions of the soil community. The soil solutions were added to microcosms filled with sterilised degraded grassland soil (from recipient site without soil inoculation). S-low: Soil legacy collected from field plots that received 1 cm of steppe soil, S-high: Soil legacy collected from field plots that received 3 cm of steppe soil, M-low: Soil legacy collected from field plots that received 1 cm of meadow soil, M-high: Soil legacy collected from field plots that received 3 cm of meadow soil, DonorM: Soil legacy collected from meadow donor soil, DonorS: Soil legacy collected from steppe donor soil, Control: Only sterilised degraded grassland soil without soil inoculation.

2.2 | Soil collection

In 2021, we collected soils (0–10 cm) from the previously mentioned 12 field plots and from the same three locations at both donor sites where the soil originally was collected. Hence, six soils, each with three replicate plots, were sampled to prepare the soil solutions (Figure 1b), that is, M-low and M-high (soil collected from experimental plots where 1 cm (low) and 3 cm (high) meadow soil was added), S-low and S-high (soil collected from experimental plots where 1 cm (low) and 3 cm (high) steppe soil was added), DonorM and DonorS (soil collected from the donor meadow and steppe). In each experimental plot or donor location, 25 soil cores (10 cm deep, 5 cm diameter) were collected in a 25 × 50 cm regular rectangular grid. The soil from the donor sites was collected adjacent to the area where the soil was collected for the soil addition field experiment 4 years earlier.

In the laboratory, the soil samples were pooled per plot and sieved through a 1 cm mesh to remove large stones and roots, thus obtaining a well-mixed composite soil sample for each plot, for a total of 18 composite soil samples. Then the soil samples were kept at 4°C for 7 days before preparing soil solutions for the microcosm experiment. We also collected soil from the recipient site from an area without soil inoculation to be used as bulk soil in the microcosms. After soil collection, this bulk soil was prepared similarly to the other samples and then sterilised by gamma irradiation (>35 kGy). In total, there were 18 soil samples (6 soils (S-low, S-high, M-low, M-high, DonorS and DonorM) × 3 replicates (three different soil samples)).

2.3 | The design of the microcosm experiment

To evaluate how different size fractions of the soil biome affect soil legacy effects in the microcosm experiment, four types (<1000, <100, <38 and <20 μm soil inocula) of watery soil inocula were prepared from each soil sample (Figure 1c). We expected that 1000 μm sieving would remove only larger soil macrofauna, 100 μm sieving would remove soil macrofauna and mesofauna, 38 μm sieving would remove soil macrofauna, mesofauna and microfauna, and that 20 μm sieving would remove part of fungi, resulting in lower richness of fungi (Wagg et al., 2014, 2019). However, our results showed that sieving through 1000, 100 and 38 μm had little effect on nematode, protist, fungal and bacterial DNA, while 20 μm sieving basically removed all nematodes, most fungi and a few bacteria. This is different from other studies (Wagg et al., 2014, 2019), probably because the soil samples used to prepare the watery inocula contained more larvae or eggs of small sizes. The soil suspensions were prepared by adding 150 mL phosphate buffer (pH 6.51, 1 g/L KH_2PO_4) to 150 g soil (water:soil = 1:1, w/w) in a 1-L beaker (Wang et al., 2019). This mixture of soil and water is sufficient to obtain 100 mL of solution for each size fraction after sieving and filtering. After stirring and resting for 30 seconds, soil suspensions were filtered through 1000 μm, 1000 μm followed by 100 μm and 1000 μm followed by 100 μm and then 38 μm mesh sieves into beakers to obtain soil inocula containing different size biotic fractions. For the 20 μm soil inoculum, soil solution that had been passed through a 38 μm mesh sieve was then filtered over a filter paper of 20 μm.

After the preparation of soil solutions, 100 mL soil inoculum was inoculated into each microcosm (d = 13.5 cm, h = 9.5 cm) containing 450 g homogenised and sterilised degraded grassland soil. For each soil type, this was repeated three times with different soil samples. In total, we obtained 72 microcosms with water inocula. Therefore, the experiment contained 24 treatments consisting of watery inocula, with four size fractions, inocula from field plots inoculated 4 years earlier with two amounts of soil from two ecosystems, and inocula from two donor ecosystems. We also directly inoculated 100 mL phosphate buffer to six pots with 450 g sterilised degraded grassland soil as a control. In total, there were 78 pots [6 soils (S-low, S-high, M-low, M-high, DonorS and DonorM) × 3 replicates × 4 biotic fractions (1000, 100, 38 and 20 μm) + 6 buffer only control pots] (Table 1 for replication statement). The experiment lasted 3 months, and more details are provided in Appendices S1 and S2 in the Supporting Information. Soil total carbon, total nitrogen, total phosphorus, dissolved organic carbon, nitrogen mineralisation, and available phosphorus were measured after plant harvest. The detailed measurements of soil physicochemical properties are provided in Appendix S3.

2.4 | Soil DNA sequencing and bioinformatic analysis

The community compositions of soil bacteria, fungi, protists and invertebrates were determined by amplicon sequencing using the Illumina MiSeq platform. Soil DNA was extracted using the ALFA-SEQ Advanced Soil Kit (mCHIP, China) based on standard

TABLE 1 Replication Statement.

Scale of inference	Scale at which factor of interest is applied	Number of replicates at the appropriate scale
Site type (steppe and meadow)	Site	Three replicates for each site type
Legacy type (high, low and donor)	Plots	Three replicates for each legacy type
Biotic fraction type (<1000, <100, <38 and <20 μm)	Pots	Three replicates for each fraction type
Control (without biotic inoculation)	Pots	Six replicates

protocols. The bacterial 16S, the fungal ITS and the eukaryotic 18S rRNA were amplified with primer sets 515F/907R (Biddle et al., 2008), ITS86F/ITS4R (De Beeck et al., 2014) and Euk1391f/EukBr (Delgado-Baquerizo et al., 2020), respectively. We analysed the MiSeq sequences with an amplicon sequence variant analysis pipeline known as EasyMicrobiome v1.14 (Liu et al., 2021). Bioinformatic processing of raw sequencing data, clustering and taxonomic assignment were performed using VSEARCH (2.15.0) (Rognes et al., 2016), USEARCH (10.0.240) (Edgar, 2010) and UNOISE (7.0.1090) (Edgar & Flyvbjerg, 2015). We assigned bacterial, fungal and eukaryotic sequences to taxonomic groups using the RDP 18 (Cole et al., 2014), UNITE 8.2 (Nilsson et al., 2019) and

