Plant Soil (2023) 485:333–347
https://doi.org/10.1007/s11104-022-05829-z

RESEARCH ARTICLE

Phagotrophic protist-mediated control of *Polymyxa graminis* in the wheat rhizosphere

Chuanfa Wu · Chaonan Ge · Fangyan Wang · Haoqing Zhang · Zhenke Zhu · Didier Lesueur · Jian Yang · Jianping Chen · Tida Ge

Received: 3 August 2022 / Accepted: 30 November 2022 / Published online: 5 December 2022

Abstract

Purpose Uncovering potential biocontrol agents that suppress soil-borne pathogens is an important step toward developing sustainable management strategies for disease control and to maintain plant health. Plant cultivars influence rhizosphere microorganism-mediated soil-borne disease control. However, the disease-resistance mechanisms and microbial taxa involved in the control of soil-borne mosaic virus are largely unknown.

Methods We designed a field experiment on wheat cultivars for virus-resistance identification and conducted metagenomic analysis to determine the potential mechanisms used by rhizosphere microbial communities that affect the density of the mosaic virus vector-*Polymyxa graminis*, and to identify potential microbes that inhibit virus transmission.

Results We found high *P. graminis* abundance and microbial diversity in the susceptible wheat cultivar rhizosphere. The relative abundance of indicative

Responsible Editor: Sven Marhan.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s11104-022-05829-z.

C. Wu · F. Wang · H. Zhang · Z. Zhu · J. Yang · J. Chen (✉) · T. Ge (✉)
State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Key Laboratory of Biotechnology in Plant Protection of Ministry of Agriculture and Zhejiang Province, Institute of Plant Virology, Ningbo University, 818 Fenghua Road, Jiangbei District, 315211 Ningbo, China
e-mail: jianpingchen@nbu.edu.cn

T. Ge
e-mail: getida@nbu.edu.cn

C. Ge
Ninghai General Station of Agricultural Technical Extension, Ninghai, Zhejiang 315600, China

D. Lesueur
Centre de Coopération Internationale en Recherche Agronomique pour le Développent (CIRAD), UMR Eco&Sols, Hanoi, Vietnam

D. Lesueur
Eco&Sols, University of Montpellier (UMR), CIRAD, Institut National de la Recherche Agronomique (INRAE), Institut de Recherche pour le Développent (IRD), Montpellier SupAgro, Montpellier 34060, France

D. Lesueur
Common Microbial Biotechnology Platform (CMBP), Alliance of Bioversity International and International Center for Tropical Agriculture (CIAT), Asia hub, Hanoi, Vietnam

D. Lesueur
School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment, Deakin University, 3125 Melbourne, VIC, Australia

D. Lesueur
Chinese Academy of Tropical Agricultural Sciences, Rubber Research Institute, Haikou, China

phagotrophs showed a strong negative correlation to *P. graminis* abundance during disease onset, indicating that predator–prey interactions influenced *P. graminis* activity. Moreover, we found strong and negative associations between the relative abundance of key ecological cluster, hub phagotrophic species and *P. graminis* abundance in multitrophic ecological networks. A structural equation model analyses indicated that phagotrophic protists were the main predictors of *P. graminis* abundance upon disease onset.
Conclusion Our results demonstrate the important role of phagotrophic protists as top-down controllers for plant defense against pathogens. Our findings highlight the complexity of rhizosphere networks, reflecting the co-occurrence patterns of multi-trophic level microbes in virtual networks and strengthening the association between soil microbial diversity and plant health.

Keywords Phagotrophic protists · Rhizosphere soil · *Polymyxa graminis* · Co-occurrence networks · Plant health

Introduction

Polymyxa graminis is a soil-borne protist of the order *Plasmodiophorida* that transmits over ten distinct plant viruses (Adams and Jacquier 1994). Wheat crops in China are susceptible to several soil-borne pathogenic viruses, such as Chinese wheat yellow mosaic virus (CWMV) (Diao et al. 1999), and wheat yellow mosaic virus (WYMV) (Ye et al. 1999), which severely affect grain yield and quality, resulting in 70–80% yield loss during severe epidemics (Guo et al. 2019; Xu et al. 2018). The viral vector, *P. graminis* exhibits strong resistance to environmental stress (including low temperature and drought), thereby maintaining infectivity for over ten years (Adams 1991; Adams and Jacquier 1994). The protist vector relies on water for transportation and infection of hosts (Adams 1991). Conventional tillage and watering can significantly accelerate virus transmission and host infection by *P. graminis*, thereby posing a long-term threat for wheat production (Guo et al. 2019).

Chemical agents such as pesticides and fungicides have been mostly ineffective in suppressing the spread of *P. graminis* which eventually leads to the spread of wheat mosaic disease (Guo et al. 2019). Presently, cultivation of disease-resistant wheat varieties is the most effective strategy to control wheat mosaic disease (Cui et al. 2017; Wu et al. 2017). The resistance toward WYMV-infection in the resistant wheat cultivars is exhibited by the roots throughout the infected culture system (Liu et al. 2016). The secretion of abscisic acid by the root caps into the plant rhizosphere could be attributed to the resistance of plants to wheat mosaic virus (He et al. 2021).

The rhizosphere is the first line of defense against pathogenic infection, and it is important for the plants to be successfully colonized by beneficial microorganisms to keep the pathogen populations under check (Mendes et al. 2013). The rhizosphere is colonized by a complex and diverse array of microorganisms, including protists, bacteria, nematodes, and fungi. Plants rely on some of these microbial taxa for specific functions, including maintenance of homeostasis during growth. Upon pathogen infection, plants alter the production of root exudates to recruit beneficial rhizosphere microbial communities that in turn suppress pathogenic infections (Bakker et al. 2013; Fu et al. 2020; Liu et al. 2021; Wang et al. 2022). Therefore, a comparative characterization of the rhizosphere microbes between healthy and infected plants would help identify beneficial microbes that maintain and enhance plant health (Bongiorno 2020; Gao et al. 2019). Research on rhizosphere microbiome assembly has adopted a bottom-up perspective to determine how resources such as root metabolites influence bacteria or fungi to support plant growth and protect against stresses (Duran et al. 2018; Manici and Caputo 2009; Sanguin et al. 2009; Wang et al. 2020; Wen et al. 2021). Till date, the role of protists as drivers of microbial community structure has been largely overlooked (Gao et al. 2019); few studies have elucidated the key microbial taxa and their potential interactions in the plant rhizosphere (Hassani et al. 2018). Therefore, a complete microbial analysis is required to determine the role of the rhizosphere microbiota and their multitrophic interactions to benefit plant health.

Protists are highly diverse and abundant soil eukaryotes and have long been recognized as sensitive biomarkers of soil health, which influence rhizosphere bacterial and fungal communities through nutrient-predator-prey interactions (Fournier et al. 2022; Kramer et al. 2016). However, protist communities have rarely been linked to plant health (Gao et al. 2019). Protists, especially phagotrophic protists such as *Glissomonadida* and Cercomonadida influence

agroecosystems through diverse functions (Sapp et al. 2018); *euglyphids* and *Rhogostomidae* regulate paddy soil fungal community structure through strong top-down control (Huang et al. 2021). Furthermore, phagotrophic protists that ingest microorganisms influence plant health by inhibiting pathogen reproduction; they also influence bacterial function by triggering the activation of pathogen-suppressing secondary metabolic genes during plant growth (Xiong et al. 2020). Additionally, protists improve crop yield by interacting with plant-beneficial microbes (Guo et al. 2021). On the other hand, interactions with other protists, including pathogenic species such as *P. graminis*, have rarely been investigated. Given the significant influence of protists, understanding the relationship between rhizosphere protists and the viral vector *P. graminis*, as well as those among rhizosphere microbes, is critical for revealing the mechanisms underlying soil-borne disease outbreaks and harnessing native microbes for soil-borne disease control.

To investigate key microbial taxa that may inhibit the spread of mosaic virus disease, two winter wheat cultivars (one resistant and one susceptible to wheat mosaic disease) were cultivated in a field ecosystem. The experimental field has been used for studies on wheat mosaic disease over a long period of time, and the soil was naturally infected with *P. graminis*. We investigated the composition of the rhizosphere microbiome (protists, bacteria, and fungi) and the abundance of *P. graminis*, to identify potential microbial interactions in the rhizosphere of both resistant and susceptible wheat cultivars. The objectives of our study were to (1) identify changes in microbial diversity and potential interactions following disease development and (2) explore whether protists, especially phagocytic protists, can predict *P. graminis* density and plant health in a highly managed farmland ecosystem.

Materials and methods

Field site, plant material, and sampling

Since 2012, the field experiment comprised a conventional cropping system (nine-year rotation of summer maize and winter wheat) in Linyi, China (35°11′N, 118°38′E). The experimental soil was classified as arenic fluvisol according to the WRB soil classification system (ISSS, ISRIC 1998). The soil has a pH of 6.2, total nitrogen of 1.19 g/kg, and organic carbon content of 12.65 g/kg.

Winter wheat cultivars, For-susceptible wheat (FSW, Linmai 4) and For-resistant wheat (FRW, Jimai 22), were chosen based on their genetic resistance to soil-borne wheat virus pathogens. Seeds were provided by the Crop Research Institute, Shandong Academy of Agricultural Sciences, China. The field experimental design included ten blocks and two wheat cultivars. Each block consisted of two plots, each plot was 4 m long with 12 rows with a 0.2 m distance between two rows; neighboring plots were separated by 1.0 m. In October, the two cultivars were planted according to local farming practices. Before sowing, the soil was mechanically tilled to reduce soil physicochemical differences between plots. Compound fertilizer was used as a basal fertilizer at 750 kg ha^{-1} (nitrogen: phosphorus pentoxide: potassium oxide [N: P2O5:K2O] = 14: 7: 9). Manual weeding was performed periodically and no herbicides or insecticides were used throughout the growth period of wheat.

The degree of soil-borne disease progression was determined based on field observations of the wheat streak mosaic in the infected leaves as described by Kojima et al. (2015). In March 2020, wheat mosaic virus disease occurred in the susceptible wheat cultivar (returning green period), while resistant varieties did not develop the disease. Rhizosphere soil (defined as those tightly attached to the roots) samples were collected from each of the cultivars by shaking the roots; the corresponding root samples were clipped and immediately placed into ice bags. Five rhizosphere soil samples from each plot were randomly selected and were pooled into one sample. A total of 20 rhizosphere soil samples were obtained (2 wheat cultivars × 10 blocks). The samples to be used for microbial analysis were stored at −80 °C. Soil physicochemical characteristics (Table S1) were determined as described (Wu et al. 2021a) and are outlined in "Method S1".

DNA extraction, quantitative PCR analysis, and amplicon sequencing

Rhizosphere soil DNA was extracted using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany)

according to manufacturer's instructions. DNA concentration and quality were assessed both by 1.5% agarose (w/v) gel electrophoresis and spectrophotometry (NanoDrop Technologies, Wilmington, DE, USA). DNA concentration and quality (ratio of absorbance, A260: A280) were in the range of 15–65 ng/μL and 1.8–2.0, respectively.

The abundance of *P. graminis* was determined using real-time quantitative polymerase chain reaction (RT-qPCR) with specific primer sets, Pg.F2-F and Pg.R2-R (Xu et al. 2018). The primer sets used were: 515 F/806R, targeting the V4 region of bacterial 16 S rRNA gene (Walters et al. 2016), ITS5-1737 F/ITS2-2043R, targeting fungal internal transcribed spacer 1 regions (Jiao et al. 2018)d TAReuk454FWD1/R-TAReukREV3, targeting the V4 region of eukaryotic 18 S rRNA gene (Stoeck et al. 2010). PCR amplification conditions and primer sequences are given in Table S1. Sequencing was performed at Novogene Biopharm Technology Co., Ltd. (Tianjing, China) using Illumina MiSeq PE250 (Illumina, San Diego, CA, USA) with a paired-end protocol. Raw DNA sequence data have been submitted to the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) Sequence Read Archive (SRA) database under the BioProject accession number PRJNA825734. Details of quantitative PCR analysis and amplicon sequencing are described in the supplementary material "Method S1."

Bioinformatics analysis

The raw sequences were merged and filtered using barcodes from Quantitative Insights into Microbial Ecology (QIIME2) (Bolyen et al. 2019) as per previously established protocols (Wu et al. 2021a). Briefly, raw sequence data were demultiplexed and quality filtered using the q2-demux plugin followed by denoising with DADA2 (Callahan et al. 2016) (via q2-dada2) to remove the errors and chimeric sequences and identify all observed amplicon sequence variants (ASVs). Next, all ASVs were aligned with MAFFT (Katoh and Standley 2013) (via q2-alignment) and used to construct a phylogenetic tree with fasttree2 (Price et al. 2010) (via q2-phylogeny). Bacterial, fungal, and protistan representative sequences were analyzed using the q2-feature-classifier (Bokulich et al. 2018) and the classify-sklearn naive Bayes taxonomy classifier (Pedregosa et al. 2011) trained and classified based on the silva132 database (McDonald et al. 2012), UNITE (v8.0, https://unite.ut.ee/) database, and Protist Ribosomal Reference (PR2) database (Guillou et al. 2013), respectively. To obtain an equivalent sequencing depth for later analyses, all samples were rarefied to 56,271 reads in bacteria, 52,863 reads in fungi, and 14,383 reads in protists using the "Vegan" package in R v.4.0.2 (Dixon 2003). We further assigned protistan ASVs to different functional groups, including phagotrophs, phototrophs, parasites, plant pathogens, saprotrophs, mixotrophs, and unknown protists, according to their nutritional mode (Dumack et al. 2020; Xiong et al. 2019).

Statistical analysis

Differences in physicochemical properties and *P. graminis* abundance in the FSW and FRW rhizosphere soils were assessed via a nonparametric *t*-test using R software. The alpha diversity indices (Richness and Shannon) of microbial communities were measured using the "Vegan" package and further analyzed for significance using the nonparametric *t*-test in R software. Beta diversity was visualized with Bray–Curtis similarity matrices using principal coordinate analysis (PCoA); analysis of similarities (ANOSIM) was applied to assess the differences in microbial community structures between FSW and FRW rhizosphere soils using the "anosim" function in R. We selected the microbial community (including richness, Shannon index, and structure (PCoA1)) index of bacteria, fungi, and protists to reduce dimensionality by factor analysis (KMO > 0.8, Bartlett test $p < 0.05$) in SPSS 22.0. We extracted three variables as microbial predictors and used multiple regression by lineal models in R to calculate the significance of the correlation between microbial predictors and *P. graminis* abundance (all data was standardized by "scale" function in R). Multiple regression analyses were performed using "relaimpo" package in R to infer the relative importance of microbial predictors on *P. graminis* abundance (Groemping 2006). Within-cultivar community dissimilarity of microbial populations (bacteria, fungi, and protist) for each cultivar was calculated using mean Bray-Curtis distance of all pairwise comparisons within a cultivar; the relationship between community dissimilarity with *P. graminis* abundance was calculated using linear correlation. Redundancy analysis was performed

to examine correlations between physicochemical properties and microbial communities (based on ASV level) using the "ggrepel" and "ggpubr" packages in R. A forward selection procedure was used to select significant variables. Linear discriminant analysis (LDA) effect size (LEfSe) was determined (Kruskal–Wallis test $p < 0.05$, logarithmic LDA score > 2.0) to identify microbial biomarkers for different wheat cultivars (Segata et al. 2011).

We then developed co-occurrence networks to infer the potential interactions between protistan, bacterial, and fungal taxa and identified ecological modules of strongly correlated taxa. The network was produced using a Spearman correlation matrix calculated with the "psych" package in R (Langfelder and Horvath 2012). Network nodes represented ASVs, and edges connecting different nodes corresponded to significant correlations between ASVs. To reduce false positive results, we adjusted all *p*-values for multiple correlations using Benjamini and Hochberg false discovery rate (FDR) (Benjamini and Hochberg 1995). Robust correlations with the Spearman correlation coefficients > 0.80 and FDR adjusted *p*-values < 0.01 were selected to construct the co-occurrence networks, which was visualized using Gephi 0.9.2 (Bastian et al. 2009). The algorithm of fast greedy modularity optimization was used to detect and isolate modules via directly optimizing the Modularity score (Deng et al. 2012). The nodes with high degree and closeness centrality values were identified as hub nodes in multitrophic networks (Agler et al. 2016; van der Heijden and Hartmann 2016). The relative abundance of each module was calculated by averaging the standard relative abundances (z-score) of all taxa under each module using "scale" function in R (Delgado-Baquerizo et al. 2018). The relationships between ecological modules, hub nodes, and *P. graminis* abundance were then tested using linear regression.

Structural equation modeling (SEM) was performed using Amos 20.0 to explore and quantify links between physicochemical soil properties, microbial community composition (including phagotrophic protists, bacteria, and fungi), and *P. graminis* abundance. All of the measured soil physicochemical property indices were reduced in dimensions by factor analysis (KMO > 0.8, Bartlett test $p < 0.05$) to the obtained physicochemical variable. Microbial community composition, such as alpha diversity (including richness and Shannon indices) and structure (PCoA1 value) indices of bacterial communities, were reduced in dimensions to form bacterial variables. All variables were standardized via Z transformation to improve normality using the "scale" function in R 4.0.2 (Zhao et al. 2019). The covariance matrix was fitted to the model using maximum likelihood estimation. The following parameter ranges were measured to ensure model fitting: chi-square ($P > 0.05$), goodness-of-fit index (GFI > 0.90), and root mean square error of approximation (RMSEA < 0.05) (Grace and Keeley 2006).

Results

Rhizosphere soil physiochemical properties and *polymyxa graminis* abundance

Comparative analysis showed that the rhizosphere soil of the FSW contained higher levels of phosphorus (AP), total phosphorus (TP), and soil organic carbon (SOC) than FRW plants ($p < 0.05$, Table S2), whereas the rhizosphere soil of the FRW plants contained higher total magnesium (TMg) and total iron (TFe) contents than FSW plants ($p < 0.05$, Table S2) upon disease onset. The rhizosphere soil pH value and other nutrient element contents did not differ significantly between the two cultivars.

RT-qPCR analysis showed that the abundance of *P. graminis* in the rhizosphere soil of the susceptible cultivar ranged from 6.60×10^6 to 2.72×10^7 during disease onset, and was significantly higher than that in the resistant cultivar (4.56×10^5 to 5.35×10^6) ($p = 2.097\text{e-}5$, Fig. 1a). However, Multiple linear regression analysis showed that none of the physiochemical soil properties correlated significantly with *P. graminis* abundance in the rhizosphere soil samples ($p > 0.1435$, Table S3).

The relationship between *Polymyxa graminis* abundance and microbial diversity in rhizosphere soil

Microbial diversity of the rhizosphere soil differed significantly between the resistant and susceptible cultivars ($p < 0.05$, Fig. S1). Rhizosphere alpha diversity (including bacteria, fungi, and protists) in FRW plants was lower than that in FSW plants ($p < 0.05$, Fig. S1a, b). After the disease outbreak, we detected significant differences in rhizosphere microbial communities

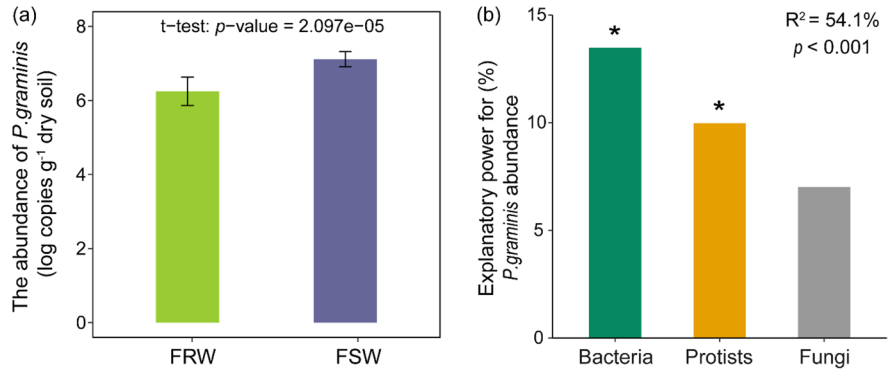

Fig. 1 *Polymyxa graminis* abundance in wheat rhizosphere soil from susceptible and resistant cultivars (**a**). Prediction of the *Polymyxa graminis* abundance by microbial parameters (**b**). FRW, For-resistant wheat; FSW, For-susceptible wheat. The *p* value indicates the significance between FRW and FSW based on the Student's *t* test. Bacteria, fungi, and protist includes alpha diversity indices (Shannon and Richness) and beta diversity (PCoA1), respectively. * $p < 0.05$

containing bacteria, fungi, and protists between the two wheat cultivars, with the strongest differences observed in the protistan community (ANOSIM test, protist: R = 0.4837, $p = 0.00021$; Fig. S1c). Furthermore, redundancy analysis revealed that the physicochemical parameters of SOC level exerted the strongest influence on the bacterial and protistan communities (Fig. S2a, c); NO_3^- content had the greatest influence on the fungal community in the wheat rhizosphere soil upon disease onset (Fig. S2b).

In this study, we found that microbial alpha diversity (including bacteria, fungi, and protist) was positively correlated with *P. graminis* abundance (Fig. S3a, b), whereas the dissimilarity in bacterial and protistan communities had a negative effect on *P. graminis* abundance (Fig. S3c). It is worth mentioning that the microbial diversity and community dissimilarity indexes of protists strongly correlated with *P. graminis* abundance (Fig, S3), indicating that *P. graminis* abundance was highly influenced by the protistan community. Further prediction analysis showed that bacterial and protistan diversity significantly predicted *P. graminis* abundance ($p < 0.001$) in all rhizosphere soil samples from both cultivars, accounting for 13.47% and 9.97% of the observed variations, respectively (Fig. 1b). In contrast, fungal diversity was not significantly associated with the abundance of *P. graminis* ($p > 0.05$; Fig. 1b).

The phagotrophic protist community was significantly different between the resistant and susceptible cultivars (ANOSIM test, R = 0.4637, $p < 0.001$; Fig. 2). Moreover, *P. graminis* abundance was negatively correlated with relative total phagotroph abundance in rhizosphere soil (R = −0.52, $p = 0.018$; Fig. 2). The above results suggest that rhizosphere protistan communities (including phagotrophic

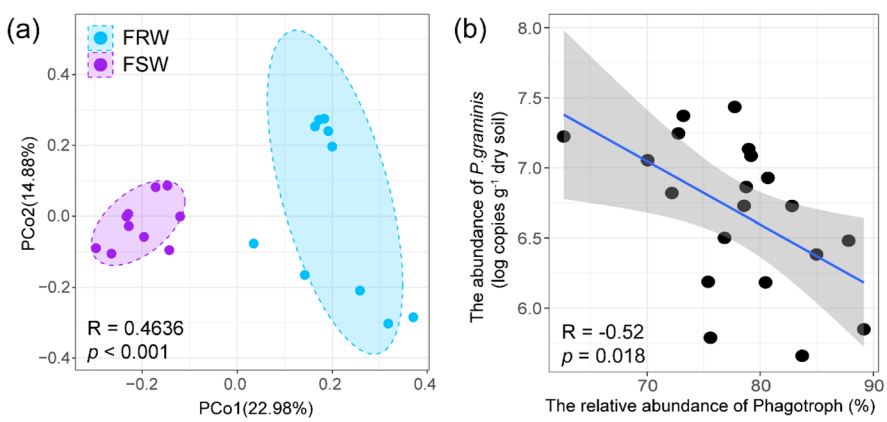

Fig. 2 Community structure of wheat rhizosphere phagotrophs (**a**). Linear relationships between *Polymyxa graminis* abundance and the relative abundance of phagotrophic protists in diseased and healthy plants across the two wheat cultivars (**b**). FRW, For-resistant wheat; FSW, For-susceptible wheat. The ellipses represent 80% confidence intervals of each cultivar

protists) showed greater dissimilarity among cultivars than bacterial and fungal communities.

LDA effect size (LEfSe) analysis revealed that 47 protistan ASVs were significantly enriched in the rhizosphere community of the FSW, and 36 protistan biomarkers were found in the FRW (Fig. S4); 39 protistan ASVs indicative of diseased plants and 33 protistan ASVs indicative of healthy plants were identified as phagotrophs, most of which were predators of other microbes. Subsequently, correlation-based network analysis revealed that nine biomarker ASVs were significantly correlated with *P. graminis* abundance in the FSW plants (Fig. 3a). The analysis also revealed seven biomarker ASVs exhibiting strong links with *P. graminis* abundance in the rhizosphere community of the FRW (Fig. 3b). Of these, seven protistan ASVs indicative of infected plants (with only four in healthy plants) were classified as phagotrophs with significant negative links to *P. graminis* abundance during disease onset (Fig. 3). This means that overall, some phagotrophs tend to only be present in susceptible cultivars, and when they are present, their relative abundance is negatively associated with that of *P. graminis*. The above results indicate that the phagotrophic protist community, as well as some phagotrophic taxa, determine virus-vector abundance, based on the community structure of phagotrophs and phagotrophic indicator ASVs; significant negative correlations exist between phagotrophic protistan ASVs and *P. graminis* in correlation-based networks upon disease outbreaks.

Ecological module linking of *P. graminis* abundance in multitrophic networks

The multitrophic network was dominated by protists, bacteria, and fungi, accounting for 46%, 39%, and 15% of total microbial nodes, respectively (Fig. 4a). We used the ecological network to identify modules of microbial taxa highly correlated with each other, and therefore, potentially linked to *P. graminis* abundance. We detected six ecological modules divided from the multitrophic network (Fig. 4b and Fig. S5). Over these modules, we found that the relative abundance of Module #4 was negatively correlated with the *P. graminis* abundance, and more interestingly, the proportion of protistan ASVs in Module 4 was greater than that of other microbial taxa (Fig. 4g and Fig. S5). The ecological module #4 was dominated by bacteria (e.g., *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*), fungi (e.g., *Ascomycota, Basidiomycota, Chytridiomycota*, and unknown taxa), and protists (e.g., *Phagotroph, Phototroph*, and *Parasite*). A complete list with microbial taxa within the module can be found in the supplementary Table S4.

We further selected some "Hub nodes" (nodes with high values of degree (>60) and closeness centrality (>0.42)) to illustrate the link between hub microbes

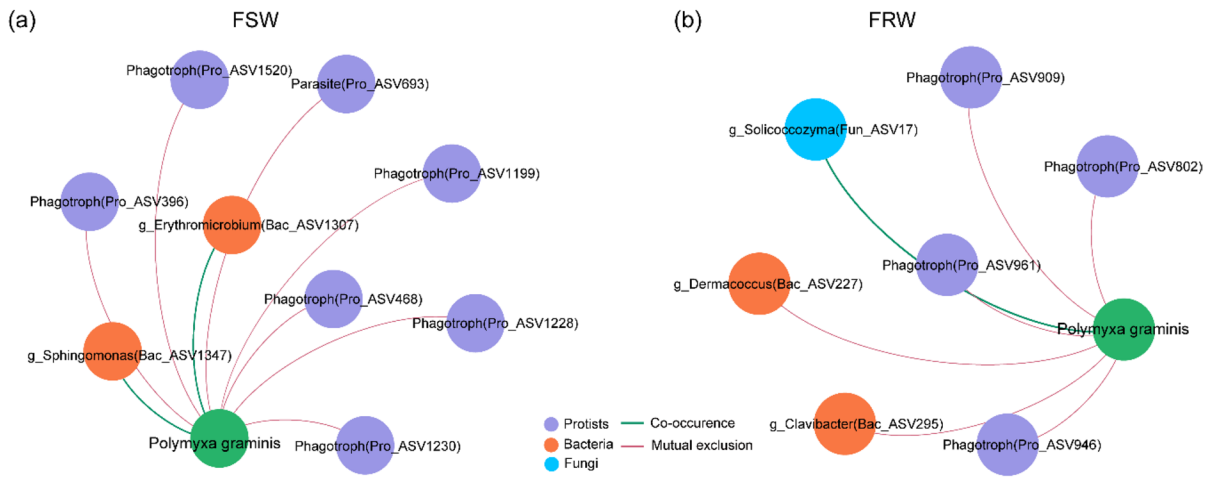

Fig. 3 Networks of indicator amplicon sequence variants (ASVs) linked with *Polymyxa graminis* vector in FSW cultivar (**a**) and FRW cultivar (**b**). Circles represent microbial ASVs, and the circle colors correspond to taxonomic features. Green and red solid lines indicate significantly positive and negative correlations ($p < 0.05$), respectively

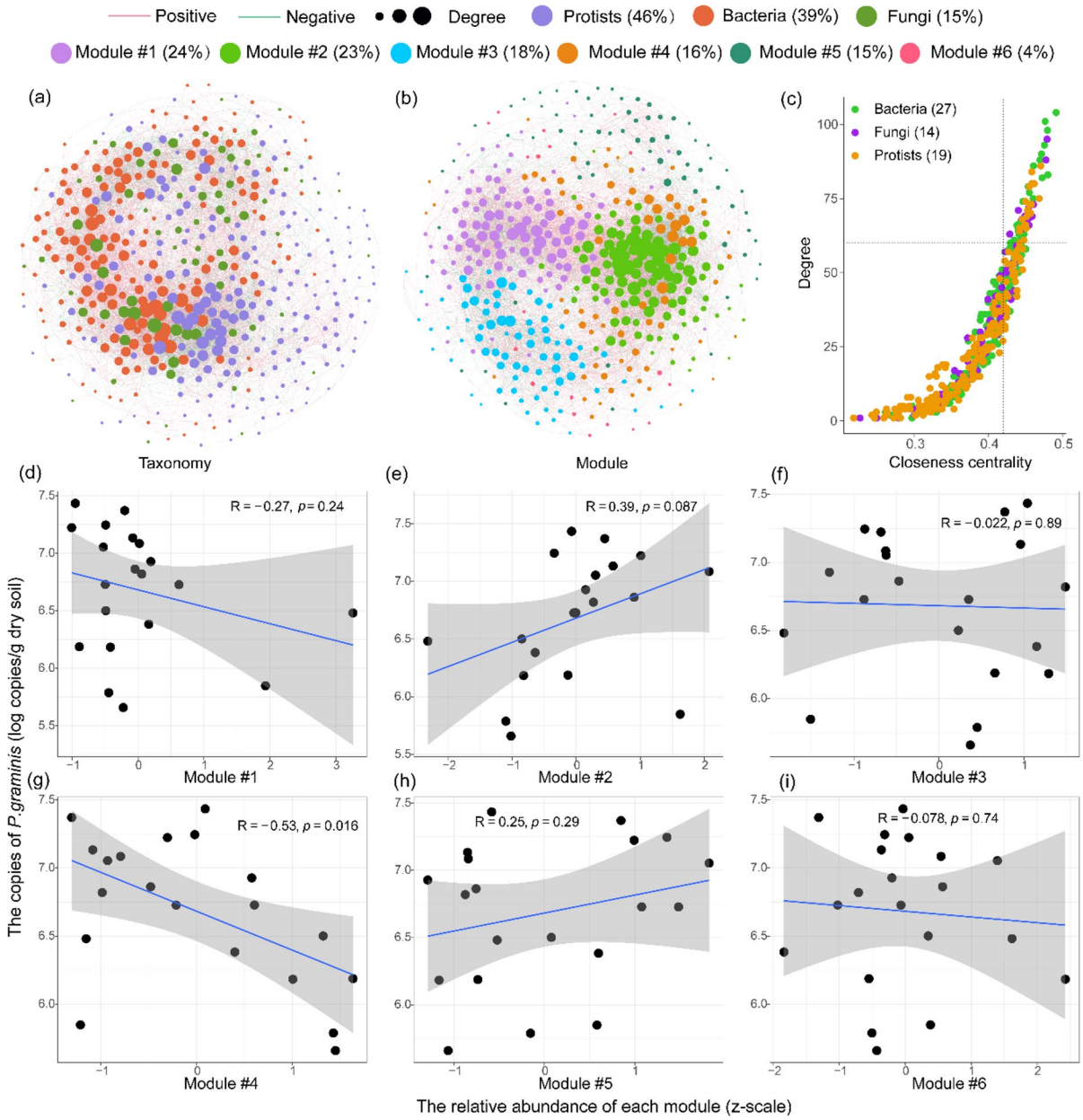

Fig. 4 Distribution patterns of the "hub nodes" and ecological modules based on multitrophic networks. **a** Network diagram with nodes colored according to each of the three taxonomic taxa (bacteria, fungi, and protists). **b** Network diagram with nodes colored according to each of the six ecological module (Modules #1–6). **c** Distribution patterns of the "hub nodes" of multitrophic networks based on all rhizosphere soil samples. **d**-**i** The regression relationships between the relative abundance of ecological module (Module #1–6) and *Polymyxa graminis* abundance. "Hub" nodes were identified as those which were significantly more central and more connected than other nodes within multitrophic networks. The relative abundance of each module was calculated by averaging the standard relative abundances (z-score) of all taxa belonging to each module

and *P. graminis* abundance in the multitrophic network (Fig. 4c and Table S5). We found these hub nodes belonged to modules #1–4, and were mainly composed of the bacterial phyla *Proteobacteria*, *Actinobacteria*, fungal *Ascomycota*, and protistan functional guild *Phagotroph* (Table S5). Specifically, we found that *P.*

graminis abundance was positively correlated with the relative abundance of Bac_ASV1173, Bac_ASV1307, Bac_ASV1312, Bac_ASV1566, Fun_ASV10, Fun_ASV18, Pro_ASV460, and Pro_ASV693 in module #2 (Fig. S6). It is worth noting that Pro_ASV693 was classified as *P. graminis*, indicating that *P. graminis* could occupy a higher living space in the rhizosphere soil of diseased plants to propagate and infect wheat roots. The *P. graminis* abundance was negatively linked to the relative abundance of Bac_ASV316, Bac_ASV317, and Bac_ASV319 (all belong to *Actinobacteria*) in module #2 (Fig. S6). The relative abundance of these phagotrophs (e.g., Pro_ASV1069, Pro_ASV1183, Pro_ASV818, and Pro_ASV971) in module #4 were also negatively associated with *P. graminis* abundance (Fig. S6). The results suggest that the significant negative correlation between module #4 and *P. graminis* abundance may be due to the regulation of phagocytic protists in the multitrophic network.

Underlying drivers of *P. graminis* abundance

SEM was performed to determine the contribution of each potential influencing factor (including soil properties, phagotrophic protistan community, and bacterial and fungal communities) to the difference in *P. graminis* abundance among the different wheat cultivars upon disease onset (Fig. 5a, b). These predictors explained 71.6% of the variance in *P. graminis* abundance (Fig. 5a). The model showed that phagotrophic protists had direct or indirect negative correlations (via negative interactions with fungi) with *P. graminis* abundance, whereas fungi had a direct positive correlation. Soil properties also had an indirect correlation with *P. graminis* abundance by negatively affecting the phagotrophic protists (Fig. 5a). The standard total effects for *P. graminis* abundance indicated that phagotrophic protists exerted a stronger correlation with *P. graminis* abundance than other factors in the rhizosphere communities between FSW and FRW cultivars (Fig. 5b). Our SEM findings show that the phagotrophic protistan community may be the primary regulator of *P. graminis* abundance.

Discussion

Sensitivity of protistan communities and other microbes to wheat cultivar during disease onset

Elucidating the assembly of the crop rhizosphere microbiome and the mechanisms by which it interacts with the environment is a central requirement for maximizing agricultural production (Singh and Trivedi 2017). To achieve this goal, we first analyzed the alpha diversity and structure within rhizosphere communities of wheat cultivars with different disease resistance levels. The results showed significant differences in the rhizosphere communities of both cultivars; particularly, higher alpha diversity was observed

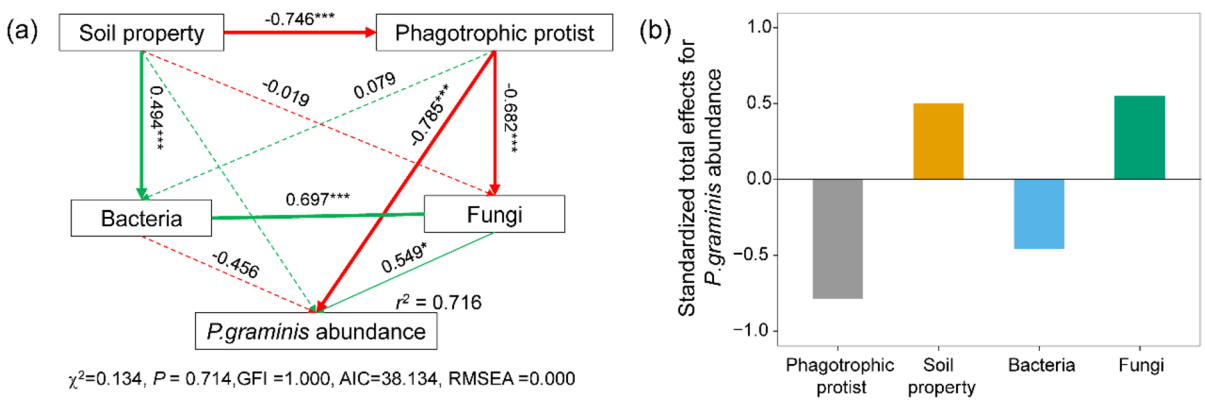

Fig. 5 Path analysis illustrating the link across microbial communities affecting *Polymyxa graminis* abundance (**a**). Contribution of biotic and abiotic factors to the changes of *Polymyxa graminis* abundance (**b**). Continuous and dashed arrows represent significant and nonsignificant relationships, respectively. Green and red arrows indicate positive and negative relationships, respectively. r^2 values indicate the proportion of variance explained by each variable. Standardized total effects (direct plus indirect effects) calculated using SEM. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$

in the rhizosphere community of the susceptible cultivar. Plant-associated microbiomes were shaped by multiple host and environmental factors, such as host genetics and edaphic factors. It is in accordance with Wen et al. (2020) who showed how plant phenotype changed the root exudate profiles which significantly affected the rhizosphere microbial diversity. We propose that the incidence of diseases weakens the effects of the host plant and reduces its ability to filter rhizosphere microorganisms, which leads to an increase in rhizosphere microbial diversity in susceptible cultivars. Soil nutrients are also important driving factors of change in rhizosphere communities, as confirmed by soil ecosystem studies (Shi et al. 2018; Xiong et al. 2021). In this study, in addition to the influence of plant genetics, the higher AP and SOC contents in rhizosphere soil of susceptible cultivars could be contributing factors to the high microbial diversity of susceptible cultivars.

Protists are taxonomically the most diverse eukaryotes and occupy all key functional roles in soil food webs (Geisen et al. 2018). We found that the planting of wheat cultivars with different levels of resistance (FSW and FRW) led to greater variation in protist community structure than in that of other microbial communities (bacteria and fungi) in rhizosphere soils. This may have resulted from the involvement of protists in the hormonal regulation of plants, strongly affecting the plant metabolome (Gao et al. 2019). Protists stimulate lateral root branching in plants by promoting auxin production, increasing cytokinin concentration (Krome et al. 2010), and stimulating the secretion of antimicrobial substances (Brazelton et al. 2008). In addition, in this study, environmental factors had a stronger impact on the community structure of protists than bacteria and fungi (Fig. S2). the niche breadth of protists may be lower, suggesting that protists are less tolerant to environmental changes than other microbes. The infection of wheat mosaic virus caused by *P. graminis* might also affect host rhizosphere communities. Infection of host plant with *Polymyxa betae* promoted the development of *plasmodia* and *sporangia*, and stimulated the expression of glutathione S-transferase gene (Decroës et al. 2022), which is involved in the regulation of protist communities interacting with plants (Gullner et al. 2018). These observations suggest that rhizosphere community assembly is co-regulated by the host, viral infection, and soil factors; the protists respond more strongly to environmental differences than fungi and bacteria.

In our study, we identified protistan predatory taxa and their importance in regulating *P. graminis*. Since there was no significant correlation between soil physicochemical properties and the abundance of *P. graminis*, we only analyzed the association between biotic factors and *P. graminis*, and used microbial communities as biological indicators to predict *P. graminis* abundance (Fig. 1). We also showed that the protist community, especially that of phagotrophic taxa, is likely involved in the suppression of *P. graminis* abundance (Fig. 2). These findings confirm the pivotal role of rhizosphere phagotrophs, a major functional group of protists in soil as a key microbiome link in agricultural systems responsible for plant health (Guo et al. 2021). The role of predatory protists inhibiting viral transmission vectors might be a disease suppression phenomenon in rhizosphere soils; such predatory protists act as keystone species and are worth exploring as biocontrol agents to increase sustainable soil management.

The important ecological role of phagotrophic taxa in the co-occurrence network

Microbial communities could also be grouped into assemblies with particular trait combinations based on different co-occurrence or association patterns, offering new insights into complex microbial community structure and soil function; rhizosphere microbial networks are extremely sensitive to pathogen infection (Barberan et al. 2012; Carrion et al. 2019; Fan et al. 2021, Fernandez-Gonzalez et al. 2020). In this study, we used multitrophic ecological networks to detect microbial clusters and hub species. The relative abundance of key ecological module and hub phagotrophic taxa potentially determine *P. graminis* abundance following disease onset (Fig. 4 and Fig. S6). In particular, the relative abundance of module #4 showed a negative correlation with *P. graminis* abundance, indicating that the decrease in *P. graminis* abundance probably resulted from the increase in relative abundance of taxa within module #4. Most of ASVs within module #4 were from *Burkholderiales*, *Actinobacteria*, and *Cercozoa* (Phagotrophic protist) (Table S4) play an important role in maintain plant health. For instance, *Burkholderiales* showed significant inhibition of pathogen growth through the production of various secondary metabolites (Depoorter et al. 2016) such as volatile organic compounds (VOCs), or *Actinobacteria* as antagonistic bacteria producing antifungal compounds to compete for resources through high niche

overlapping (Essarioui et al. 2017). In particular, *Cercozoa*, which are microbe-consuming protists, improve plant growth by preying on plant pathogen; they also increase the performance of plant growth-promoting rhizobacteria by preying on their competitors (Gao et al. 2019, Jousset 2017). Interestingly, the relative abundance of some hub species belonging to phagocytic protists from module #4 also showed negative correlations with *P. graminis* abundance, and could be classified as microbe-consuming protists for plant health. For instance, the hub protistan species Pro_ASV1069, Pro_ASV1183, Pro_ASV818, and Pro_ASV971 belong to *Cercozoa* (phagocytic protists), which are known to feed on bacteria, fungi, and even some eukaryotes (Dumack et al. 2020); the microbes with strong competitiveness screened out by this protozoan predation stress may also effectively resist the pathogen infection (Guo et al. 2022). These results further suggest that key ecological clusters and phagotrophic protists potentially inhibit the growth of the viral vector and help maintain host health.

Role of phagotrophic protists toward *P. graminis* abundance

As a vector of plant viruses, *P. graminis* infects the roots of plants and exist in the soil for extended periods in the form of dormant spores (Adams 1991). One sustainable biological strategy is to mobilize indigenous microorganisms and antagonizing pathogens to prevent a disease outbreak. Notably, *P. graminis* abundance was higher in FSW rhizosphere soils than in FRW soils. However, relative phagotroph abundance was negatively correlated with *P. graminis* abundance among all rhizosphere samples, possibly due to predation by phagotrophic protists (Geisen et al. 2016). Previous studies have indicated that phagotrophs prey on or lyse the hyphae and spores of a variety of pathogens, thereby decreasing the risk of pathogenic infection (Chakraborty and Old 1982; Nikoljuk 1969). LDA effect size and network analyses showed more negative correlations between *P. graminis* and protistan ASVs in FRW than in FSW soil during disease onset. Most protistan ASVs have been identified as phagotrophs, which are predators of other microbes (Dumack et al. 2020). Alternatively, the negative impacts of phagotrophic protists on *P. graminis* could result from interactions with competitors, as the niche breadth of protists is relatively low in complex agro-ecosystems (Wu et al. 2018). Our SEM results showed a significant link between phagotrophic protists and fungi, as shown previously (Guo et al. 2021; Huang et al. 2021), highlighting phagotrophic protists as the key determinants of *P. graminis* abundance. Overall, our results suggest that phagotrophic protists represent keystone taxa, as they exhibit a strong negative correlation, and are therefore potential drivers of *P. graminis* abundance.

We demonstrate that phagotrophic protists may regulate virus-vector development throughout plant growth, as the decline in relative abundance of rhizosphere phagotrophs coincided with soil-borne disease outbreaks. Although the viral vector was present in the rhizosphere soil of healthy plants, a stable and high relative abundance of phagotrophic protists might have helped mitigate the transmission of the disease. Future studies should focus on the isolation of phagotrophic protists and *P. graminis* and assess the relationship between the two taxa. In future studies, we intend to utilize phagotrophic protists to prevent and control the spread of soil-borne mosaic virus in the rhizosphere. Overall, this study suggests that phagotrophic protists may control pathogen vector and improve plant health by influencing changes in rhizosphere microbial community composition and function through multitrophic network regulatory strategies in complex farmland ecosystems. Herein, we reported the potential ecological functions of phagotrophic protists to control pathogen spread and improve plant health in highly managed agro-ecosystems. Our findings highlight the complexity of rhizosphere community networks, reflecting the co-occurrence patterns of multi-trophic level microbes in virtual networks and strengthening the association between soil microbial diversity and plant health.

Acknowledgements We thank Mr. Liu and Yufei Ning for their assistance in field work and soil sampling, and Dr. Anhui Ge and Dr. Chao Xiong for their generous help in data analysis. This study was financially supported by National key research and development program (2022YFA1304400), Ningbo Science and Technology Bureau (2021Z101), China Agriculture Research System from the Ministry of Agriculture of P.R. China (CARS-03) and sponsored by the K.C. Wong Magna Fund of Ningbo University.

Author contribution Tida Ge, Jian Yang, and Jianping Chen conceived and designed the study. Chuanfa Wu conducted the experiments. Chuanfa Wu, Fangyan Wang, and Haoqing Zhang

conducted the field investigation. Zhenke Zhu performed data analysis. Chuanfa Wu, Didier Lesueur and Tida Ge wrote the manuscript. Chaonan Ge participated in the revision and discussion of the manuscript. All authors read and approved the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Adams M (1991) Transmission of plant viruses by fungi. Ann Appl Biol 118:479–492. https://doi.org/10.1111/j.1744-7348.1991.tb05649.x

Adams MJ, Jacquier C (1994) Infection of cereals and grasses by isolates of *Polymyxa graminis* (*Plasmodiophorales*). Ann Appl Biol 125:53–60. https://doi.org/10.1111/j.1744-7348.1994.tb04946.x

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM (2016) Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biol 14:e1002352. https://doi.org/10.1371/journal.pbio.1002352

Bakker PA, Berendsen RL, Doornbos RF, Wintermans PC, Pieterse CM (2013) The rhizosphere revisited: root microbiomics. Front Plant Sci 4:165. https://doi.org/10.3389/fpls.2013.00165

Barberan A, Bates ST, Casamayor EO, Fierer N (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J 6:343–351. https://doi.org/10.1038/ismej.2011.119

Bastian M, Heymann S, Jacomy M (2009) Gephi: an open-source software for exploring and manipulating networks. Proceedings of the international AAAI conference on web and social media, pp 361–362

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate - a practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory Caporaso J (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6:1–17. https://doi.org/10.1186/s40168-018-0470-z

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodriguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS 2, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vazquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9

Bongiorno G (2020) Novel soil quality indicators for the evaluation of agricultural management practices: a biological perspective. Front Agric Sci Eng 7:257–274. https://doi.org/10.15302/j-fase-2020323

Brazelton JN, Pfeufer EE, Sweat TA, Gardener BB, Coenen C (2008) 2,4-diacetylphloroglucinol alters plant root development. Mol Plant Microbe Interact 21:1349–1358. https://doi.org/10.1094/MPMI-21-10-1349

Callahan B, McMurdie P, Rosen M, Han W, Johnson A, Holmes S (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

Carrion VJ, Perez-Jaramillo J, Cordovez V, Tracanna V, de Hollander M, Ruiz-Buck D, Mendes LW, van Ijcken WFJ, Gomez-Exposito R, Elsayed SS, Mohanraju P, Arifah A, van der Oost J, Paulson JN, Mendes R, van Wezel GP, Medema MH, Raaijmakers JM (2019) Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. Science 366:606–612. https://doi.org/10.1126/science.aaw9285

Chakraborty S, Old K (1982) Mycophagous soil amoeba: interactions with three plant pathogenic fungi. Soil Biol Biochem 14:247–255. https://doi.org/10.1016/0038-0717(82)90034-7

Cui Z, Li P, Gao G, Kan H, Du Z, Zhang F, Sun M, Li X (2017) Effect of wheat yellow mosaic viral disease on biological traits and grain yield of different wheat cultivars. J Triticeae Crops (in Chinese with English abstract) 37:1175–1180

Decroës A, Mahillon M, Genard M, Lienard C, Lima Mendez G, Gilmer D, Bragard C, Legreve A (2022) *Rhizomania*: hide and seek of *Polymyxa betae* and the *Beet necrotic yellow vein virus* with Beta vulgaris. Mol Plant Microbe Interact. https://doi.org/10.1094/MPMI-03-22-0063-R

Delgado-Baquerizo M, Reith F, Dennis PG, Hamonts K, Powell JR, Young A, Singh BK, Bissett A (2018) Ecological drivers of soil microbial diversity and soil biological networks in the Southern Hemisphere. Ecology 99:583–596. https://doi.org/10.1002/ecy.2137

Depoorter E, Bull MJ, Peeters C, Coenye T, Vandamme P, Mahenthiralingmam E (2016) *Burkholderia*: an update on taxonomy and biotechnological potential as antibiotic producers. Appl Microbiol Biotechnol 100:5215–5229. https://doi.org/10.1007/s00253-016-7520-x

Deng Y, Jiang YH, Yang Y, He Z, Luo F, Zhou J (2012) Molecular ecological network analyses. BMC Bioinform 13:113. https://doi.org/10.1186/1471-2105-13-113

Diao A, Chen J, Ye R, Zheng T, Yu S, Antoniw JF, Adams MJ (1999) Complete sequence and genome properties of *chinese wheat mosaic virus*, a new furovirus from China. J Gen Virol 80(Pt 5):1141–1145. https://doi.org/10.1099/0022-1317-80-5-1141

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

Dumack K, Fiore-Donno AM, Bass D, Bonkowski M (2020) Making sense of environmental sequencing data: ecologically important functional traits of the protistan groups *Cercozoa* and *Endomyxa* (*Rhizaria*). Mol Ecol Resour 20:398–403. https://doi.org/10.1111/1755-0998.13112

Duran P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S (2018) Microbial interkingdom interactions in roots promote *Arabidopsis* survival. Cell 175:973-983e914. https://doi.org/10.1016/j.cell.2018.10.020

Essarioui A, LeBlanc N, Kistler HC, Kinkel LL (2017) Plant community richness mediates inhibitory interactions and resource competition between *Streptomyces* and *Fusarium* populations in the rhizosphere. Microb Ecol 74:157–167. https://doi.org/10.1007/s00248-016-0907-5

Fan KK, Delgado-Baquerizo M, Guo XS, Wang DZ, Zhu YG, Chu HY (2021) Biodiversity of key-stone phylotypes determines crop production in a 4-decade fertilization experiment. ISME J 15:550–561. https://doi.org/10.1038/s41396-020-00796-8

Fernandez-Gonzalez AJ, Cardoni M, Gomez-Lama Cabanas C, Valverde-Corredor A, Villadas PJ, Fernandez-Lopez M, Mercado-Blanco J (2020) Linking belowground microbial network changes to different tolerance level towards *Verticillium* wilt of olive. Microbiome 8:11. https://doi.org/10.1186/s40168-020-0787-2

Fournier B, Steiner M, Brochet X, Degrune F, Mammeri J, Carvalho DL, Siliceo SL, Bacher S, Peña-Reyes CA, Heger TJ (2022) Toward the use of protists as bioindicators of multiple stresses in agricultural soils: a case study in vineyard ecosystems. Ecol Indic 139:108955. https://doi.org/10.1016/j.ecolind.2022.108955

Fu L, Xiong W, Dini-andreote F, Wang B, Tao C, Ruan Y, Shen Z, Li R, Shen Q (2020) Changes in bulk soil affect the disease-suppressive rhizosphere microbiome against Fusarium wilt disease. Front Agric Sci Eng 7:307–316. https://doi.org/10.15302/j-fase-2020328

Gao Z, Karlsson I, Geisen S, Kowalchuk G, Jousset A (2019) Protists: Puppet masters of the rhizosphere microbiome. Trends Plant Sci 24:165–176. https://doi.org/10.1016/j.tplants.2018.10.011

Geisen S, Koller R, Hunninghaus M, Dumack K, Urich T, Bonkowski M (2016) The soil food web revisited: diverse and widespread mycophagous soil protists. Soil Biol Biochem 94:10–18. https://doi.org/10.1016/j.soilbio.2015.11.010

Geisen S, Mitchell EAD, Adl S, Bonkowski M, Dunthorn M, Ekelund F, Fernández LD, Jousset A, Krashevska V, Singer D, Spiegel FW, Walochnik J, Lara E (2018) Soil protists: a fertile frontier in soil biology research. FEMS Microbiol Rev 42:293–323. https://doi.org/10.1093/femsre/fuy006

Grace JB, Keeley JE (2006) A structural equation model analysis of postfire plant diversity in California shrublands. Ecol Appl 16:503–514. https://doi.org/10.1890/1051-0761(2006)016[0503:asemao]2.0.co;2

Groemping U (2006) Relative importance for linear regression in R: the package relaimpo. J Stat Softw 17:1–27. https://doi.org/10.18637/jss.v017.i01

Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, Boutte C, Burgaud G, de Vargas C, Decelle J, Del Campo J, Dolan JR, Dunthorn M, Edvardsen B, Holzmann M, Kooistra WH, Lara E, Le Bescot N, Logares R, Mahe F, Massana R, Montresor M, Morard R, Not F, Pawlowski J, Probert I, Sauvadet AL, Siano R, Stoeck T, Vaulot D, Zimmermann P, Christen R (2013) The Protist Ribosomal reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41:D597-604. https://doi.org/10.1093/nar/gks1160

Gullner G, Komives T, Király L, Schröder P (2018) Glutathione S-transferase enzymes in plant-pathogen interactions. Front Plant Sci 9:1836. https://doi.org/10.3389/fpls.2018.01836

Guo LM, He J, Li J, Chen JP, Zhang HM (2019) *Chinese wheat mosaic virus*: a long-term threat to wheat in China. J Integr Agric 18:821–829. https://doi.org/10.1016/S2095-3119(18)62047-7

Guo S, Tao CY, Jousset A, Xiong W, Wang Z, Shen ZZ, Wang BB, Xu ZH, Gao ZL, Liu SS, Li R, Ruan YZ, Shen QR, Kowalchuk GA, Geisen S (2022) Trophic interactions between predatory protists and pathogen-suppressive bacteria impact plant health. ISME J, 1–12. https://doi.org/10.1038/s41396-022-01244-5

Guo S, Xiong W, Hang X, Gao Z, Jiao Z, Liu H, Mo Y, Zhang N, Kowalchuk GA, Li R, Shen Q, Geisen S (2021) Protists as main indicators and determinants of plant performance. Microbiome 9:64. https://doi.org/10.1186/s40168-021-01025-w

Hassani MA, Duran P, Hacquard S (2018) Microbial interactions within the plant holobiont. Microbiome 6:58. https://doi.org/10.1186/s40168-018-0445-0

He L, Jin P, Chen X, Zhang TY, Zhong KL, Liu P, Chen JP, Yang J (2021) Comparative proteomic analysis of *Nicotiana benthamiana* plants under *chinese wheat mosaic virus* infection. BMC Plant Biol 21:51. https://doi.org/10.1186/s12870-021-02826-9

Huang X, Wang JJ, Dumack K, Liu WP, Zhang QC, He Y, Di HJ, Bonkowski M, Xu JM, Li Y (2021) Protists modulate fungal community assembly in paddy soils across climatic zones at the continental scale. Soil Biol Biochem 160:108358. ARTN 108358. https://doi.org/10.1016/j.soilbio.2021.108358

ISSS, ISRIC FAO (1998) World reference base for soil resources, Wageningen/Rome, 1–68

Jiao S, Chen W, Wang J, Du N, Li Q, Wei G (2018) Soil microbiomes with distinct assemblies through vertical soil profiles drive the cycling of multiple nutrients in reforested ecosystems. Microbiome 6:146. https://doi.org/10.1186/s40168-018-0526-0

Jousset A (2017) Application of protists to improve plant growth in sustainable agriculture. In: Mehnaz S (ed) Rhizotrophs: plant growth promotion to bioremediation. Springer, Singapore, pp 263–273

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Kojima H, Nishio Z, Kobayashi F, Saito M, Sasaya T, Kiribuchi-Otobe C, Seki M, Oda S, Nakamura T (2015) Identification and validation of a quantitative trait locus associated with *wheat yellow mosaic virus* pathotype I resistance in a japanese wheat variety. Plant Breed 134:373–378. https://doi.org/10.1111/pbr.12279

Kramer S, Dibbern D, Moll J, Huenninghaus M, Koller R, Krueger D, Marhan S, Urich T, Wubet T, Bonkowski M, Buscot F, Lueders T, Kandeler E (2016) Resource partitioning between bacteria, fungi, and protists in the detritusphere of an agricultural soil. Front Microbiol 7:1524. https://doi.org/10.3389/fmicb.2016.01524

Krome K, Rosenberg K, Dickler C, Kreuzer K, Ludwig-Muller J, Ullrich-Eberius C, Scheu S, Bonkowski M (2010) Soil bacteria and protozoa affect root branching via effects on the auxin and cytokinin balance in plants. Plant Soil 328:191–201. https://doi.org/10.1007/s11104-009-0101-3

Langfelder P, Horvath S (2012) Fast R functions for robust correlations and hierarchical clustering. J Stat Softw 46:1–17

Liu C, Suzuki T, Mishina K, Habekuss A, Ziegler A, Li C, Sakuma S, Chen G, Pourkheirandish M, Komatsuda T (2016) *Wheat yellow mosaic virus* resistance in wheat cultivar madsen acts in roots but not in leaves. J Gen Plant Pathol 82:261–267. https://doi.org/10.1007/s10327-016-0674-7

Liu H, Li J, Carvalhais LC, Percy CD, Prakash Verma J, Schenk PM, Singh BK (2021) Evidence for the plant recruitment of beneficial microbes to suppress soil-borne pathogens. New Phytol 229:2873–2885. https://doi.org/10.1111/nph.17057

Manici LM, Caputo F (2009) Fungal community diversity and soil health in intensive potato cropping systems of the east Po valley, northern Italy. Ann Appl Biol 155:245–258. https://doi.org/10.1111/j.1744-7348.2009.00335.x

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J 6:610–618. https://doi.org/10.1038/ismej.2011.139

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663. https://doi.org/10.1111/1574-6976.12028

Nikoljuk V (1969) Some aspects of the study of soil protozoa. Acta Protozool 7:1–37

Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Olivier G, Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas J, Passos A (2011) Scikit-learn: machine learning in Python. J Mach Learn Res 12:2825–2830

Price MN, Dehal PS, Arkin AP (2010) FastTree 2 - approximately maximum-likelihood trees for large alignments. PLoS ONE 5. https://doi.org/10.1371/journ.pone.0009490

Sanguin H, Sarniguet A, Gazengel K, Moenne-Loccoz Y, Grundmann GL (2009) Rhizosphere bacterial communities associated with disease suppressiveness stages of take-all decline in wheat monoculture. New Phytol 184:694–707. https://doi.org/10.1111/j.1469-8137.2009.03010.x

Sapp M, Ploch S, Fiore-Donno AM, Bonkowski M, Rose LE (2018) Protists are an integral part of the *Arabidopsis thaliana* microbiome. Environ Microbiol 20:30–43. https://doi.org/10.1111/1462-2920.13941

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12:R60. https://doi.org/10.1186/gb-2011-12-6-r60

Shi Y, Li Y, Xiang X, Sun R, Yang T, He D, Zhang K, Ni Y, Zhu YG, Adams JM, Chu H (2018) Spatial scale affects the relative role of stochasticity versus determinism in soil bacterial communities in wheat fields across the North China Plain. Microbiome 6:27. https://doi.org/10.1186/s40168-018-0409-4

Singh BK, Trivedi P (2017) Microbiome and the future for food and nutrient security. Microb Biotechnol 10:50–53. https://doi.org/10.1111/1751-7915.12592

Stoeck T, Bass D, Nebel M, Christen R, Jones MD, Breiner HW, Richards TA (2010) Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol 19(Suppl 1):21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x

Van der Heijden MGA, Hartmann M (2016) Networking in the plant microbiome. PLoS Biol 14:e1002378

Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, Gilbert JA, Jansson JK, Caporaso JG, Fuhrman JA, Apprill A, Knight R (2016) Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. Msystems 1:e00009-00015. https://doi.org/10.1128/mSystems.00009-15

Wang E, He D, Zhao Z, Smith CJ, Macdonald BC (2020) Using a systems modeling approach to improve soil management and soil quality. Front Agr Sci Eng 7(3):289–295. https://doi.org/10.15302/J-FASE-2020337

Wang H, Wu C, Zhang H, Xiao M, Ge T, Zhou Z, Liu Y, Peng S, Peng P, Chen J (2022) Characterization of the belowground microbial community and co-occurrence networks of tobacco plants infected with bacterial wilt disease. World J Microbiol Biotechnol 38:155. https://doi.org/10.1007/s11274-022-03347-9

Wen T, Yuan J, He X, Lin Y, Huang Q, Shen Q (2020) Enrichment of beneficial cucumber rhizosphere microbes mediated by organic acid secretion. Hortic Res 7:154. https://doi.org/10.1038/s41438-020-00380-3

Wen T, Zhao M, Yuan J, Kowalchuk GA, Shen Q (2021) Root exudates mediate plant defense against foliar pathogens by recruiting beneficial microbes. Soil Ecol Lett 3:42–51. https://doi.org/10.1007/s42832-020-0057-z

Wu B, Jiang S, Zhang M, Wang S, Zhao J, Xin X (2017) Resistance of wheat cultivars to *wheat yellow mosaic virus* in shandong. J Triticeae Crops (in Chinese with English abstract) 37:332–336. https://doi.org/10.7606/j.issn.1009-1041

Wu W, Lu HP, Sastri A, Yeh YC, Gong GC, Chou WC, Hsieh CH (2018) Contrasting the relative importance of species

sorting and dispersal limitation in shaping marine bacterial versus protist communities. ISME J 12(2):485–494. https://doi.org/10.1038/ismej.2017.183

Wu CF, Wang FY, Ge AH, Zhang HQ, Chen GX, Deng YW, Yang J, Chen JP, Ge TD (2021a) Enrichment of microbial taxa after the onset of wheat yellow mosaic disease. Agric Ecosyst Environ 322:107651. ARTN 107651. https://doi.org/10.1016/j.agee.2021.107651

Wu CF, Wang FY, Zhang HQ, Chen GX, Deng YW, Chen JP, Yang J, Ge TD (2021b) Enrichment of beneficial rhizosphere microbes in chinese wheat yellow mosaic virus-resistant cultivars. Appl Microbiol Biotechnol 105:9371–9383. https://doi.org/10.1007/s00253-021-11666-4

Xiong C, Zhu YG, Wang JT, Singh B, Han LL, Shen JP, Li PP, Wang GB, Wu CF, Ge AH, Zhang LM, He JZ (2021) Host selection shapes crop microbiome assembly and network complexity. New Phytol 229:1091–1104. https://doi.org/10.1111/nph.16890

Xiong W, Li R, Guo S, Karlsson I, Jiao ZX, Xun WB, Kowalchuk GA, Shen QR, Geisen S (2019) Microbial amendments alter protist communities within the soil microbiome. Soil Biol Biochem 135:379–382. https://doi.org/10.1016/j.soilbio.2019.05.025

Xiong W, Song Y, Yang K, Gu Y, Wei Z, Kowalchuk GA, Xu Y, Jousset A, Shen Q, Geisen S (2020) Rhizosphere protists are key determinants of plant health. Microbiome 8:27. https://doi.org/10.1186/s40168-020-00799-9

Xu Y, Hu L, Li L, Zhang Y, Sun B, Meng X, Zhu T, Sun Z, Hong G, Chen Y, Yan F, Yang J, Li J, Chen J (2018) Ribotypes of *Polymyxa graminis* in wheat samples infected with soilborne wheat viruses in china. Plant Dis 102:948–954. https://doi.org/10.1094/PDIS-09-17-1394-RE

Ye R, Zheng T, Chen J, Diao A, Adams MJ, Yu S, Antoniw JF (1999) Characterization and partial sequence of a new furovirus of wheat in China. Plant Pathol 48:379–387. https://doi.org/10.1046/j.1365-3059.1999.00358.x

Zhao ZB, He JZ, Geisen S, Han LL, Wang JT, Shen JP, Wei WX, Fang YT, Li PP, