Title: Soil inoculum identity and rate jointly steer plant and belowground communities in the field

Authors: Xu Han[1, 2]†, Yingbin Li[1]†, Yuhui Li[1, 2], Xiaofang Du[1], Bing Li[1, 2], Qi Li[1]* and T. Martijn Bezemer[3, 4]

Affiliations:

[1]Erguna Forest-Steppe Ecotone Research Station, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.

[2]University of Chinese Academy of Sciences, Beijing 100049, China.

[3]Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO- KNAW), P.O. Box 50, Wageningen 6700 AB, Wageningen, The Netherlands.

[4]Institute of Biology, Above-Belowground Interactions group, Leiden University, P.O. Box 9505, 2300 RA Leiden, The Netherlands.

*Corresponding author. Email: liq@iae.ac.cn

† Han Xu, and Yingbin Li should be considered joint first author.

Abstract: Inoculation with soil from different ecosystems can induce directional changes in plant and soil communities, however, it is unknown how specific these inoculations are, how much inoculum is needed, and whether different inocula collected from a similar ecosystem will steer differential ecosystem development at the recipient site. We conducted a soil inoculation experiment at a degraded grassland site and used two different soil inocula both from grasslands and three inoculation depths. We then measured the development of plant and soil communities over a period of three years. Our results show that soil and plant communities diverged and that these effects were stronger with increasing inoculation amount. Inoculation with upland meadow soil resulted in more complex biotic networks at the degraded site than inoculation with meadow steppe soil. Our experiment highlights that soil inoculation can promote restoration of degraded ecosystems but that the direction and speed of ecosystem development depend on the origin of the inoculum and the amount of inoculum used.

One-Sentence Summary: Soil inocula can promote restoration of degraded ecosystems but the direction and speed of ecosystem development depends on the soil identity and rate.

Main Text:

Grasslands cover about 40% of the earth's surface, providing a large number of ecosystem services (*1*, *2*). However, the intensification of land use has resulted in a worldwide degradation of species-rich forage grasslands (*3*, *4*). Recent studies have shown that inoculation with soil from a late successional stage can facilitate the establishment and growth of late-successional plant species and suppress early successional plant species at ruderal or degraded sites (*5* - *9*). This is particularly efficient when the inoculation is carried out after the top-soil layer has been removed (*8*, *10*). For example, Wubs *et al.* (*8*) recently showed that in a former arable field the composition of both the plant and soil community was steered towards a predefined target community when using soil inoculum collected from a semi-restored grassland and from a heathland. While there is now evidence that inoculation with soil from a two distinctly different ecosystems can induce directional changes in both the plant and the soil community in the field (*11*), a major outstanding question is how specific these inoculations are and whether inoculation with soil originating from underneath different plant communities but from a similar ecosystem will also lead to differential ecosystem development at the recipient site.

In empirical soil inoculation field studies, so far, greatly varying amounts of donor soil were used ranging from 0.016 cm up to 40 cm layers of donor soil (*6*, *12*). We may expect that there is a positive relationship between the establishment of plants and soil biota and hence the success of the inoculation, and the amount of soil that is used. Results from a controlled pot experiment show that indeed there was a positive effect between the amount of inoculated soil and plant biomass (*13*). However, in the field, and over longer time periods, it is also possible that due to positive feedbacks between the plant and soil

community, inoculation with a small amount of donor soil will give similar "steering power" of the plant and soil communities as larger amounts of donor soil (*8*, *11*). How the amount of donor soil that is used for inoculation influences the establishment of plant and soil communities in the field is still not known. In restoration projects, the donor soil typically originates from a nature area, and collecting this soil will inevitably cause some destruction of the donor area. Hence it is essential to better understand the minimum amount of soil inoculum required for successful restoration to avoid over-exploitation of the donor area.

We conducted a soil inoculation experiment in a degraded grassland and measured the development of plant and soil communities over a period of three years following soil inoculation. In soil from the experimental fields we identified nematode communities and analyzed bacterial and fungal microbiomes with next generation sequencing. We used two donor grasslands: a meadow steppe and an upland meadow, and inoculated three different amounts of donor soil in experimental plots that were established after removing the top soil layer at the degraded site (Fig. 1). We hypothesized that inoculation with the different donor grassland soils would result in different soil and plant communities; and that the effect of soil inoculation would increase with increasing amounts of donor soil used to inoculate the field plots.

Soil identity and depth steer belowground communities

Inoculation with the two donor soils and inoculation depth both significantly affected the biotic composition in the recipient plots (Fig. 2; Table. 1). Already after three years, the bacterial and fungal communities had diverged towards those of the donor soil and inoculation depth increased this divergence (Fig. 2). Soil inoculation effects on the nematode

community were limited in the first year but significant in the second and third year (Table. S1). Richness of bacterial and fungal communities were also affected by soil inoculation (Fig. S1, Fig. S2). Inoculation depth had a positive effect on fungal richness (F = 15.26, $P < 0.001$) but a negative effect on bacterial richness (F = 3.21, $P = 0.044$). Additionally, richness of the bacterial community was lower in soil inoculation with upland meadow than that with meadow steppe (F = 5.6, $P = 0.02$). Together, these results show that two donor soils originating from a similar ecosystem can steer the biotic communities in the recipient site towards the specific donor community but also, for the first time that in the field, this is accelerated when increasing amounts of inocula are used.

Soil identity and depth steer plant communities

Soil inoculation also significantly affected plant community composition (Fig. 2; Table. 1) and steered the composition of plant communities towards the specific grassland donor sites (Fig. 2). The proportional cover of perennials increased strongly over time (F = 168.82, $P = 0.006$) and was higher in plots inoculated with upland meadow soil than in plots that received meadow steppe soil (F = 92.57, $P < 0.001$) (Fig. S3). With increasing inoculation depth, species richness of the plant community also increased and plant species richness was higher in upland meadow than in meadow steppe inoculated plots (Fig. S2). When compared to the uninoculated control (top soil removed only, TSR), soil inoculation suppressed the growth of degenerate indicator species, and increased the relative cover and abundance of the target species *Leymus chinensis* (Fig. S3). The total cover of all target species increased over time (meadow steppe: F = 92.4, $P = 0.011$; upland meadow: F = 79.37, $P = 0.012$) but this was not affected by inoculation depth (Fig. S4). More upland meadow target species colonized in plots inoculated with upland meadow soil (F = 15.62, $P < 0.001$) but meadow

steppe target species did not colonize more successfully in plots inoculated with meadow steppe soil than in control plots (Fig. S4). In the third year, belowground (root) biomass was significantly higher in inoculated than in control plots, and this effect increased with increasing inoculation depth but no significant effect was observed for aboveground biomass. Concentrations of soil nutrients and soil moisture also increased with increasing inoculation depth (Fig. 3, Fig. S5).

Similarity to the control and the donor sites

We further analyzed the effectiveness of inoculation by comparing the similarity of the plant and soil communities of inoculated and uninoculated plots and of inoculated plots to the two donor sites in the third year (Fig. 4). With increasing soil inoculation depth, inoculated plots became more dissimilar from the control (uninoculated) plots, and this was particularly so for bacteria and plants. Inoculation with meadow steppe soil increased the similarity to this donor more than inoculation with upland meadow for bacterial, fungal and nematode communities and a positive inoculation depth effect was found for bacterial and fungal communities. Similarly, inoculation with upland meadow soil resulted in higher similarity to the upland meadow donor site for fungi and plants and a positive depth effect was found for bacterial, fungal and plant communities.

Soil biotic networks

Soil genera with higher relative abundance in the inoculation plots than in the control were selected (Fig. S6). These may be directly introduced by soil inoculation or indirectly promoted following inoculation. To confirm whether these genera play an important role in

the soil community, we explored the distributions of bacterial, fungal and nematode communities in co-occurrence networks (Fig. 5A, B). The ten most important genera at the center of the network (higher node degree values, i.e. related with more other genera) were selected (Fig. 5C). Most of those genera significantly correlated with plant and soil traits (Fig. 5D). We also found that eight out of the ten of these central genera were increased after inoculation with upland meadow soil, and this also positively correlated with the amount of soil inoculation, and plant and soil traits. However, the genera that increased after inoculation with meadow steppe soil did not occupy the central position in the biotic network. Network complexity was also higher after inoculation with upland meadow (1.86) than with meadow steppe soil (1.55) (Table. S2).

This soil inoculation experiment at a degraded grassland with two different soil inocula and three inoculation depths shows that in the experimental plots soil and plant communities diverged and that these effects were stronger when more soil was used for inoculation. Moreover, inoculation with upland meadow soil introduced more genera playing important roles in biotic networks than inoculation with meadow steppe soil. These findings emphasize that there are specific effects of donor soil on the newly developing above- and belowground communities and that these effects depend on the identity of the donor even when comparing two grassland donors.

The importance of the role of soil organisms in the restoration of plant communities is becoming widely acknowledged now (*14*, *15*). Soil inoculation can be used to promote the colonization of soil organisms at the start of restoration. This study now shows that inoculation with soil communities that originate from different donor grasslands steers the

soil and plant communities of the inoculated degraded grassland site into different directions. This result highlights the importance of selecting the correct donor site in restoration projects. In our study, inoculation with both meadow steppe and upland meadow soil led to a higher similarity to the specific donor but plant community inoculation with upland meadow soil caused much stronger effects than inoculation with meadow steppe soil. More target plant species from the upland meadow colonized the inoculated site after inoculation with upland meadow soil, while colonization of meadow steppe target plant species after inoculation with meadow steppe soil was less successful. These findings suggest that there are distinct differences in colonization or establishment success of plant and soil organisms among different donor soils. Interestingly, these findings also show that differences in the initial composition of the whole soil inoculum, can alter interactions in the soil networks at the recipient site and this suggests that the choice of donor can have important consequences for the functioning of soil communities and ultimately for the functioning of the entire ecosystem that is restored. To what extend the inoculum-specific changes in the plant communities were directly caused by introducing different species of plants (e.g. as seeds or root pieces) in the inoculated soil or whether this was due to different rates of establishment of the plants mediated by the altered soil community requires further work.

