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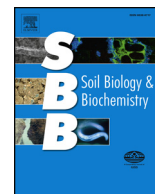
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Short Communication

Microbial amendments alter protist communities within the soil microbiome

Wu Xiong^{a,b}, Rong Li^{a,b}, Sai Guo^a, Ida Karlsson^{b,c}, Zixuan Jiao^a, Weibing Xun^a,
George A. Kowalchuk^b, Qirong Shen^{a,*}, Stefan Geisen^d

^a Jiangsu Provincial Key Lab of Solid Organic Waste Utilization, Jiangsu Collaborative Innovation Center of Solid Organic Wastes, Educational Ministry Engineering Center of Resource-saving Fertilizers, Nanjing Agricultural University, Nanjing 210095, Jiangsu, PR China

^b Ecology and Biodiversity Group, Department of Biology, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

^c Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden

^d Department of Terrestrial Ecology, Netherlands Institute for Ecology, (NIOO-KNAW), 6708 PB, Wageningen, The Netherlands

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ABSTRACT

Plant-beneficial microbes improve while pathogens reduce plant performance. When introduced in soils, such microbes can induce entire microbiome changes. However, the impact of those microbial introductions on protists – key predators within the soil microbiome – remain unknown. Here, we tracked how soil protists respond to bacterial (*Bacillus* and *Ralstonia*) and fungal (*Trichoderma* and *Fusarium*) introductions, with both microbial groups represented by one beneficial and one pathogenic taxon. We found that plant-beneficial *Bacillus* bacteria change the protist community structure. This community-shift was likely induced by an increased fungi/bacteria ratio, supported by a negative correlation of the fungi/bacteria ratio with the relative abundance of phagotrophic protists across all treatments. Our results indicate that microbial introductions can impact protist communities, thereby altering microbiome-derived multi-functionality.

Plant beneficial organisms have the potential to improve plant performance (Berendsen et al., 2012; Toju et al., 2018), while plant pathogens can be detrimental for plant health. Both plant beneficial and pathogenic microbes interact with, and thereby change, the community composition and functioning of other organisms in soil (Chapelle et al., 2016; Mallon et al., 2015). Among these soil organisms, protists represent the most diverse and abundant eukaryotes (Adl et al., 2005; Geisen et al., 2018). Protists function as dynamic hubs within soil communities that drive microbiome composition and turnover (Gao et al., 2018; Geisen et al., 2018). However, we have little knowledge on how plant beneficial microbes and plant pathogens affect protist communities as the few studies that examine these links are generally confounded by the fact that microbial amendments are typically applied in combination with organic fertilizers (Xiong et al., 2018). This makes it difficult to tease apart impacts induced by microbes themselves and those of abiotic factors. In this study, we monitored the temporal impact of two well-studied plant-beneficial microbes, consisting of one bacterium (*Bacillus amyloliquefaciens*) and one fungus (*Trichoderma guizhouense*) and two plant pathogens (the bacterium *Ralstonia solanacearum* and the fungus *Fusarium oxysporum*) and control (sterilized water) on protist communities after inoculation to bulk soil (without plants) over four weeks using 18S rRNA gene sequencing.

In doing so, we first compared the exact sequence variants (ESVs) approach “Unoise3” (Edgar, 2016) with “Swarm v2” (Mahé et al., 2015) and the commonly used Operational Taxonomic Units (OTUs) clustering by “Uparse” (Edgar, 2013), to identify an appropriate criterion to study eukaryotic community. In line with Glassman and Martiny (2018), our results suggest that ecological patterns provided by the three approaches are similar (Fig. S1a and Fig. S1b). However, the Unoise and Swarm approaches artificially over-estimated eukaryotic diversity by an order of magnitude, while only the Uparse method with a 97% similarity threshold provided expected OTU numbers (Table S1, Fig. S1c and Fig. S1d). We therefore utilized Uparse clustered sequences in all further analyses. We also traced the performance of each inoculated microbe and the total abundance of bacteria and fungi using qPCR approaches (see Supplementary Information for additional details).

The abundances of two beneficial microorganisms (*Bacillus* and *Trichoderma*) and the pathogenic *Fusarium* showed no decline over the course of the experiment (Fig. S2), while the abundance of the pathogen *Ralstonia* significantly ($P < 0.05$, Tukey's test) decreased by 87.96% within 4 weeks (Fig. S2c). These results may be related to the fact that both fungi (*Trichoderma* and *Fusarium*) can produce spores (Gordon, 2017; Papavizas et al., 1982) and *Bacillus* can form endospores (Pérez-

* Corresponding author.

