


Rhizosphere shapes the associations between protistan predators and bacteria within microbiomes through the deterministic selection on bacterial communities

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Abstract

The assembly of bacterial communities in the rhizosphere is well-documented and plays a crucial role in supporting plant performance. However, we have limited knowledge of how plant rhizosphere determines the assembly of protistan predators and whether the potential associations between protistan predators and bacterial communities shift due to rhizosphere selection. To address this, we examined bacterial and protistan taxa from 443 agricultural soil samples including bulk and rhizosphere soils. Our results presented distinct patterns of bacteria and protistan predators in rhizosphere microbiome assembly. Community assembly of protistan predators was determined by a stochastic process in the rhizosphere and the diversity of protistan predators was reduced in the rhizosphere compared to bulk soils, these may be attributed to the indirect impacts from the altered bacterial communities that showed deterministic process assembly in the rhizosphere. Interestingly, we observed that the plant rhizosphere facilitates more close interrelationships between protistan predators and bacterial communities, which might promote a healthy rhizosphere microbial community for plant growth. Overall, our findings indicate that the potential predator–prey relationships within the microbiome, mediated by plant rhizosphere, might contribute to plant performance in agricultural ecosystems.

INTRODUCTION

The rhizosphere microbiome plays an important role in supporting plant growth (Berendsen et al., 2012). Bacterial community assembly in the rhizosphere, determined by the genetic basis and root exudates of host plants (Oyserman et al., 2022; Zhalnina et al., 2018), has also been extensively studied in various ecosystems, including grassland (Ning et al., 2020), farmland (Jiao et al., 2020) and desert (Marasco et al., 2018). The complex interactions within the microbiome in the rhizosphere are crucial for bacterial assembly (Rossmann

et al., 2020; Zhang & Lueders, 2017), with predator–prey interactions being one of the most important factors (Thakur & Geisen, 2019). Predatory protists (traditionally termed protozoa) are primary consumers of bacterial communities in rhizosphere soils (Gao et al., 2019; Jousset et al., 2008). They can also feed on fungi, algae, and even nematodes (Geisen et al., 2018), thus serving as the key determinants of microbial compositions and functions. However, few studies explored the microbial assembly of protistan predators in plant rhizosphere (Ceja-Navarro et al., 2021), with even the potential interrelationships between protistan predators and bacterial prey within the rhizosphere microbiome being largely unknown. Community assembly involves both

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deterministic and stochastic processes (Stegen et al., 2012). Investigating the contributions of deterministic and stochastic processes to microbial community assembly might help to elucidate the ecological strategies between microbiome predators and bacterial prey (Thakur & Geisen, 2019). Such research for microbial community assembly processes widely depends on amplicon sequencing data and phylogenetic analyses, with the two main approaches being the β -nearest taxon index (β NTI) (Dini-Andreote et al., 2015; Stegen et al., 2012) and normalized stochasticity ratio (NST) (Jiao et al., 2020; Ning et al., 2019).

In this study, we propose the hypothesis that the assembly of plant rhizosphere bacterial communities might be driven by deterministic processes and further influence the associations between protistan predators and bacteria. Here, we focused on 443 agricultural soils including bulk soils and rhizosphere soil samples with amplicon sequencing-based datasets of bacterial and protistan communities (Xiong et al., 2021). We aim to examine the richness and relative abundance of the main bacterial and protistan taxonomic/functional groups, to compare community assembly processes of bacteria and protistan predators through the β -nearest taxon index (β NTI), and to explore the potential interrelationships between bacteria and protistan predators in bulk and rhizosphere soils.

EXPERIMENTAL PROCEDURES

Dataset collection for the bacterial (via 16S rRNA genes) and protistan (via 18S rRNA genes) communities was performed in our previous study (Xiong et al., 2021). In short, this dataset was obtained through literature screening by collecting bacterial and protistan amplicon datasets from Web of Science (<https://webofknowledge.com/>) and Google Scholar (<https://scholar.google.com/>). The V4 region of the 16S rRNA gene with the primer sets: 520F and 802R was used to generate bacteria, and the V4 region of the 18S rRNA gene with the primer sets: 616*f and TAReukREV3 was used to analyse eukaryotic community. To acquire the protistan Amplicon Sequence Variants (ASVs) table, we eliminated sequences affiliated with Rhodophyta, Streptophyta, Metazoa, Fungi, unclassified Opisthokonta and other unknown taxa from the eukaryotic community. Furthermore, we categorized the protistan ASVs into different functional groups based on their nutrient-uptake modes (Bjorbækmo et al., 2020; Xiong et al., 2020) mainly including predators, phototrophs, parasites and plant pathogens. In this study, we selected soil samples from the agricultural ecosystems including 323 bulk soil samples and 120 rhizosphere soil samples of different crops (Xiong et al., 2021). We used the Wilcoxon test in R (version 4.2.2) to compare the differences in the richness of bacteria, entire