An important result from this study is that the changes in the soil and plant communities and the similarity to the donor sites increased almost linearly with the amount of inoculum used. These findings support our second hypothesis that the effect of soil inoculation on plant and soil communities is accelerated with increasing amounts of soil used for inoculation. In an experiment with trees, stronger inoculation effects were observed when

higher amounts of soil inoculum were used on the height and diameter of ectomycorrhizal tree species (*16*). It is likely that with the increasing amount of soil, the density of seeds, root pieces and soil organisms linearly increased and that this results in increased establishment of the introduced organisms (*17*). However, it is important to note that for many plant species, germination of seeds from the seedbank only occurs at the soil surface (*8*) and in our experiment for all inoculation depths the soil surface was covered with a layer of inoculated soil. Inoculation depth can also influence the establishment and survival of the introduced soil biota. It is likely that the introduced soil community survives and establishes best in the local habitat and that increasing the amount of inoculated soil at the recipient site increased resemblance to the original habitat conditions, e.g. by preventing the effects of desiccation and by better resembling the original abiotic conditions such as nutrient availability and pH (*6*, *11*). A better functioning new soil community could, in turn, be important for the establishment of target plant species. Many functions that soil biota provide are density dependent (*13*) and the density of soil organisms should be sufficiently high to avoid mortality and inbreeding depression (*6*, *11*). In this study, upland meadow soil inoculation introduced soil genera such as *Acidobacteriota*, *Proteobacteria* and *Bacteroidota* and the abundance of those genera positively correlated with the amount of inoculum. These bacteria play important roles in soil carbon and nitrogen cycling especially when nutrient availability is low (*18* - *20*), which may, in turn, influence the biogeochemical processes at the recipient site.

Over the first three years following soil inoculation, the restoration of the degraded grassland soil and plant communities (i.e. the similarity to the donor sites) improved considerably. Importantly, the effects that we observed were not strongest immediately after

inoculation and then declined, but instead the effects increased over time (*16*). For example, soil inoculation effects on the nematode community were limited in the first year but significant in the second and third year. This clearly shows that the effects of inoculation were not remnants of what was left behind in the inoculated soil, but instead, that soil inoculation initiated the development of new soil and plant communities at the degraded site. Nematodes occur at different positions in the soil food web, and our results show that also higher trophic levels of the soil food web are influenced by soil inoculation, already two to three years after inoculation, even though organisms at these trophic levels are typically slow-growing and have low dispersal (*21*, *22*). These results show that whole soil inoculation is a method by which entire soil food webs can be molded rapidly in the field and as such can be a very useful tool in ecosystem restoration projects.

Via soil inoculation we created new plant and soil communities in the field. It is plausible that these new aboveground and belowground communities will interactively influence each other. A recent long term study in a species rich grassland on a former arable field showed that it can take up to a decade before such interactions become apparent (*15*). Long term measurements are needed at the experimental field site where after three years both plant and soil communities changed considerably, to determine how the new aboveground and belowground communities will interact and how this will change the functioning of these grassland ecosystems and the composition of species in those ecosystems.

Overall, the experiment highlights that soil inoculation can promote restoration of degraded ecosystems but that the way and direction the ecosystem develops towards after inoculation depends strongly on the origin of the inoculum and the amount of inoculum

used. Long-term measurements are now needed to determine whether the ecosystems that received small amounts of inoculum, in the longer term, will exhibit a similar but slower pattern of development as the ecosystems that received larger amounts of inoculum or whether instead the former will become similar to the uninoculated controls over time. Such long term data will also reveal whether and for how long the ecosystems that were inoculated with different donor soils will continue to develop into different communities (e.g. via different aboveground-belowground interaction loops), or whether they will converge over time.

REFRENCES AND NOTES

1. Z. M. Hu, S. G. Li, Q. Guo, S. L. Niu, N. P. He, L. H. Li, G. R. Yu, A synthesis of the effect of grazing exclusion on carbon dynamics in grasslands in China. *Global Change Biol.* **22**, 1385-1393 (2016).
2. X. Lyu, X. B. Li, J. R. Gong, H. Wang, D. L. Dang, H. S. Dou, S. K. Li, S. Y. Liu, Comprehensive Grassland Degradation Monitoring by Remote Sensing in Xilinhot, Inner Mongolia, China. *Sustainability* **12**, 3682 (2020).
3. B. A. Bryan, L. Gao, Y. Q. Ye, X. F. Sun, J. D. Connor, N. D. Crossman, M. Stafford-Smith, J. G. Wu, C. Y. He, D. Y. Yu, Z. F. Liu, A. Li, Q. X. Huang, H. Ren, X. Z. Deng, H. Zheng, J. M. Niu, G. D. Han, X. Y. Hou, China's response to a national land-system sustainability emergency. *Nature* **559**, 193-204 (2018).
4. J. F. Chang, P. Ciais, T. Gasser, P. Smith, M. Herrero, P. Havlik, M. Obersteiner, B. Guenet, D. S. Goll, W. Li, V. Naipal, S. S. Peng, C. J. Qiu, H. Q. Tian, N. Viovy, C. Yue, D. Zhu, Climate warming from managed grasslands cancels the cooling effect of carbon sinks in sparsely grazed and natural grasslands. *Nat. Commun.* **12**, 118 (2021).

5. E. L. Middleton, J. D. Bever, Inoculation with a Native Soil Community Advances Succession in a Grassland Restoration. *Restor. Ecol.* **20**, 218-226 (2012).
6. J. Moradi, F. Vicentini, H. Simackova, V. Pizl, K. Tajovsky, J. Stary, J. Frouz, An investigation into the long-term effect of soil transplant in bare spoil heaps on survival and migration of soil meso and macrofauna. *Ecol. Eng.* **110**, 158-164 (2018).
7. M. P. Vecrin, S. Muller, Top-soil translocation as a technique in the re-creation of species-rich meadows. *Appl. Veg. Sci.* **6**, 271-278 (2003).
8. E. R. J. Wubs, W. H. van der Putten, M. Bosch, T. M. Bezemer, Soil inoculation steers restoration of terrestrial ecosystems. *Nat. Plants* **2**, 16107 (2016).
9. E. R. J. Wubs, T. van Heusden, P. D. Melchers, T. M. Bezemer, Soil Inoculation Steers Plant-Soil Feedback, Suppressing Ruderal Plant Species. *Front. Ecol. Evol.* **7**, 451 (2019).
10. R. Jaunatre, E. Buisson, T. Dutoit, Topsoil removal improves various restoration treatments of a Mediterranean steppe (La Crau, southeast France). *Appl. Veg. Sci.* **17**, 236-245 (2014).
11. P. Contos, J. L. Wood, N. P. Murphy, H. Gibb, Rewilding with invertebrates and microbes to restore ecosystems: Present trends and future directions. *Ecol. Evol.* **11**, 7187-7200 (2021).
12. T. Emam, Local soil, but not commercial AMF inoculum, increases native and non-native grass growth at a mine restoration site. *Restor. Ecol.* **24**, 35-44 (2016).
13. V. Carbajo, B. den Braber, W. H. van der Putten, G. B. De Deyn, Enhancement of Late Successional Plants on Ex-Arable Land by Soil Inoculations. *Plos One* **6**, e21943 (2011).
14. P. Kardol, D. A. Wardle, How understanding aboveground-belowground linkages can assist restoration ecology. *Trends Ecol. Evol.* **25**, 670-679 (2010).
15. E. R. J. Wubs, W. H. van der Putten, S. R. Mortimer, G. W. Korthals, H. Duyts, R. Wagenaar, T. M. Bezemer, Single introductions of soil biota and plants generate long-term

legacies in soil and plant community assembly. *Ecol. Lett.* **22**, 1145-1151 (2019).

16. A. St-Denis, D. Kneeshaw, N. Belanger, S. Simard, I. Laforest-Lapointe, C. Messier, Species-specific responses to forest soil inoculum in planted trees in an abandoned agricultural field. *Appl. Soil Ecol.* **112**, 1-10 (2017).
17. J. A. J. Kitto, D. P. Gray, H. S. Greig, D. K. Niyogi, J. S. Harding, Meta-community theory and stream restoration: evidence that spatial position constrains stream invertebrate communities in a mine impacted landscape. *Restor. Ecol.* **23**, 284-291 (2015).
18. T. A. Conradie, K. Jacobs, Distribution patterns of Acidobacteriota in different fynbos soils. *Plos One* **16**, e0248913 (2021).
19. B. U. Foesel, V. Nagele, A. Naether, P. K. Wust, J. Weinert, M. Bonkowski, G. Lohaus, A. Polle, F. Alt, Y. Oelmann, M. Fischer, M. W. Friedrich, J. Overmann, Determinants of Acidobacteria activity inferred from the relative abundances of 16S rRNA transcripts in German grassland and forest soils. *Environ. Microbiol.* **16**, 658-675 (2014).
20. V. Chaudhry, A. Rehman, A. Mishra, P. S. Chauhan, C. S. Nautiyal, Changes in Bacterial Community Structure of Agricultural Land Due to Long-Term Organic and Chemical Amendments. *Microb. Ecol.* **64**, 450-460 (2012).
21. T. E. Brewer, K. M. Handley, P. Carini, J. A. Gilbert, N. Fierer, Genome reduction in an abundant and ubiquitous soil bacterium 'Candidatus Udaeobacter copiosus'. *Nat. Microbiol.* **2**, 16198 (2017).
22. A. Kulmatiski, K. H. Beard, Long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community structure. *Soil Biol. Biochem.* **43**, 823-830 (2011).

ACKNOWLEDGMENTS:

We thank X.G. Han and Z.W. Wang for discussion and selection of the degraded grassland. We thank J. Guo for the help of vegetation investigation.

Funding: Q.L. acknowledges funding from International cooperation program of Chinese Academy of Sciences (151221KYSB20200014) and National Natural Science Foundation of China (41877047). T.M.B. acknowledges funding from The Netherlands Organization for Scientific Research (NWO VICI grant 865.14.006).

Author contributions:

T.M.B, Q.L. designed the study. X.H., Y.B.L set up the experiment and analyzed the data. X.H., Y.H.L, X.F.D., B.L. and Y.B.L collected the samples and conducted the measurements. X.H., Q.L. and T.M.B. wrote the first draft, and all authors contributed to the editing of the paper.

Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data needed to evaluate the conclusions in the paper are presented in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the corresponding author.

SUPPLEMENTARY MATERIALS

Materials and Methods

Supplementary Text

Figs. S1 to S6

Tables S1 to S2

References

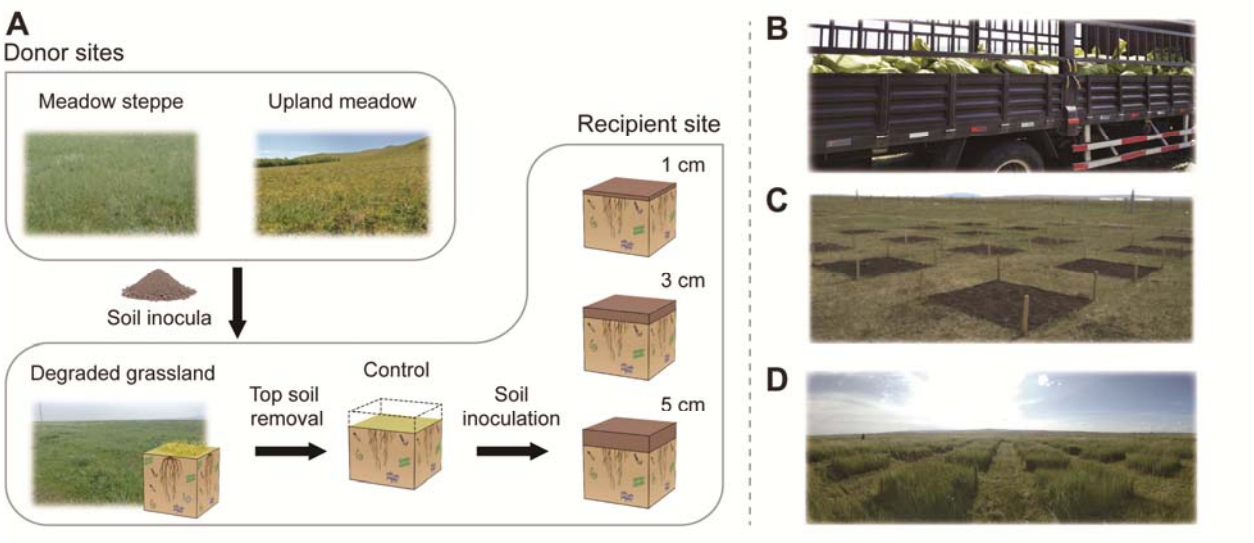

Fig. 1. Graphical illustration of the experimental design. A, Three replicate meadow steppes and upland meadows were selected as donor sites. At each donor location, the soil of the top 10 cm was excavated, homogenized and transported to the recipient site. A degraded grassland was selected as recipient site. The top 5 cm of the soil was removed before inoculation. The experiment was set up with three fully replicated blocks. In each block, one plot without inoculation (TSR) was also established. We established 19 experimental plots of 2 × 2 m^2 (2 soil types × 3 replicate sites × 3 inoculation depths + TSR) in each block and a total of 57 plots. **B**, the transfer of soil inoculum in 2018 to the field; **C**, the experimental field site after inoculation in 2018; **D**, and three years later, in 2021.

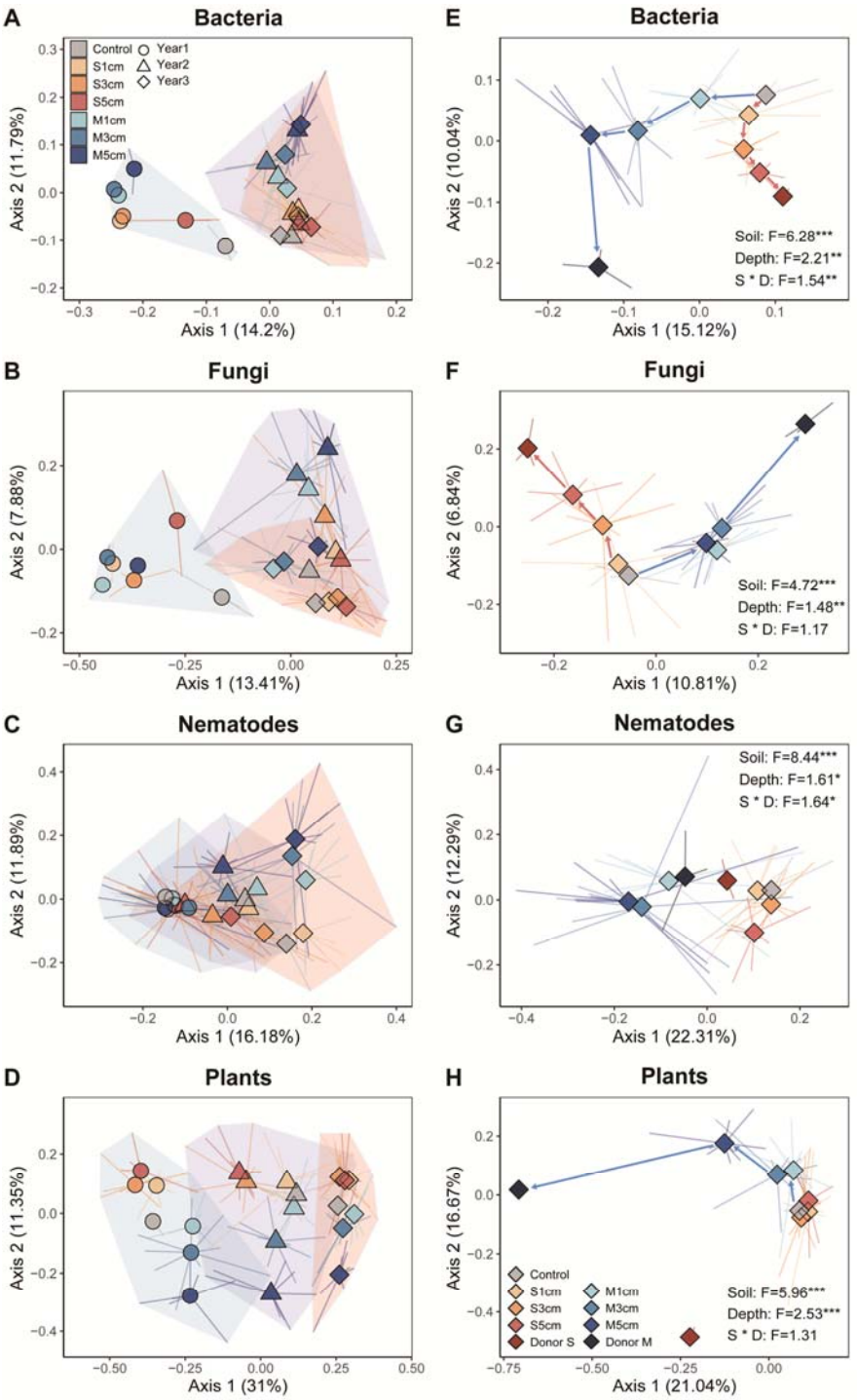

Fig. 2. Effects of soil inoculation on soil bacteria, fungi and nematodes, and plant communities. Separate PCoA ordinations using Bray-Curtis distance are presented in three years (**A**, **B**, **C** and **D**) and the third years (**E**, **F**, **G** and **H**). Percentage of variation given on each axis refers to the explained fraction of total variation. The colors depict soil inoculation treatments:

Control, the top soil removed treatment (TSR); S 1cm, meadow steppe soil inoculation with 1 cm depth; S 3cm, meadow steppe soil inoculation with 3 cm depth; S 5cm, meadow steppe soil inoculation with 5 cm depth; M 1cm, upland meadow soil inoculation with 1 cm depth; M 3cm, upland meadow soil inoculation with 3 cm depth; M 5cm, upland meadow soil inoculation with 5 cm depth; Donor S, donor meadow steppe; Donor M, donor upland meadow. The different shapes and shaded areas jointly represent plots in different years: circle, for the first year; triangle, for the second year and rhombus, for the third year. The result of PERMANOVA for three years were showed in table 1 and for the third year were listed above, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

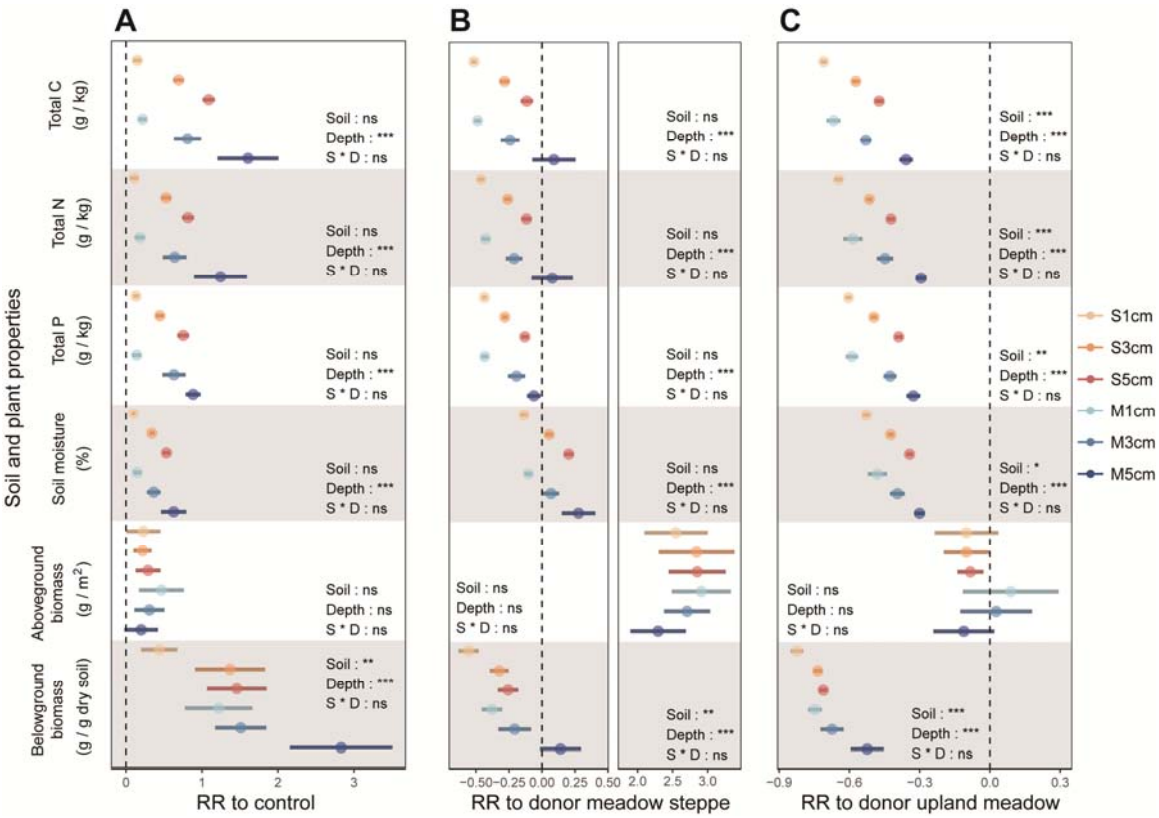

Fig. 3. Response ratio of soil and plant traits to control, donor meadow steppe and donor upland meadow. Error bars represent ± SE. The category definitions are as follows: S 1cm, meadow steppe soil inoculation with 1 cm depth; S 3cm, meadow steppe soil inoculation with 3 cm depth; S 5cm, meadow steppe soil inoculation with 5 cm depth; M 1cm, upland meadow soil inoculation with 1 cm depth; M 3cm, upland meadow soil inoculation with 3 cm depth; M 5cm, upland meadow soil inoculation with 5 cm depth. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

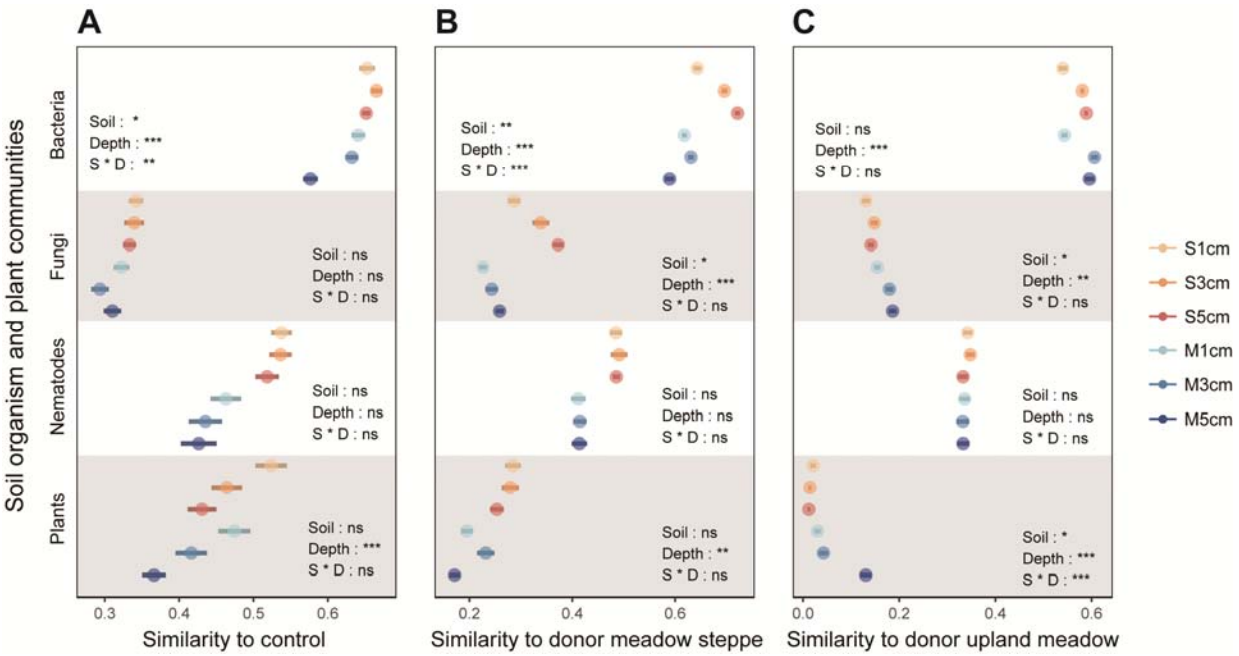

Fig. 4. Similarity between soil inoculation treatments to TSR or donor treatments. Error bars represent ± SE. The category definitions are as follows: S 1cm, meadow steppe soil inoculation with 1 cm depth; S 3cm, meadow steppe soil inoculation with 3 cm depth; S 5cm, meadow steppe soil inoculation with 5 cm depth; M 1cm, upland meadow soil inoculation with 1 cm depth; M 3cm, upland meadow soil inoculation with 3 cm depth; M 5cm, upland meadow soil inoculation with 5 cm depth. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

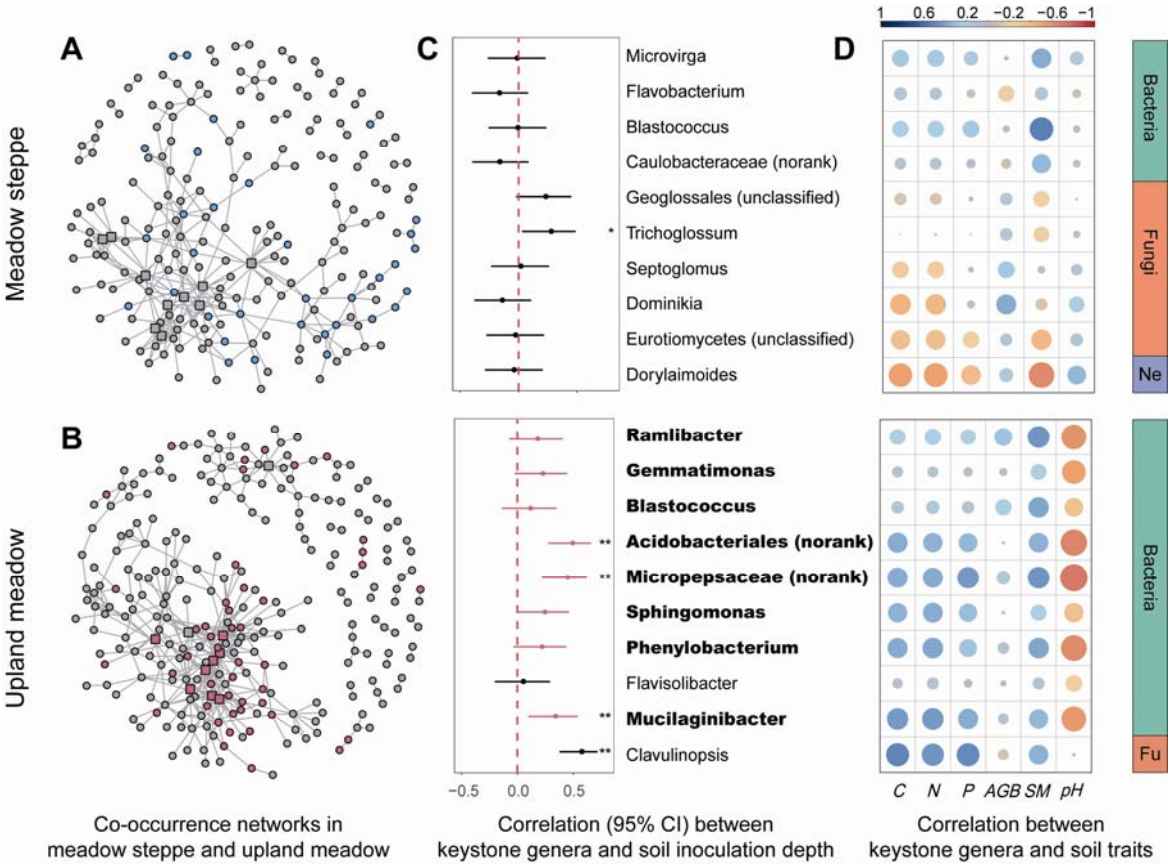

Fig. 5. The network analysis and the increased genera after inoculation with different donor soil. Co-occurrence networks at genus level based on meadow steppe (**A**) and upland meadow (**B**) soil inoculation. Correlation between the relative abundance of 10 central genera with the highest node degree and soil inoculation depth (**C**) and plant and soil traits (**D**). Blue (in **A**) and pink points (in **B**) indicated the genera significantly increased after inoculation meadow steppes and upland meadow soil than control plot. Square in networks (in **A** and **B**) and bold genera names (in **C**) indicated the genera significantly increased after soil inoculation. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. C: soil total carbon, N: soil total nitrogen, P: soil total phosphorus, AGB: aboveground biomass, SM: soil moisture, Ne: nematodes, Fu, fungi.

Table 1. Adonis test of the effect of year, soil type, depth and their interactions on soil and plant communities.

	Bacteria		Fungi		Nematodes		Plants	
	F	*P*	F	*P*	F	*P*	F	*P*
Year	13.4	<0.001	11.89	<0.001	18.24	<0.001	71.64	<0.001
Soil	10.53	<0.001	7.33	<0.001	5.41	<0.001	19.48	<0.001
Depth	3.55	<0.001	2.09	<0.001	1.91	0.008	6.7	<0.001
Year * Soil	1.1	0.305	1.13	0.237	4.07	<0.001	4.67	<0.001
Year * Depth	0.89	0.656	0.63	0.998	1.21	0.11	2.17	0.011
Soil * Depth	2.07	<0.001	1.51	0.016	1.35	0.131	4.18	<0.001
Year * Soil * Depth	0.84	0.786	0.69	0.992	0.97	0.417	1.01	0.432