E-mail address: shenqirong@njau.edu.cn (Q. Shen).

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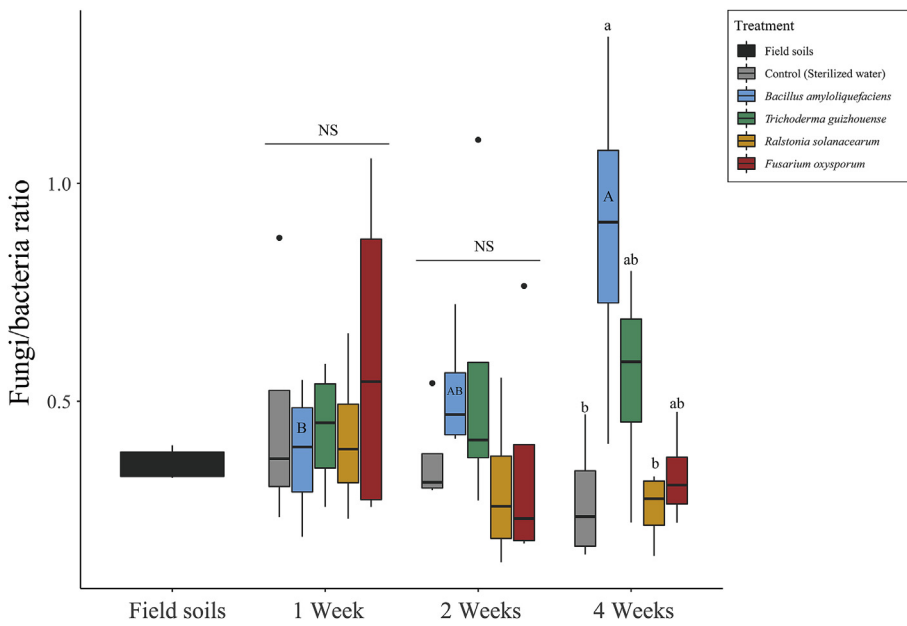


Fig. 1. Fungi/bacteria ratio as a function of the different microbial inoculation treatments. Inoculation consisted of two plant-beneficial microbes (bacterial *Bacillus amyloliquefaciens* and fungal *Trichoderma guizhouense*) and two plant pathogenic microbes (bacterial *Ralstonia solanacearum* and fungal *Fusarium oxysporum*). Sterilized water as control. Different capital letters inside the bars indicate a significant difference between the different time points for each treatment and different lowercase letters above the bars indicate a significant difference between the treatments for each time point at the 0.05 probability level according to Tukey's post-hoc tests. NS = not significant.

García et al., 2011), contributing to long-term survival in bulk soil, while *Ralstonia solanacearum* is a non-spore forming bacterium. Strikingly, we found that the plant-beneficial *Bacillus* bacteria, significantly increased ($P < 0.05$, Tukey's test) the fungi/bacteria ratio over time (Fig. 1). This shift in fungi/bacteria ratio was mostly due to the marginal ($P > 0.05$) decrease in bacterial abundance after *Bacillus* inoculation (Fig. S3a). Although it is tempting to speculate that the ability to influence fungi/bacteria ratio may be a more general property of plant-beneficial microorganisms, our study only examined two microbial amendments, with observed effects being driven to a large extent by *Bacillus amyloliquefaciens* bacteria. Future research is therefore required to examine the extent to which other plant-beneficial organisms may affect fungi/bacteria ratio.