protists, and protistan predators as well as the relative abundance of the main (with average relative abundance >1%) bacterial and protistan taxonomic/functional groups between bulk soils and rhizosphere soils, we used STAMP software package (Parks et al., 2014) to compare the differences of the main bacterial and protistan genera between bulk soils and rhizosphere soils. We calculated microbial community assembly processes by the β -nearest taxon index (β NTI) (Dini-Andreote et al., 2015; Stegen et al., 2012) with the 'bNTI.big' function through the 'iCAMP' package in R (Ning et al., 2020). The absolute value of β NTI >2 denotes the dominance of deterministic processes, with β NTI <-2 or >+2 indicating homogeneous or heterogeneous selection respectively (Dini-Andreote et al., 2015), while the absolute value of β NTI <2 represents stochastic community assembly. Only the abundant bacterial and predatory protists ASVs with relative abundance over 0.1% were selected for the β NTI calculation. We also used the 'NST' package in R to calculate the taxonomic normalized stochasticity ratio (tNST) with the default setting to quantify the contributions of deterministic and stochastic processes to microbial community assembly (Ning et al., 2019). We examined the linear correlations between protistan predators (including both the richness and relative abundance parameters) and bacterial richness by the 'lm' function in R. We calculated the relative importance of the abundant bacterial genera predicting the richness and relative abundance of protistan predators through the 'relaimpo' package in R (all data was standardized by 'scale' function) (Groemping, 2006). We tested the Spearman's rank correlations between the abundant bacterial genera and the abundant protistan genera, the p -values were adjusted with the false discovery rate method.

RESULTS AND DISCUSSION

Our results showed that bacterial diversity of richness in plant rhizosphere soils was significantly lower (Figure 1A; $p < 0.001$, Wilcoxon test) compared to bulk soils, supporting that host plants recruit a subset of microbial communities from bulk soil reservoir (Shi et al., 2015; Zhalnina et al., 2018). However, the richness of the entire protists did not differ (Figure 1B; $p = 0.750$, Wilcoxon test) between bulk and rhizosphere soils, indicating plant rhizosphere has little selecting effects on the diversity of entire protists. By focusing on protistan predators, we found that the richness of protistan predators was also lower (Figure 1C; $p = 0.026$) in the rhizosphere compared to bulk soils. The richness of the entire protists/bacteria ratio (Figure S1a) and protistan predators/bacteria ratio (Figure S1b) were not significantly different between bulk and rhizosphere soils. Furthermore, our findings