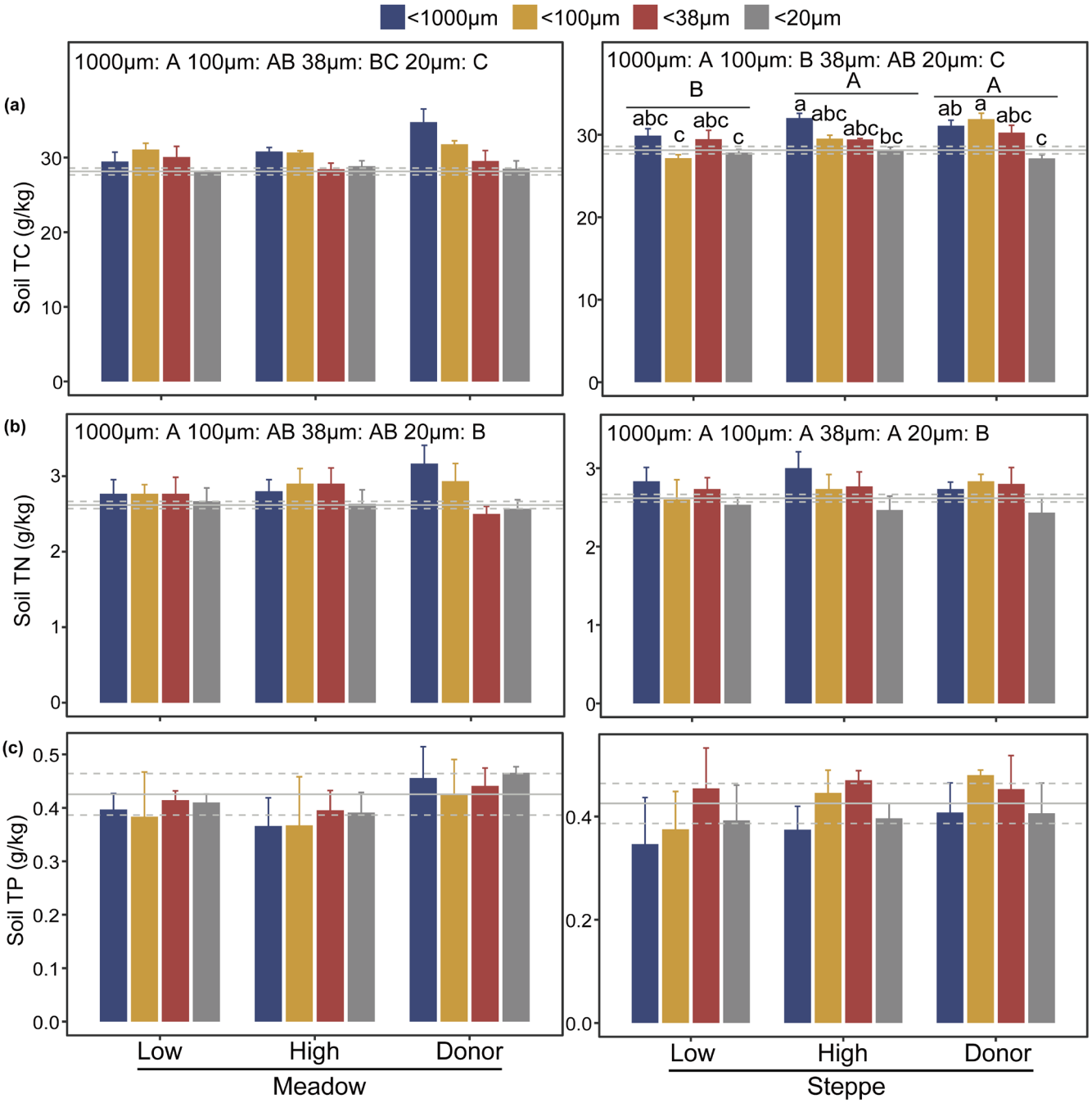

FIGURE 2 Mean (±S.E.) (a) Soil total carbon, (b) total nitrogen and (c) total phosphorus in microcosms inoculated with watery extracts from soil collected from the soil inoculation plots that received low or high amounts of meadow soil and steppe soil and from donor sites. The soil solutions were prepared by sieving through different mesh sieves. The grey line represents the mean of the control that contained only sterilized degraded grassland soil without solutions inoculation, and the two dashed lines represent the mean ± standard error, respectively. Bars without letters or with identical letters are not significantly different based on a *Tukey* post hoc *test* ($p < 0.05$). Capital letters represent differences among main effects, such as different biotic fractions or legacy types ($p < 0.05$).

PR2 (Guillou et al., 2013) databases. As only eight ASVs of mesofauna were identified from the 18S sequencing dataset and only in nine samples (Table S1), the final 18S data were analysed only for protists and nematodes. To account for differences in sequencing depth, ASV abundance tables were rarefied to 7572 (bacteria), 554 (fungi), 887 (protists) and 165 (nematodes) ASVs using the iterative rarefication method based on 10,000 iterations (Beckers et al., 2017) to ensure an even sampling depth among samples within each group of organisms.

2.5 | Statistical analyses

All statistical analyses were performed using R v.4.0.4 (R Core Team, 2021). The microcosms inoculated with biotic fractions from donor soils were used to compare whether the legacy effects of the one-time soil addition persisted 4 years after the event. Next to this, the control containing only sterilised degraded grassland soil without inoculated biotic fractions is provided only as a background value and was not included in the statistical analyses. Data normality and homoscedasticity were assessed before statistical analyses. Logarithmic (i.e. root/shoot ratio and soil TP) or square-root (i.e. soil TC and TN) transformation was performed to meet the assumption of normality for each variable. All plant and soil data (i.e. plant root biomass, shoot biomass, root/shoot ratio, soil TC, TN, TP, the richness of soil bacteria, fungi, protists and nematodes) from the microcosm experiment were analysed using linear mixed-effect models with the *lmer* function from the *lme4* package (Bates et al., 2015). Soil legacy treatment (low or high of added soil in the field experiment and donor soil), size fractions of the soil biome and their interaction were included as fixed factors. To identify the true replication level, donor site replication was included as a random factor. Tukey's post hoc tests were used for multiple comparisons, with the 'Bonferroni' correction method (FWER = 0.05). Soil legacy effects from the two different donor ecosystems (steppe and meadow) were analysed separately.

Unconstrained principle coordinates analysis (PCoA) and permutational analysis of variance (PERMANOVA) were performed based on ordination of Bray-Curtis+ distances, to assess the effects of soil legacy, size fractions of the soil biome and their interaction on the composition of the soil communities (i.e. soil bacteria, fungi, protists and nematodes). In order to compare the effects of soil legacies on soil communities, we also calculated the similarity of soil communities between soil legacies collected from the inoculated field plots and the respective donor sites based on Bray-Crutis similarity, for example, S-high and donorS. We subsequently assessed the effect of soil legacies with previously described linear mixed-effect models. The richness of each soil biome (soil bacteria, fungi, protists and nematodes) was also calculated to assess the effectiveness of the sieving treatments similar to earlier studies (Wagg et al., 2014, 2019). Redundancy analyses (RDA) was performed to assess how changes in the microbial community were correlated to soil abiotic variables (i.e. soil TC, TN, TP, DOC and AP) and plant growth (i.e. root and shoot biomass). To assess whether the effect of plant biomass on the composition of soil communities is dependent on soil legacy effects, we used the ANOVA model with the interaction between plant biomass and soil legacy effects as explanatory variable, while plant biomass was also a covariate (Petr & Jan, 2014). Then we chose any two levels of data from the soil legacy effects, including 'S-low, S-high and DonorS' or 'M-low, M-high and DonorM' were paired, to use the above model, to compare whether the effect of plant biomass on community composition differed significantly between the two levels. For example, for the combination 'S-low and S-high', we used the model to explore whether there was a significant difference in how well plant biomass explained community composition for S-low and S-high treatments. In addition, whether the effect of plant biomass on soil communities depended on the sieving treatments and whether the effect of soil abiotic variables on soil communities depended on soil legacy effects or sieving treatments were also assessed by the above analysis. Richness, PCoA, RDA and PERMANOVA were performed using the *vegan* package (Dixon, 2003).

TABLE 2 Effects of mixed-effects models testing the effects of different soil legacies, biotic fractions and their interactions on soil nutrients.

	Legacy type (L)			Biotic fraction type (F)			L: F		
	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Meadow									
TC	2, 22	2.74	0.087	3, 22	6.59	**0.002**	6, 22	1.90	0.127
TN	2, 22	0.35	0.712	3, 22	3.80	**0.025**	6, 22	2.30	0.071
TP	2, 22	2.76	0.085	3, 22	0.30	0.823	6, 22	0.06	0.999
Steppe									
TC	2, 22	6.34	**0.007**	3, 22	14.28	**<0.01**	6, 22	3.95	**0.008**
TN	2, 22	0.55	0.587	3, 22	9.42	**<0.01**	6, 22	1.24	0.324
TP	2, 22	0.95	0.403	3, 22	1.87	0.165	6, 22	0.29	0.936

Note: Values in bold are significant at $p < 0.05$.