Amendment with *Bacillus* bacteria further affected protist community structures (Fig. 2), with protist Bray-Curtis distance increasing ($P < 0.05$, Tukey's test) in comparison to control over time (Fig. 2b). The larger effects of *Bacillus* in comparison to the other three introduced microorganisms may be attributed to the antibiotic

compounds produced by *Bacillus amyloliquefaciens*. This strain, which produces the antibiotics bacillomycin D and difficidin, has been shown to inhibit a range of soil-borne pathogens (Xu et al., 2014, 2013). When examining taxonomic shifts within the protist community, we found that Rhizaria (mostly composed of Cercozoa, see Table S2 for details) was lower ($P < 0.05$, Tukey's test) in the *Bacillus* treatment as compared to the *Ralstonia* treatment after one week (Fig. S4g), and Amoebozoa decreased over time in the *Bacillus* treatment (Fig. S4b). Both Rhizaria and Amoebozoa are numerically dominant protist groups in soils (Fig. S4) and largely consist of phagotrophic protists (Geisen et al., 2015; Grossmann et al., 2016), predominantly bacterivores (Table S2). In addition, the *Bacillus* treatment increased ($P < 0.05$, Tukey's test) the proportion of Macrobia (mostly phototrophic Cryptophyta) within 4 weeks (Fig. S4e and Table S2), suggesting that newly available habitat niches (spatial niches) resulting from a relative decrease of phagotrophs can be filled by functionally different protists, such as phototrophs. Thus, inoculated *Bacillus* bacteria may induce bottom-up changes in higher trophic level protists, which can critically affect the soil

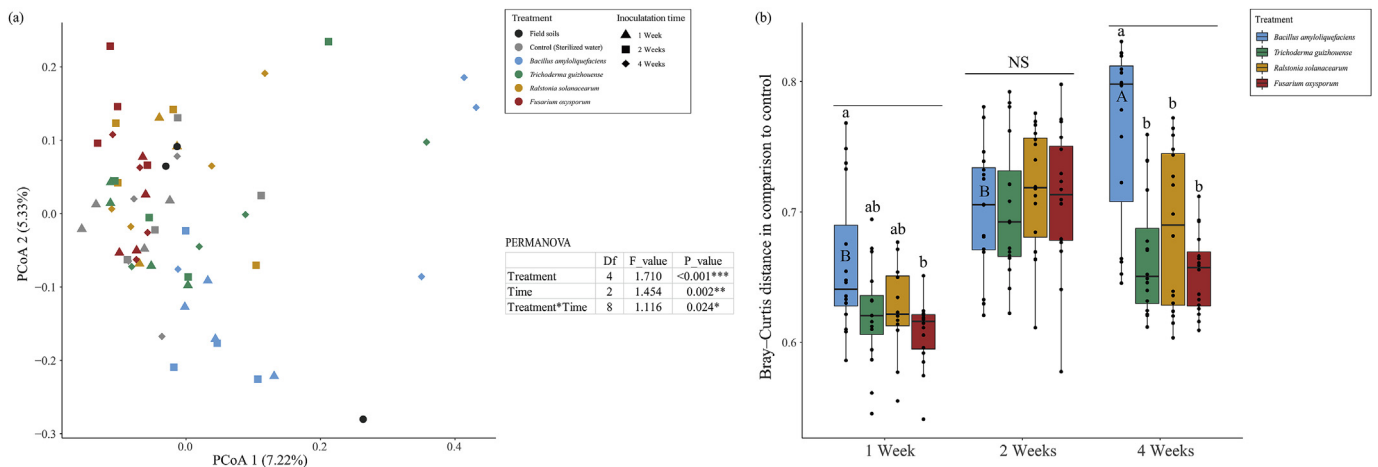


Fig. 2. Protist community structures based on Bray–Curtis distances as a function of the different microbial inoculation treatments (a), and Bray–Curtis distances of protist communities between the inoculated microorganisms and the control treatment (inoculated with the sterilized water) (b). Inoculation consisted of two plant-beneficial microbes (bacterial *Bacillus amyloliquefaciens* and fungal *Trichoderma guizhouense*) and two plant pathogenic microbes (bacterial *Ralstonia solanacearum* and fungal *Fusarium oxysporum*). Sterilized water as control. Different capital letters inside the bars indicate a significant difference between the different time points for each treatment and different lowercase letters above the bars indicate a significant difference between the treatments for each time point at the 0.05 probability level according to Tukey's post-hoc tests. NS = not significant. PERMANOVA means Permutational multivariate analysis of variance test.