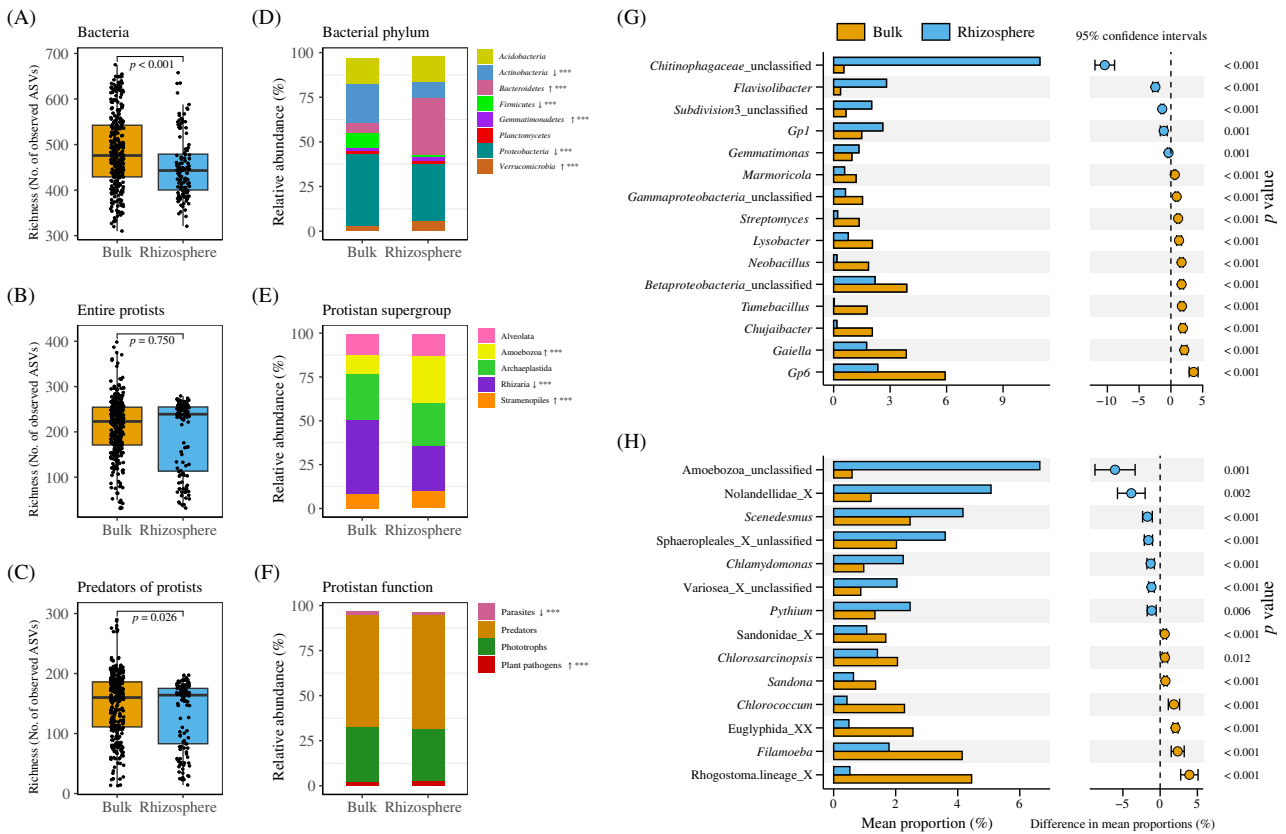


FIGURE 1 Richness and the main community composition of bacteria and protists in bulk and rhizosphere soils. The richness (number of observed ASVs) of bacteria in bulk and rhizosphere soils (A); the richness of entire protists in bulk and rhizosphere soils (B); the richness of protistan predators in bulk and rhizosphere soils (C); the main (with average relative abundance >1%) bacterial phyla in bulk and rhizosphere soils (D); the main protistan taxonomic groups in bulk and rhizosphere soils (E); the main protistan functional groups in bulk and rhizosphere soils (F). The differences of the main bacterial genera in bulk and rhizosphere soils with STAMP analysis (G); the differences of the main protistan genera in bulk and rhizosphere soils with STAMP analysis (H). In panels (D), (E), and (F), * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ by the Wilcoxon test; the '↑' indicates the enrichment, and '↓' indicates the deletion of bacterial and protistan taxonomic/functional groups in the rhizosphere compared with bulk soils. In panels (G) and (H), only the significant (Wilcoxon test, $p < 0.050$) differential bacterial and protistan genera between bulk and rhizosphere soils were shown.

showed disparate patterns of rhizosphere microbial assembly in bacteria and protistan predators. Bacterial community assembly was driven by a deterministic process in the rhizosphere (absolute value of β NTI >2 with 58.82% heterogeneous selection, Figure 2A), likely to be directly mediated by root metabolites (Wen et al., 2022) and determined by the increasing spatial and temporal heterogeneities in the rhizosphere soils during plant growth (Hinsinger et al., 2005). In contrast, community assembly of protistan predators was determined by a stochastic process in both bulk and rhizosphere soils (absolute value of β NTI <2), but the relative importance of heterogeneous selection increased from 24.62% in bulk soils to 37.51% in rhizosphere soils (Figure 2B). The contributions of deterministic and stochastic processes to community assembly of bacterial communities and protistan predators in bulk and rhizosphere soils were also validated by tNST analysis (Figure S4). These results suggest that the distinct patterns of diversity and community assembly

of bacteria and protistan predators in the plant rhizosphere may be attributed to different rhizosphere microbiome selection strategies. The indirect rhizosphere selection effects on microbiome predators might be explained by the rhizosphere-modified bacterial communities, as the majority of the protistan predators do not directly utilize root nutrients but serve as the main consumers in soil food webs (Crotty et al., 2012; Geisen et al., 2018), while most bacteria can directly utilize carbon sources derived from root exudates (Preece & Peñuelas, 2020) that showing a deterministic process assembly in the rhizosphere.