Abbreviations: df, degrees of freedom; *F*, *F*-value; *p*: *p*-value; TC, soil total carbon; TN, soil total nitrogen; TP, soil total phosphorus.

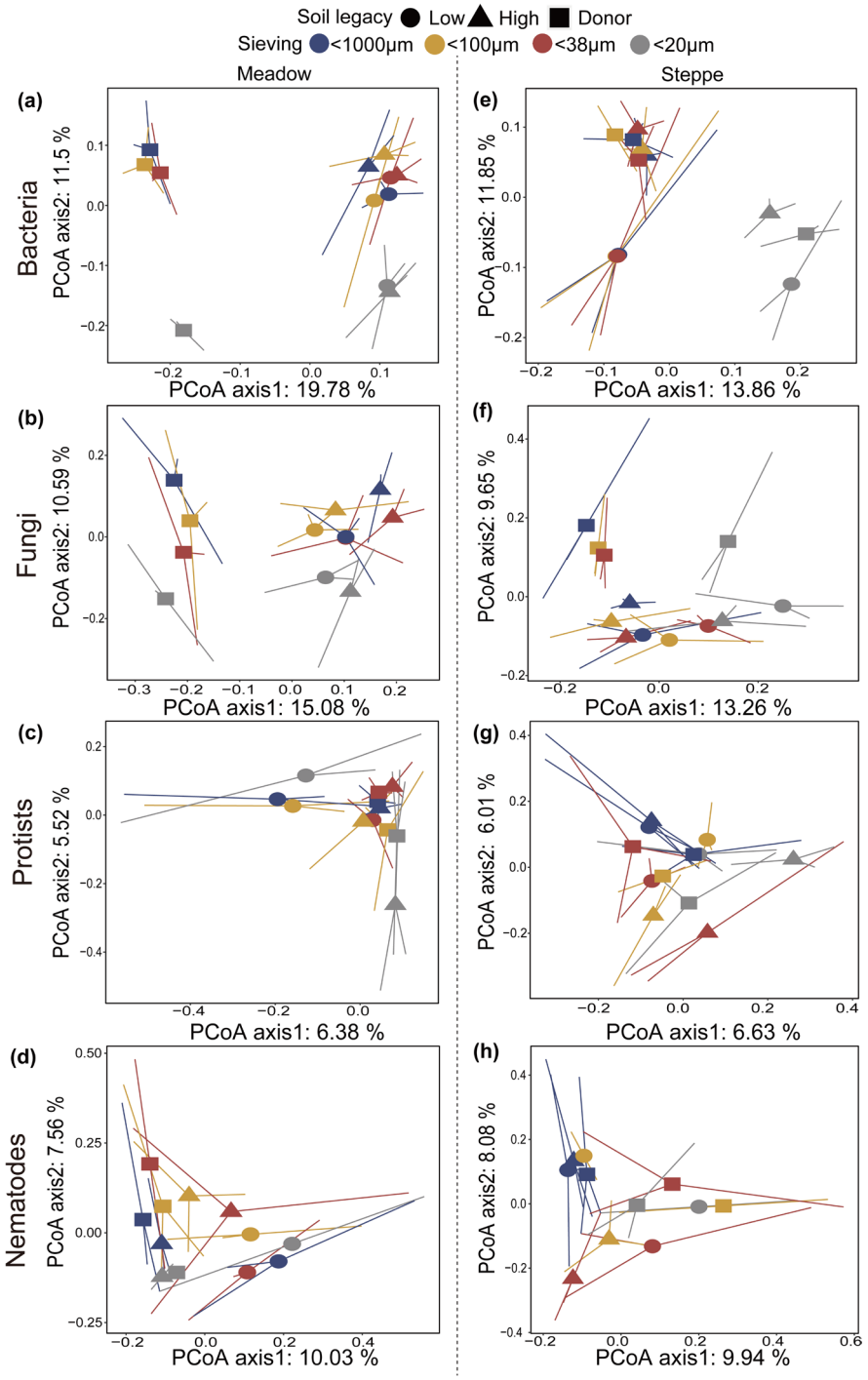

FIGURE 3 Biplots of a PCoA analysis of the community composition of soil (a, e) bacteria, (b, f) fungi, (c, g) protists and (d, h) nematodes in microcosms inoculated with different size fractions of the soil community collected from field plots that received different amounts of meadow or steppe soil. PCoA with the control treatment that contained only sterilised degraded grassland soil without solutions inoculation; see Figure S3.

3 | RESULTS

3.1 | Effects of soil legacies and addition of biotic fractions on soil nutrients

For both site types, there were small but significant effects of biotic fractions on soil total carbon and nitrogen (Figure 2a,b; Table 2). In general, soil total carbon and total nitrogen in the 20 μm sieving treatment were lower than those in other biotic fractions inoculation treatments ($p < 0.05$; Figure 2a,b). For steppe soil, the pattern was more evident in the microcosms with inocula from donor steppe and from field plots inoculated with the high amount of steppe soil. In steppe soil, the effect of biotic fraction type on soil total carbon was also influenced by site type ($p < 0.05$; Table 2). Specifically, compared to the microcosms with inocula from field plots that received the low amount of soil, soil total carbon in microcosms with inocula from plots that received the higher amount of soil was more similar to that in microcosms that received inocula from the donor sites, especially in the treatments inoculated with larger sized soil organisms (i.e. 1000 and

TABLE 3 Effects of different soil legacies, biotic fractions and their interactions on the composition of soil communities tested using PERMANOVA.

	Legacy type (L)				Biotic fraction type (F)				L: F			
	df	*F*	*p*	%	df	*F*	*p*	%	df	*F*	*p*	%
Meadow												
Bacterial community	2, 35	5.04	**0.001**	22.7	3, 35	0.95	**0.001**	13.2	6, 35	0.73	0.995	9.8
Fungal community	2, 35	3.31	**0.001**	17.2	3, 35	1.23	0.095	9.6	6, 35	0.7	0.999	10.9
Protists community	2, 35	1.14	**0.048**	6.5	3, 35	1.04	0.317	8.8	6, 35	0.97	0.696	16.6
Nematodes community	2, 35	1.26	**0.050**	8.2	3, 35	0.97	0.564	9.5	6, 35	0.90	0.895	17.5
Steppe												
Bacterial community	2, 35	2.73	**0.001**	13.4	3, 35	2.25	**0.001**	16.5	6, 35	0.76	0.999	11.2
Fungal community	2, 35	2.31	**0.001**	12.2	3, 35	1.59	**0.001**	12.6	6, 35	0.74	0.997	11.8
Protists community	2, 35	1.03	0.403	6.0	3, 35	1.02	0.397	8.9	6, 35	1.01	0.424	17.8
Nematodes community	2, 35	0.91	0.733	6.1	3, 35	1.09	0.223	11.0	6, 35	1.14	0.084	19.2

Note: Values in bold are significant at $p < 0.05$.

Abbreviations: %, percentage explained variation; df, degrees of freedom; *F*, *F*-value; *p*, *p*-value.

100 μm; $p < 0.05$; Figure 2a,b; Table 2). Soil total phosphorus and soil available nutrients (i.e. soil DOC and AN) were not affected by both site type and biotic fractions ($p > 0.05$; Figure 2c; Figure S1; Table 2).