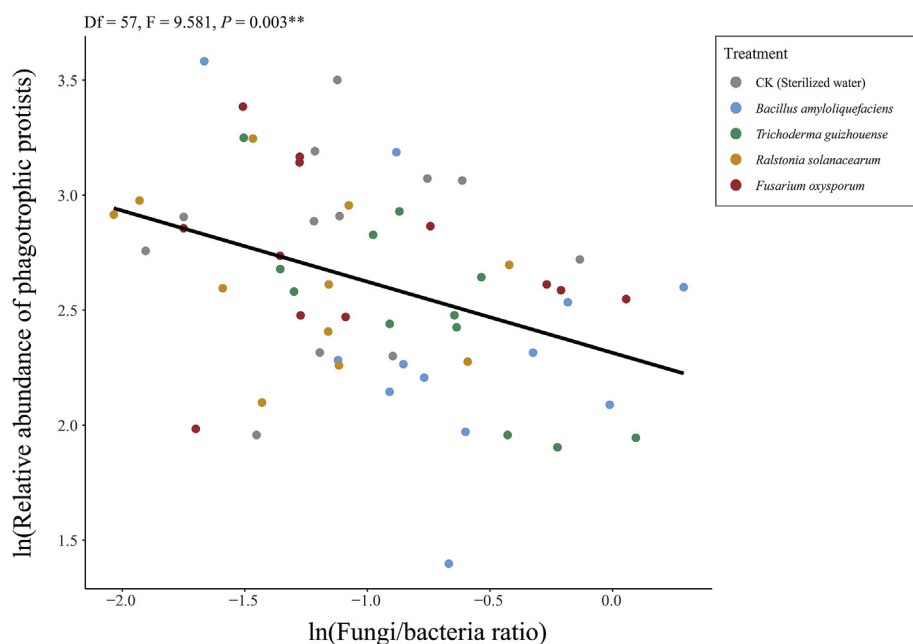


Fig. 3. The relationship between the fungi/bacteria ratio and the relative abundance of phagotrophic protists in relation to total protist reads. Inoculation consisted of two plant-beneficial microbes (bacterial *Bacillus amyloliquefaciens* and fungal *Trichoderma guizhouense*) and two plant pathogenic microbes (bacterial *Ralstonia solanacearum* and fungal *Fusarium oxysporum*). Sterilized water as control. The black solid line shows the significantly ($P < 0.01^{**}$; $R^2 = 0.14$) negative relationship between the fungi/bacteria ratio and the relative abundance of phagotrophic protists across all the treatments. “ln” denotes the natural logarithm.

microbiome composition and associated functions (Schuldt et al., 2018; Soliveres et al., 2016). Furthermore, we found that *Colpoda* was positively ($P < 0.05$) correlated with bacterial abundance, and *Chlorella* was positively correlated ($P < 0.05$) with fungal abundance (Table S3), indicating that some protist taxa are linked with overall bacterial and fungal community properties. Overall, bacterial abundance, but not fungal abundance, positively ($P < 0.01$) correlated with protist Shannon diversity (Fig. S5). This provides additional evidence for a tight link of protist and bacterial communities through predator-prey interactions (Geisen et al., 2018), pointing to the importance of top-down control of protists on bacterial communities. This suggestion is further strengthened by the fact that most protists were putatively identified as phagotrophic consumers, particularly bacterivores (Table S2). We further found that the fungi/bacteria ratio was negatively ($F = 9.581$, $P = 0.003^{**}$) correlated with the relative abundance of phagotrophic protists (Fig. 3). The possible underlying reason for this observed effect is that the antibiotics-producing *Bacillus* increased fungi/bacteria ratio by the decrease of bacterial abundance (although not significant) leading to reductions of bacterivorous protists – the dominant phagotrophic protists in soils (Geisen, 2016; Murase, 2017). Together, we found that changes in protist communities were related to the addition of the inoculated bacteria and fungi and correlated with fungi/bacteria ratio.

Understanding the interactions between soil bacteria and fungi with higher trophic level protists has recently gained increasing scientific attention (Gao et al., 2018; Hassani et al., 2018; Zhao et al., 2019). Our results reveal that inoculation with the plant-beneficial *Bacillus amyloliquefaciens* strain increased the fungi/bacteria while impacting the structure of the protist community, with a pronounced effect on phagotrophic protists over time. We thus highlight that the tight interactions within soil microbiomes, such as bottom-up bacterial-driven effects on higher trophic level protists, can be altered through microbial amendments. In sum, microbial amendments could therefore serve as leverage for targeted, bottom-up driven, modulations of soil microbiomes and entire food webs that might open new perspectives in future soil management.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2019.05.025>.

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