The rhizosphere significantly enriched the relative abundance of certain bacterial phyla, including *Bacteroidetes*, *Gemmatimonadetes* and *Verrucomicrobia* (Figure 1D; $p < 0.001$, Wilcoxon test). Especially, the average proportion of *Bacteroidetes* in bulk soils increased substantially from 5.74% to 32.30% in the rhizosphere. The results are consistent with previous meta-analyses of datasets comparing bacterial

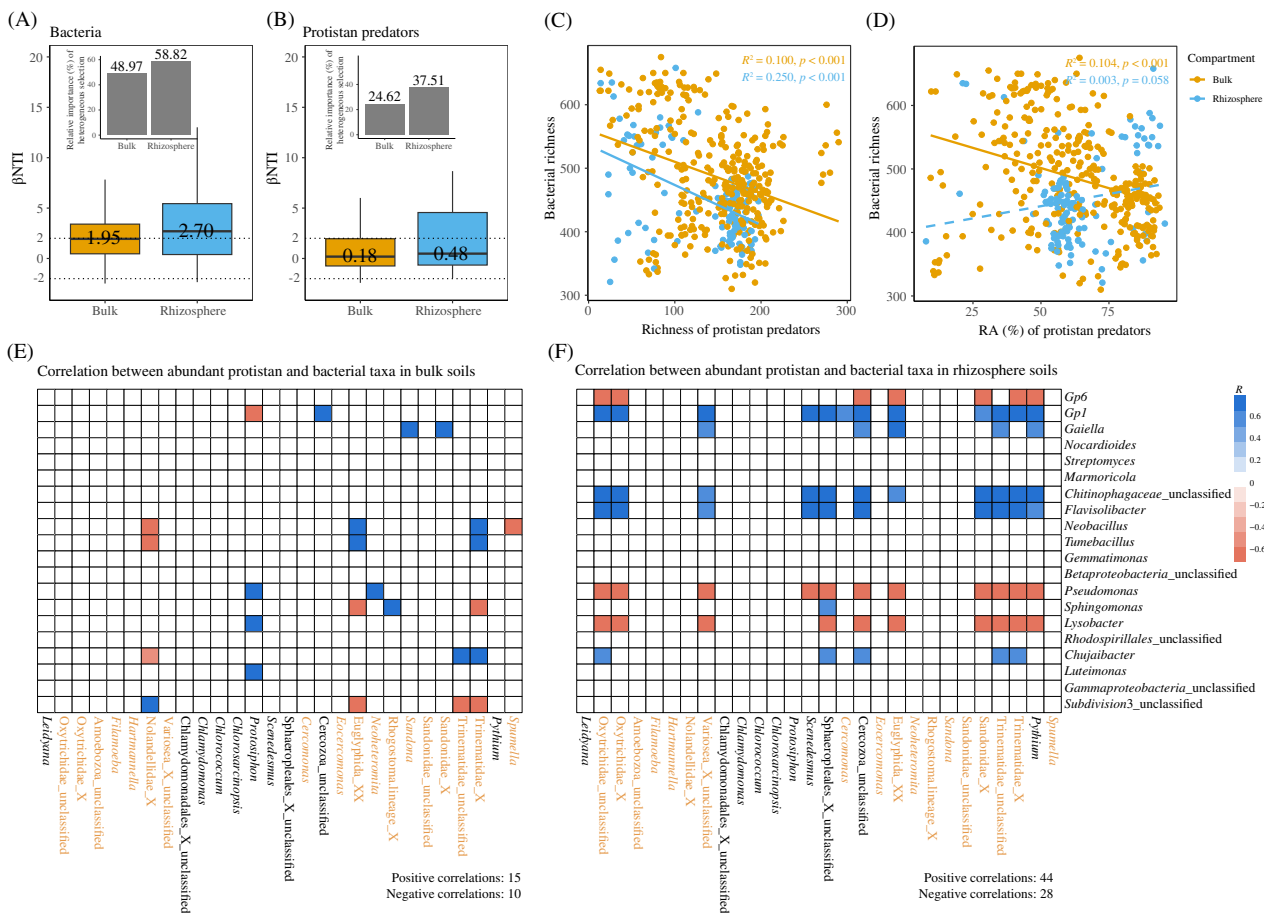


FIGURE 2 Community assembly processes of bacteria and protistan predators as well as their potential interrelationships in bulk and rhizosphere soils. The β -nearest taxon index (β NTI) and relative importance of the heterogeneous selection of bacteria in bulk and rhizosphere soils (A); the β -nearest taxon index (β NTI) and relative importance of the heterogeneous selection of protistan predators in bulk and rhizosphere soils (B). The relationship between the richness of bacteria and richness of protistan predators in bulk and rhizosphere soils (C); the relationship between the richness of bacteria and the relative abundance of protistan predators in bulk and rhizosphere soils (D). Spearman's rank correlations between abundant bacterial genera and protistan genera in bulk soils (E); Spearman's rank correlations between abundant bacterial genera and protistan genera in rhizosphere soils (F). In the panel of (A) and (B), the absolute value of β NTI > 2 denotes the dominance of deterministic processes (β NTI < -2 or $> +2$ indicating homogeneous or heterogeneous selection), while the absolute value of β NTI < 2 represents stochastic community assembly. In panels (C) and (D), the solid line indicates a significant linear correlation, while the dashed line indicates a non-significant linear correlation. In panels (E) and (F), only Spearman's rank correlation with the absolute value of $R > 0.6$ and $p < 0.050$ were shown in the heatmap plot, and the predatory protistan taxa on the x-axis were colour labelled.

communities in bulk and rhizosphere soils (Ling et al., 2022) and with global investigations of the citrus rhizosphere microbiome (Xu et al., 2018) that show *Bacteroidetes* dominated in the rhizosphere. The enrichment of *Bacteroidetes* can be attributed to their copiotrophic lifestyles utilizing diverse carbon sources available in the rhizosphere (Ho et al., 2017). Furthermore, *Bacteroidetes* are known to play a crucial role in promoting plant growth by facilitating phosphorus mobilization in the rhizosphere (Lidbury et al., 2021) and by suppressing soil-borne pathogens (Carrion et al., 2019; Pérez-Jaramillo et al., 2018). Specifically, *Chitinophagaceae*_unclassified (an unclassified genus from *Chitinophagaceae*) and *Flavisolibacter* (a genus from *Chitinophagaceae*) from *Bacteroidetes* were found to be enriched (Figure 1G; $p < 0.001$, Wilcoxon test) in rhizosphere soils. The *Chitinophagaceae* in the root

endosphere has been shown to harbour chitinase genes (Carrion et al., 2019) that are important for fungal disease suppression and plant health.

We further found that the rhizosphere exhibited distinct compositions of protists when compared to bulk soils. Specifically, the relative abundance of protistan supergroups of Amoebozoa and Stramenopiles was enriched in the rhizosphere, while the Rhizaria was reduced in comparison with bulk soils (Figure 1E; $p < 0.001$, Wilcoxon test). At the protistan functional level, however, we did not find a significant difference in the relative abundance of the most abundant functional protists, namely protistan predators, between bulk and rhizosphere soils (Figure 1F; $p > 0.050$, Wilcoxon test). This may be because protistan predators have different feeding modes and consume a wide range of prey, including bacteria, fungi, algae, other

protists, and nematodes (Bjorbækmo et al., 2020; Geisen et al., 2016, 2018). Upon closer examination at the genus level, we observed that protistan taxa such as Amoebozoa_unclassified (unclassified genus from Amoebozoa) and Nolandellidae_X (predators from Amoebozoa) dominated in the rhizosphere (Figure 1H; $p < 0.010$, Wilcoxon test). In contrast, predatory *Filamoeba* from Amoebozoa and some predatory Cercozoa taxa of Rhogostoma.lineage_X and Euglyphida_XX were reduced in the rhizosphere (Figure 1H; $p < 0.001$, Wilcoxon test). These results suggest that the distribution of protistan predator between rhizosphere and bulk soils is likely affected by their feeding preferences, as even closely related protistan strains have different preferences for their bacterial prey (Glücksman et al., 2010) which are diverse in the rhizosphere. Furthermore, we found that plant pathogens of protists (such as *Pythium*) were enriched in the rhizosphere (Figure 1F, H; $p = 0.006$, Wilcoxon test). This supports the previous findings (Asiloglu et al., 2021; Ceja-Navarro et al., 2021) that *Pythium* is favoured by the root and rhizosphere environment, which can induce root rot disease in diverse economically important crops (Martin & Loper, 1999).

In addition to the comparison of bacterial communities and microbiome predators between bulk and rhizosphere soils, we further explored the potential interrelationships between bacterial communities and protistan predators in the two soil compartments. We found that the bacterial richness significantly and negatively correlated with the richness (Figure 2C; $R^2 = 0.100$, $p < 0.001$) and the relative abundance (Figure 2D; $R^2 = 0.104$, $p < 0.001$) of protistan predators in bulk soils, suggesting protistan predators are main consumers of bacteria in soils (Geisen et al., 2018). However, the significant correlation between the bacterial richness and the relative abundance of protistan predators was not observed in rhizosphere soils (Figure 2D; $R^2 = 0.003$, $p = 0.058$), where the explanation power of bacterial composition in predicting the relative abundance of protistan predators being not high (with the proportion of explained variance of 18.03%, Figure S2d). These results indicate complex multi-trophic interactions occurring within microbial communities in this dynamic plant hotspot of the rhizosphere (de Vries & Wallenstein, 2017; Rossmann et al., 2020). We found that *Gp1* from *Acidobacteria* enriched in the rhizosphere had a positive effect (35.64%) on predicting the richness of protistan predators (Figure S2b), while decreased *Lysobacter* had a negative effect (38.06%) in the rhizosphere soils (Figure S2b). When examining the robust correlations between the abundant bacterial and protistan genera in rhizosphere and bulk soils (Figure 2E, F; Spearman's rank correlation; absolute value of $R > 0.6$, $p < 0.050$), we found more significant (including positive and negative) links between bacteria and protists in rhizosphere than bulk soils (although the fragile connections

between bacteria and protists still being higher in bulk soils compared to rhizosphere soils, Figure S3a, b). Our findings agree with previous studies that rhizosphere soils select more complex bacterial communities (Shi et al., 2016) and highly connected protistan networks (Ceja-Navarro et al., 2021), these findings further highlight that plant rhizosphere promotes close interrelationships between protistan predators and bacterial communities. Finally, we detected negative correlations (Figure 2F; Spearman's rank correlation; $R < -0.6$, $p < 0.050$) between two potential antagonistic bacteria (i.e., *Pseudomonas* and *Lysobacter*) and plant pathogen of *Pythium* in the rhizosphere soils, which may support healthy growth of plants (Folman et al., 2004; Postma & Nijhuis, 2019).

We acknowledge that the intricate predator-prey relationships between predatory protists and their microbial prey cannot be adequately drawn from exclusive amplicon sequencing investigations. Instead, our study focuses on the complexity of the microbiome, specifically on the integration of protistan predators and bacterial communities in the rhizosphere compared to bulk soils. Future works with comprehensive approaches including stable isotope analysis tracking the transfer of carbon and nitrogen sources between protistan predators and microbial preys (Frias-Lopez et al., 2009), RNA-based metatranscriptomics exploring active protistan predators and microbial preys in situ (Singer et al., 2017), metagenomics capturing the functional genes involved predator-prey interactions within microbiome (Geisen & Bonkowski, 2018; Jousset et al., 2010; Lind & Pollard, 2021) and experiment confirmation with the traditional cultivation-based method are needed to unravel the complex trophic interactions between predatory protists and their microbial preys in the terrestrial ecosystems.

In conclusion, our findings demonstrate contrasting patterns of bacteria and protistan predators in rhizosphere microbiome assembly. Plant rhizosphere reveals stronger selection effects on the bacterial communities with reduced richness and enriched potential plant-beneficial microbes from *Bacteroidetes* in a deterministic process. Although the entire protistan richness was not affected by the plants, the richness of predatory protists was reduced in the rhizosphere in a stochastic process, possibly due to indirect effects from bacterial communities. Furthermore, the plant rhizosphere promotes closer interrelationships between the microbiome predators and bacterial communities, which may contribute to healthy rhizosphere microbial communities and thereby enhance plant performance in agriculture.

AUTHOR CONTRIBUTIONS

Yang Yue: Data curation (equal); formal analysis (equal); methodology (equal); writing – original draft (equal). **Chen Liu:** Data curation (equal); formal analysis (equal); methodology (equal); writing – original draft

(equal). **Boting Xu**: Writing – review and editing (equal). **Yijin Wang**: Writing – review and editing (equal). **Qihui Lv**: Writing – review and editing (equal). **Zeyuan Zhou**: Writing – review and editing (equal). **Rong Li**: Writing – review and editing (equal). **George A. Kowalchuk**: Supervision (equal); writing – review and editing (equal). **Alexandre Jousset**: Resources (equal); supervision (equal); writing – review and editing (equal). **Qirong Shen**: Supervision (equal); writing – review and editing (equal). **Wu Xiong**: Data curation (equal); methodology (equal); resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the figshare repository: <https://doi.org/10.6084/m9.figshare.24257224.v2>.

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SUPPORTING INFORMATION

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

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