3.2 | Effects of soil legacies and biotic fractions on soil communities

After 3 months of plant growth in the microcosm experiment, the richness of soil bacteria, fungi and nematodes significantly decreased ($p < 0.05$) with decreasing mesh size used for inoculation (Figure S2; Table S2). The assembled bacterial communities in microcosms inoculated with biotic fractions from plots inoculated with meadow soil differed greatly between soils collected from the donor sites and from the experimental field plots (Figure 3a; Table 3). However, the assembled bacterial communities in microcosms inoculated with biotic fraction from plots inoculated with a high amount of steppe soil resembled greatly with the biotic fraction from the donor steppe (Figure 3e; Table 3).

A similar pattern was observed for fungal communities (Figure 3b,f). For both ecosystems, there were large differences between the two legacy treatments (soil from plots where donor soil was added and donor soil), and this was mainly due to the distinct effects of the donor soil legacies (Figure 3b,f; Table 3). However, fungal communities also differed depending on whether the field plots originally received a low or a high amount of soil. For the steppe system, fungal communities from plots that received the higher amount of soil resembled those of the donor more closely than fungal communities from plots that received the lower amount of soil (Figure 3f), while for the meadow system, the opposite effect was observed (Figure 3b; Table 3).

There were no significant differences among treatments in communities of protists and nematodes for either the meadow or steppe ecosystems ($p > 0.05$; Figure 3c,d,g,h; Table 3). However, for the meadow system, for protists there was a small significant effect of legacy type, mainly because inocula from plots that received the lower amount of soil differed from those of plots that received the higher amount of soil and from donor soil (Figure 3c; Table 3). Microbial communities originating from soil collected from experimental field plots that originally received a higher amount of soil were more similar to those from soil of the donor community than microbial communities from plots that received a lower amount of soil, especially in the steppe ecosystem ($p < 0.05$; Figure S4; Table S4).

3.3 | Effects of soil legacies and biotic fractions on *Leymus* biomass

Overall, plant shoot and root biomass, in microcosms inoculated with meadow soil inocula, were higher when inoculated with 20 μm size biotic fractions than when inoculated with other biotic fractions ($p < 0.05$; Figure 4a,b; Table 4). A similar trend was found for plant shoot biomass when inocula were from steppe soil (Figure 4a). For steppe soil, plant root biomass was higher in microcosms inoculated with 1000 μm biotic fractions than when inoculated with other biotic fractions ($p < 0.05$; Figure 4b; Table 4). For meadow soil, plant root biomass was higher in microcosms with inocula from the donor than from soil addition experimental plots, while for steppe soil root biomass was not different among different legacy types, but shoot biomass was highest with inocula from the donor ($p < 0.05$; Figure 4a,b; Table 4). The root/shoot ratio was highest in microcosms with inocula from donor soil for M soil, while it was lowest for S soil ($p < 0.05$; Figure 4c; Table 4).

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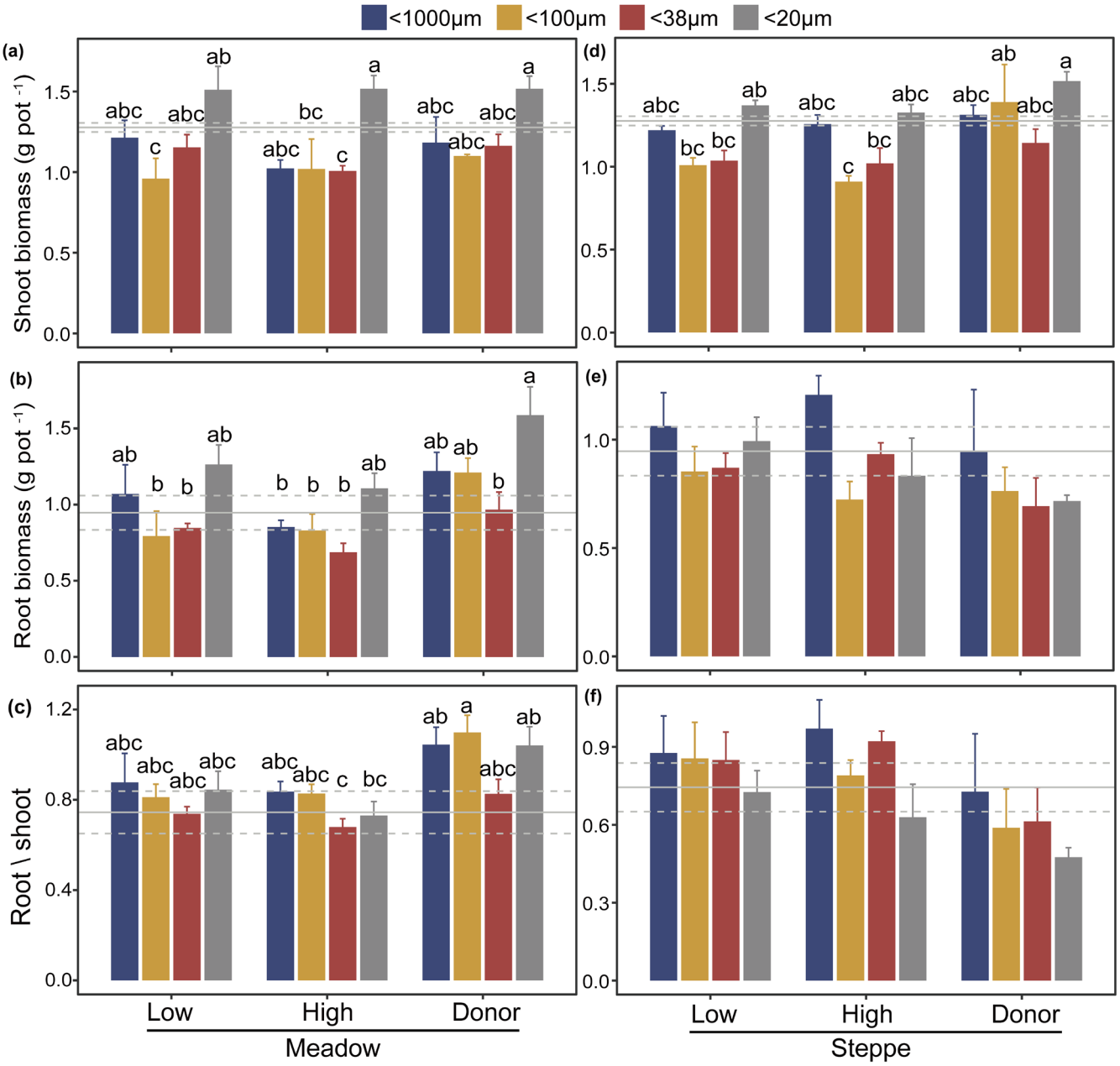

FIGURE 4 Mean (±S.E.) (a, d) Shoot biomass, (b, e) root biomass and (c, f) root/shoot ratio of *Leymus chinesis* in microcosms inoculated with watery extracts from soil collected from the soil inoculation plots that received low or high amounts of meadow soil and steppe soil and from donor sites. The soil solutions were prepared by sieving through different mesh sieves. The grey line represents the mean of the control that contained only sterilized degraded grassland soil without solutions inoculation and the two dashed lines represent the mean ± standard error, respectively. Bars without letters or with identical letters are not significantly different based on a *Tukey* post hoc *test* ($p < 0.05$).

3.4 | Relationships between soil microbiomes, plant characteristics and abiotic soil properties

For meadow soil, bacterial communities with legacies from experimental plots had a lower effect ($p < 0.001$) on plant growth than those from donor meadow soils (Figure 5a; Table 5). For steppe soil, bacterial communities from plots that received high amounts of steppe soil explained more variation ($p < 0.05$) in plant biomass than other legacy types of inoculation (Figure 5b; Table 5). In contrast, the fungal communities from plots that received low amounts of meadow soil explained more of the plant biomass ($p < 0.001$) than fungal communities from the other treatments (Figure 5e; Table 5). The assembled fungal communities in microcosms inoculated with the smallest fraction (20 μm) had a greater effect ($p < 0.001$) on plant biomass than those inoculated with the largest fraction (1000 μm) for meadow soil (Figure 5g; Table 5).

For steppe soil, the bacterial community in microcosms inoculated with donor soil and with soil from experimental plots that received the low amount of donor soil was more strongly affected ($p < 0.01$) by soil nutrients than those that received the high

TABLE 4 Effects of mixed-effects models testing the effects of soil legacies and biotic fractions on plant biomass.

	Legacy type (L)			Biotic fraction type (F)			L: F		
	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Meadow									
Shoot biomass	2, 22	1.10	0.350	3, 22	15.13	**<0.001**	6, 22	0.46	0.831
Root biomass	2, 22	11.51	**<0.001**	3, 22	10.12	**<0.001**	6, 22	0.51	0.796
Root/Shoot ratio	2, 22	12.35	**<0.001**	3, 22	3.85	**0.024**	6, 22	0.50	0.80
Steppe									
Shoot biomass	2, 22	7.24	**0.004**	3, 22	9.96	**<0.001**	6, 22	1.28	0.306
Root biomass	2, 22	2.08	0.149	3, 22	3.23	**0.042**	6, 22	0.51	0.793
Root/Shoot ratio	2, 22	4.5	**0.023**	3, 22	2.15	0.118	6, 22	0.18	0.98

Note: Values in bold are significant at $p < 0.05$.

Abbreviations: df, degrees of freedom; *F*, *F*-value; *p*, *p*-value.

amount of soil (Figure 6b; Table 6). A similar pattern was observed for fungal communities assembled from meadow water inocula ($p < 0.01$; Figure 6e; Table 6). The variance explained relationships between soil nutrients and plant biomass and the assembled protist and nematode communities showed no significant differences ($p > 0.05$) among legacy and biotic fractions (Figures 5i–p and 6i–p; Table 6).

4 | DISCUSSION

4.1 | Soil biotic effects as a driver of soil legacies

Soil legacies may persist in the soil for many years, but they may also disappear quickly (Hannula et al., 2021; Wubs et al., 2019). After 3 months of growth of a single plant species, the soil biome (i.e. bacteria, fungi, protists and nematodes) in microcosms inoculated with soil biotic fractions from donor sites and experimental plots clearly differed. This is interesting because, after 3 months of growth of a single plant species, we may expect that this species has conditioned the soil and reshaped the microbiome (Hannula et al., 2019). Clearly, irrespective of the overriding effects of the plant monocultures on the soil biome, soil legacies were still detected in our experiment. Moreover, soil bacteria and fungi in microcosms with soil collected from experimental field plots that originally received a higher amount of soil inoculum resembled that of the donor community more closely than those that had received a lower amount of soil. This indicates that there is a legacy of the original soil inoculation treatments in the field experiment that is still detectable. It highlights that the addition of soil with soil organisms in the field can have long-term impacts in the system where they have been introduced and emphasizes the potential of whole soil inoculation treatments for nature restoration (Lyons et al., 2023). One explanation for these results is that the introduced soil organisms remained present and active in the new environment and hence settled within the recipient soil food web (Han et al., 2022; Wubs et al., 2016). Alternatively, it is possible that the addition of soil and soil organisms at the recipient site influenced the plant community and that feedbacks between the plant and soil biome then resulted in persistent differences in the soil biome over time (Heinen et al., 2020).

Similar to what we detected in our study, a previous study also reported that higher amounts of added soil result in greater impacts on soil microbial communities and plant growth (Dong et al., 2022). It is possible that with a higher amount of added soil, the survival of introduced soil organisms increases as the habitat created by the addition of a thicker layer of soil may more closely resemble that of the donor site than when only a thin layer of donor soil is introduced (Han et al., 2022). Also, a higher amount of soil could transfer a larger amount of soil organisms and a larger inoculum or replacement of soil organisms could contribute to stronger or longer-lasting legacy effects. Another possible explanation is that an increased amount of added soil may also contain more seeds of the donor vegetation, and hence this may also promote the development of new plant communities, which in turn match the new soil biome, leading to positive plant–soil feedbacks and more distinct differences in soil communities over time (Radujkovic et al., 2020).

Soil legacies are the net effect of soil abiotic and biotic effects, and higher amounts of added soil result in higher abiotic and biotic effects created by the donor plant community. Considering that in our results there were almost no differences in soil nutrients, our results suggest that the legacies from the field experiment that were detected are mainly of biotic origin. Interestingly, the microbial communities arising from inoculation with inocula from experimental plots where the lower amount of soil was added were more affected (i.e. stronger correlation) by the abiotic nutrients than those from experimental plots where a higher amount of donor soil was added. This suggests that the greater impact on soil microbial communities of higher amounts of added soil is mainly from biotic effects, but that the soil abiotic context is also important for shaping microbial legacies after soil addition. Hence, the combination of alterations in soil nutrients and bio-regulation may be a promising restoration strategy.

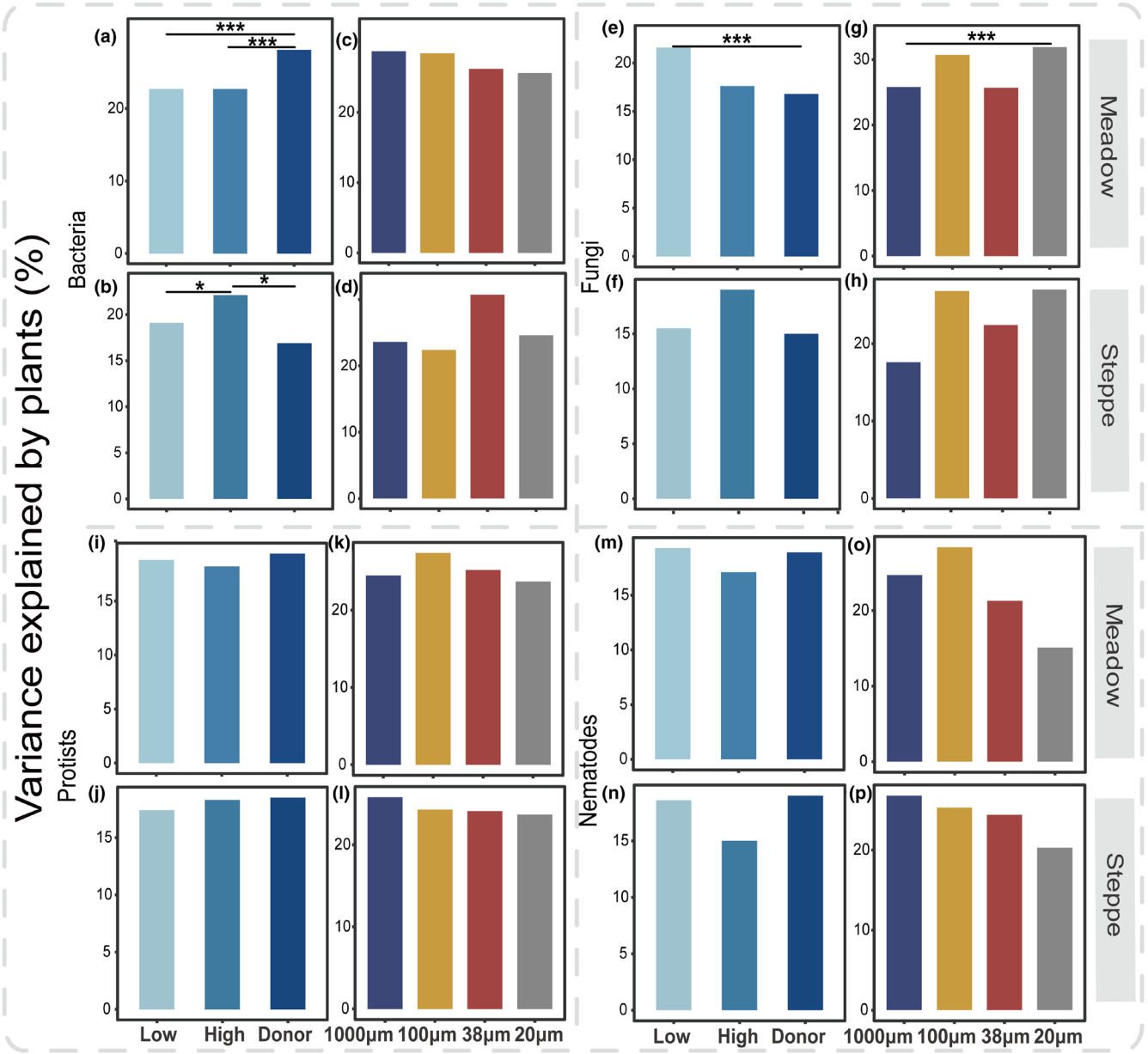

FIGURE 5 Proportion of variation of the composition of the communities of soil bacteria (a–d), fungi (e–h), protists (i–l) and nematodes (m–p) explained by the biomass of subsequently grown plant (results from RDA). (a, e, i, m) Explained variance of plant biomass for soil communities in microcosms inoculated with soil legacies from meadow donor soil and field plots received low and high amounts of meadow soil. (b, f, j, n) Explained variance of plant biomass for soil communities in microcosms inoculated with soil legacies from steppe donor soil and field plots received low and high amounts of steppe soil. (c, g, k, o) Explained variance of plant biomass for soil communities in microcosms inoculated with different biotic fractions from meadow ecosystem. (d, h, l, p) Explained variance of plant biomass for soil communities in microcosms inoculated with different biotic fractions from steppe ecosystem. $^{*}p < 0.05$; $^{***}p < 0.001$.

4.2 | Removal of soil biotic groups and the persistence of soil microbial legacies

In the microcosms, the similarity in soil biome among treatments increased when the biotic fraction used for inoculation was smaller than 20 µm, which is in line with our third hypothesis. The smaller mesh sizes removed dissimilar larger organisms, for example, mesofauna, which usually are slow-growing and have low dispersal but can directly or indirectly affect the community composition of smaller soil organisms (Kulmatiski & Beard, 2011). The results suggest that the composition of the microbial communities smaller than 20 µm is similar in the field plots that received lower and higher amounts of soil from the two donors. The similarity among microbial communities was lowest for the largest mesh size and increased with decreasing mesh size for inocula prepared from soil that received the low amount of donor soil. Interestingly, this was not the case for inocula from soil that received a high amount of donor soil and here similarity was higher overall after the stepwise

TABLE 5 Effects of different soil legacies inoculation on the explained variance of plant biomass on the composition of soil communities (results from RDA).

	Bacteria			Fungi			Protists			Nematodes		
	Variance	*F*	*p*	Variance	*F*	*p*	Variance	*F*	*p*	Variance	*F*	*p*
Meadow ecosystem												
Legacy type	0.087	2.075	**0.001**	0.11	1.43	**0.003**	0.17	1.05	0.17	0.16	1.08	0.158
Biotic fraction type	0.077	1.021	0.427	0.12	1.07	0.259	0.25	1.02	0.338	0.21	0.90	0.884
Steppe ecosystem												
Legacy type	0.062	1.38	**0.002**	0.09	1.11	0.092	0.16	1.01	0.448	0.13	0.89	0.863
Biotic fraction type	0.085	1.24	**0.011**	0.12	0.98	0.594	0.25	1.01	0.424	0.21	0.99	0.553

Note: Values in bold are significant at $p < 0.05$.

Abbreviations: *F*, *F*-value; *p*, *p*-value.

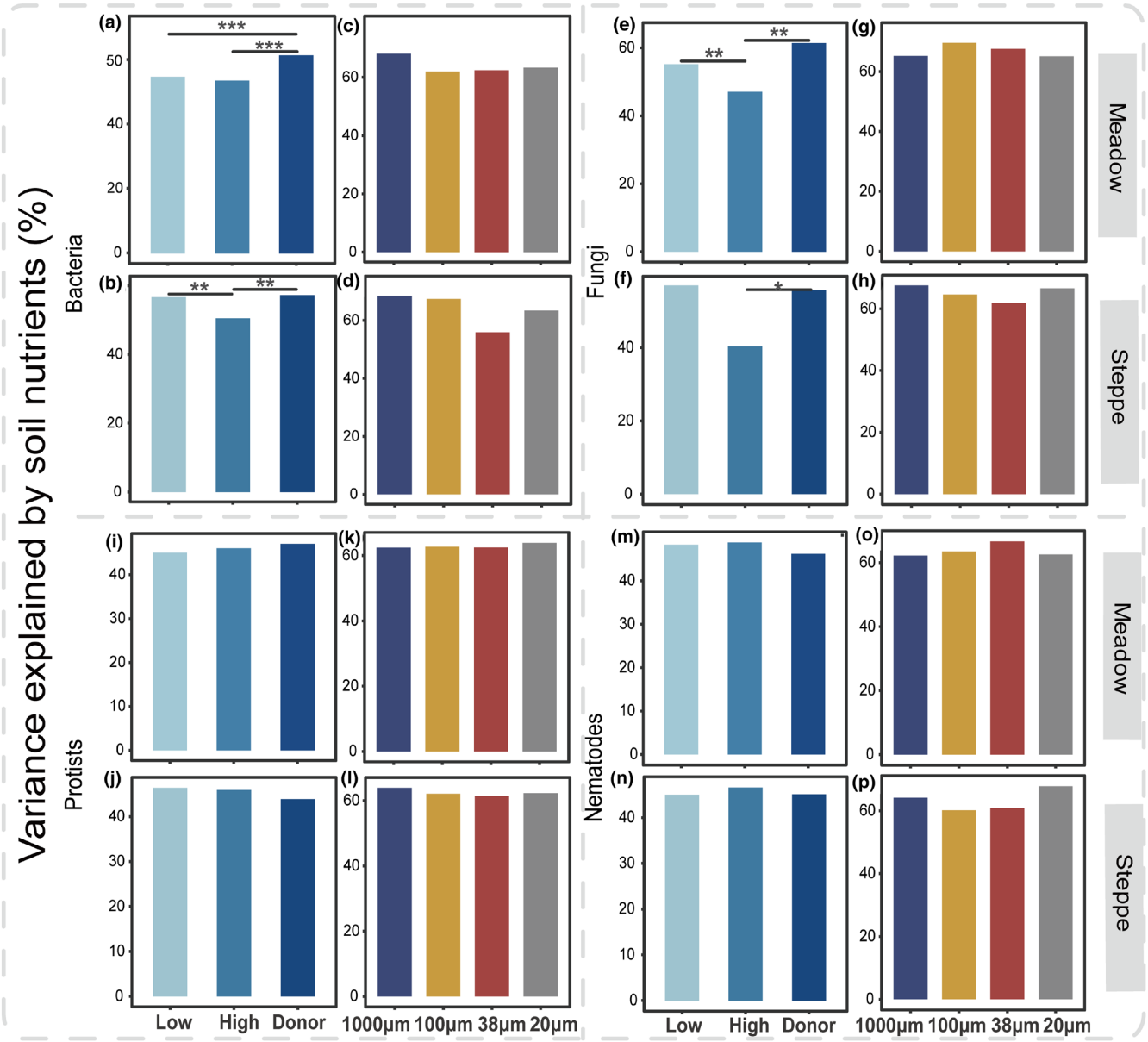

FIGURE 6 Proportion of variation of the composition of the communities of soil bacteria (a–d), fungi (e–h), protists (i–l) and nematodes (m–p) explained by soil nutrients (results from RDA). (a, e, i, m) Explained variance of soil nutrients for soil communities in microcosms inoculated with soil legacies from meadow donor soil and field plots that received low and high amounts of meadow soil. (b, f, j, n) Explained variance of soil nutrients for soil communities in microcosms inoculated with soil legacies from steppe donor soil and field plots that received low and high amounts of steppe soil. (c, g, k, o) Explained variance of soil nutrients for soil communities in microcosms inoculated with different biotic fractions from the meadow ecosystem. (d, h, l, p) Explained variance of soil nutrients for soil communities in microcosms inoculated with different biotic fractions from the steppe ecosystem. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

TABLE 6 Effects of different soil legacies inoculation on the explained variance of soil nutrients on the composition of soil communities (results from RDA).

	Bacteria			Fungi			Protists			Nematodes		
	Variance	*F*	*p*	Variance	*F*	*p*	Variance	*F*	*p*	Variance	*F*	*p*
Meadow ecosystem												
Legacy type	0.14	1.74	**0.001**	0.20	1.66	**0.001**	0.35	1.06	0.126	0.32	1.14	0.06
Biotic fraction type	0.15	0.95	0.666	0.23	1.01	0.485	0.50	1.00	0.491	0.44	1.01	0.446
Steppe ecosystem												
Legacy type	0.11	1.47	**0.001**	0.17	1.21	**0.047**	0.32	1	0.502	0.27	0.99	0.487
Biotic fraction type	0.14	1.08	0.214	0.23	1.06	0.311	0.49	1.04	0.289	0.42	1.06	0.3

Note: Values in bold are significant at $p < 0.05$.

Abbreviations: *F*, *F*-value; *p*, *p*-value.

removal of larger-sized soil organisms (Figure S4). An explanation is that, in the field, the higher amount of soil that was added not only generated a microbial community that was more similar to that of the donor soil but also generated a community with similar larger-sized soil organisms. The changes in the similarity between the field plots and donor soils after the introduction of different biotic fractions from two amounts of added soil in the microcosms indicate that inoculating a larger amount of soil directly increased the chances of transferring more larger-sized organisms from the donor to the recipient soil. It also suggested that soil inoculation in the field plots probably first altered the smaller soil microbial communities that can then influence the entire soil biome via bottom-up effects (Hassi et al., 2023).

In our study, the composition of soil microbial communities created by inoculation with the smallest (20 μm) biotic fraction differed from that of inoculation with other larger-sized biotic fractions. That is, after the removal of the larger-sized microbial groups (20–38 μm), both the composition of the fungal and the bacterial communities changed considerably. This may be due to the stronger regulation of soil microbial communities by 20–38 μm sized microbes and a weaker regulation of soil microbial communities by macrofauna, mesofauna and microfauna. Another explanation for the distinction between soil microbial communities from 20-μm and from larger sieve fractions is that the effect of the single plant species on the soil microbial community was more obvious after inoculation with the smallest biotic fraction than after inoculating other size fractions. The interactions between plant and soil biomes can be a driver of microbial community structure (Fox et al., 2020), and this role may be even stronger in simplified soil communities, as stronger biotic interactions are usually present in complex soil communities. The correlation analysis revealed a higher correlation between the soil fungal community composition and plant growth after inoculation with the smallest biotic fraction than after inoculation with other fractions (Figure 5g,h). This also suggests a stronger connection between the fungal communities that were formed after inoculation with the smallest biotic fraction. In addition, the changes in the fungal community may affect the soil bacterial community, as fungal communities play a bridging role and connect multiple biomes in nature (Yang et al., 2022). Thus, higher associations between plant and fungal communities after inoculation with the smallest mesh size ultimately may lead to differences in microbial communities between 20-μm and other size fractions (Figure 5g). In a wider perspective, our results showed that the interactions between current plant and soil fungi play a vital role in shaping microbial legacies. As we could not assess absolute abundance due to the molecular method that we used to examine the composition, our results may not reflect the real densities. Additionally, we sequenced the soil biome from only three replicate soil samples (originally from different field plots) at plant harvest. To improve the accuracy of these results, future studies should also measure absolute abundance and increase the number of replicates, as well as add direct sequencing of biota in water inocula.

5 | CONCLUSIONS

In conclusion, our study indicates that legacies in soil collected from a one-time soil addition experiment that occurred 4 years earlier in

a degraded grassland can affect the assemblage of the entire soil biome. This legacy effect is strongly influenced by the amount of soil that was added originally to the field plots. Importantly, legacies carried by small-sized soil organisms are more similar to the soil biome in donor soils and have a stronger positive effect on plant growth. Over time, we argue that these smaller-sized soil microbial legacies may gradually restore the positive interactions between the soil food web and plants through bottom-up effects. These findings improve our understanding of the interactions between plant and soil food webs, which could help to design suitable strategies for conservation and restoration in degraded ecosystems.

AUTHOR CONTRIBUTIONS

Qi Li and T. Martijn Bezemer designed the study. Yuhui Li, Yixin Sun and Yingbin Li analysed the data. Yuhui Li, Xu Han, Bing Li and Xiaofang Du collected the samples and conducted the measurements. Yuhui Li, Qi Li and T. Martijn Bezemer wrote the first draft, and all authors contributed to the editing of the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available from Figshare: https://doi.org/10.6084/m9.figshare.28227182 (Li et al., 2025). The raw sequencing data reported in this paper have been deposited in the National Microbiology Data Center under the accession number NMDC10018651.

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REFERENCES

Bardgett, R. D., & van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*, 505–511. https://doi.org/10.1038/nature13855

Bates, D., Machler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Beckers, B., De Op Beeck, M., Weyens, N., Boerjan, W., & Vangronsveld, J. (2017). Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome*, *5*, 25. https://doi.org/10.1186/s40168-017-0241-2

Biddle, J. F., Fitz-Gibbon, S., Schuster, S. C., Brenchley, J. E., & House, C. H. (2008). Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 10583–10588. https://doi.org/10.1073/pnas.0709942105

Cole, J. R., Wang, Q., Fish, J. A., Chai, B. L., McGarrell, D. M., & Tiedje, J. M. (2014). Ribosomal database project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Research*, *42*, 633–642. https://doi.org/10.1093/nar/gkt1244

Cong, W. F., van Ruijven, J., Mommer, L., Deyn, G. B., Berendse, F., Hoffland, E., & Lavorel, S. (2014). Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *Journal of Ecology*, *102*, 1163–1170. https://doi.org/10.1111/1365-2745.12280

David, J. F. (2014). The role of litter-feeding macroarthropods in decomposition processes: A reappraisal of common views. *Soil Biology & Biochemistry*, *76*, 109–118. https://doi.org/10.1016/j.soilbio.2014.05.009

De Beeck, M. O., Lievens, B., Busschaert, P., Declerck, S., Vangronsveld, J., & Colpaert, J. V. (2014). Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. *PLoS One*, *9*, e97629. https://doi.org/10.1371/journal.pone.0097629

Delgado-Baquerizo, M., Reich, P. B., Trivedi, C., Eldridge, D. J., Abades, S., Alfaro, F. D., & Singh, B. K. (2020). Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nature Ecology & Evolution*, *4*, 210–220. https://doi.org/10.1038/s41559-019-1084-y

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*, 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Dong, Y. J., Tang, B. B., He, M. M., Wang, L. L., Wu, K., Yang, S. X., Liu, J., Yang, H., Wang, C., Yin, F., & Zhang, W. D. (2022). High concentrations of antagonistic bacterial strains from diseased sanqi ginseng rhizosphere suppressed Fusarium root rot. *European Journal of Plant Pathology*, *163*, 143–153. https://doi.org/10.1007/s10658-022-02463-4

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edgar, R. C., & Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics*, *31*, 3476–3482. https://doi.org/10.1093/bioinformatics/btv401

Fox, A., Lüscher, A., & Widmer, F. (2020). Plant species identity drives soil microbial community structures that persist under a following crop. *Ecology and Evolution*, *10*, 8652–8668. https://doi.org/10.1002/ece3.6560

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., & Christen, R. (2013). The protist ribosomal reference database (PR2): A catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*, *41*, 597–604. https://doi.org/10.1093/nar/gks1160

Han, X., Li, Y., Li, Y., Du, X., Li, B., Li, Q., & Bezemer, T. M. (2022). Soil inoculum identity and rate jointly steer microbiomes and plant communities in the field. *ISME Communications*, *2*, 59. https://doi.org/10.1038/s43705-022-00144-1

Hannula, S. E., Heinen, R., Huberty, M., Steinauer, K., De Long, J. R., Jongen, R., & Bezemer, T. M. (2021). Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. *Nature Communications*, *12*, 5686. https://doi.org/10.1038/s41467-021-25971-z

Hannula, S. E., Kielak, A. M., Steinauer, K., Huberty, M., Jongen, R., De Long, J. R., & Bezemer, T. M. (2019). Time after time: Temporal variation in the effects of grass and forb species on soil bacterial and fungal communities. *MBio*, *10*, e02635. https://doi.org/10.1128/mBio.02635-19

Hassi, U., Hu, J. W., Sleutel, S., & De Neve, S. (2023). Recovery of soil microbial community structure and activity following partial sterilization with gamma irradiation. *Applied Soil Ecology*, *187*, 104839. https://doi.org/10.1016/j.apsoil.2023.104839

Heinen, R., Hannula, S. E., De Long, J. R., Huberty, M., Jongen, R., & Isbell, F. (2020). Plant community composition steers grassland vegetation via soil legacy effects. *Ecology Letters*, *23*, 973–982. https://doi.org/10.1111/ele.13497

Jing, J., Cong, W. F., & Bezemer, T. M. (2022). Legacies at work: Plant-soil-microbiome interactions underpinning agricultural sustainability. *Trends in Plant Science*, *27*, 781–792. https://doi.org/10.1016/j.tplants.2022.05.007

Kardol, P., Bezemer, T. M., & van der Putten, W. H. (2009). Soil organism and plant introductions in restoration of species-rich grassland communities. *Restoration Ecology*, *17*, 258–269. https://doi.org/10.1111/j.1526-100X.2007.00351.x

Kulmatiski, A., & Beard, K. H. (2011). Long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community structure. *Soil Biology & Biochemistry*, *43*, 823–830. https://doi.org/10.1016/j.soilbio.2010.12.018

Li, Y. H., Sun, Y. X., Li, Y. B., Han, X., Li, B., Du, X. F., Li, Q., & Martijn, T. B. (2025). Data for: Small-sized soil organisms drive the legacy of soil addition in a degraded grassland. *Figshare*. https://doi.org/10.6084/m9.figshare.28227182

Liu, Y. X., Qin, Y., Chen, T., Lu, M., Qian, X., Guo, X., & Bai, Y. (2021). A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein & Cell*, *5*, 315–330. https://doi.org/10.1007/s13238-020-00724-8

Lyons, K. G., Török, P., Hermann, J. M., Kiehl, K., Kirmer, A., Kollmann, J., & Temperton, V. M. (2023). Challenges and opportunities for grassland restoration: A global perspective of best practices in the era of climate change. *Global Ecology and Conservation*, *46*, e02612. https://doi.org/10.1016/j.gecco.2023.e02612

Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., & Abarenkov, K. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, *47*, 259–264. https://doi.org/10.1093/nar/gky1022

Petr, S., & Jan, L. (2014). *Multivariate analysis of ecological data using Canoco 5*. Cambridge University Press.

R Core Team. (2021). *R: A language and environment for statistical computing*. R foundation for statistical computing.

Radujkovic, D., van Diggelen, R., Bobbink, R., Weijters, M., Harris, J., Pawlett, M., & Verbruggen, E. (2020). Initial soil community drives heathland fungal community trajectory over multiple years through altered plant-soil interactions. *New Phytologist*, *225*, 2140–2151. https://doi.org/10.1111/nph.16226

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahe, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Teste, F. P., Kardol, P., Turner, B. L., Wardle, D. A., Zemunik, G., Renton, M., & Laliberté, E. (2017). Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *Science*, *355*, 173–176. https://doi.org/10.1126/science.aai8291

van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., & Hutchings, M. (2013). Plant-soil feedbacks: The past, the present and future challenges. *Journal of Ecology*, *101*, 265–276. https://doi.org/10.1111/1365-2745.12054

Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 5266–5270. https://doi.org/10.1073/pnas.1320054111

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E. E., & van der Heijden, M. G. A. (2019). Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nature Communications*, *10*, 4841. https://doi.org/10.1038/s41467-019-12798-y

Wang, M. G., Ruan, W. B., Kostenko, O., Carvalho, S., Hannula, S. E., Mulder, P. P. J., & Bezemer, T. M. (2019). Removal of soil biota alters soil feedback effects on plant growth and defense chemistry. *New Phytologist*, *221*, 1478–1491. https://doi.org/10.1111/nph.15485

Wubs, E. R. J., van der Putten, W. H., Bosch, M., & Bezemer, T. M. (2016). Soil inoculation steers restoration of terrestrial ecosystems. *Nature Plants*, *2*, 16107. https://doi.org/10.1038/Nplants.2016.107

Wubs, E. R. J., van der Putten, W. H., Mortimer, S. R., Korthals, G. W., Duyts, H., Wagenaar, R., & Bezemer, T. M. (2019). Single introductions of soil biota and plants generate long-term legacies in soil and plant community assembly. *Ecology Letters*, *22*, 1145–1151. https://doi.org/10.1111/ele.13271

Yang, T., Tedersoo, L., Liu, X., Gao, G. F., Dong, K., Adams, J. M., & Chu, H. (2022). Fungi stabilize multi-kingdom community in a high elevation timberline ecosystem. *iMeta*, *1*, e49. https://doi.org/10.1002/imt2.49

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Plants.

Appendix S2. Method used for detailed process of the microcosm experiment.

Appendix S3. Method used for measurement of soil physicochemical properties.

Table S1. The number of ASVs identified from sequences of each soil organism group and the sample sizes present in the ASV data.

Table S2. Effects of mixed-effects models for the different soil legacies inoculation on soil nutrients.

Table S3. Effects of mixed-effects models for the different soil legacies inoculation on the richness of soil fungi, bacteria, protists and nematodes.

Table S4. Effects of mixed-effects models for the different soil legacies inoculation on the similarity of soil fungi and bacteria to according donor soil.

Figure S1. Changes of soil dissolved organic carbon, net nitrogen mineralisation rate and available phosphorus in microcosms.

Figure S2. Changes of soil fungal richness, bacterial richness, protists richness and nematodes richness in microcosms.

Figure S3. Biplots of PCoA analysis of the community composition of soil bacteria, fungi, protists and nematodes in microcosms.

Figure S4. The similarity of soil bacteria and fungi in microcosms inoculated with watery extracts from soil addition plots and from donor sites.

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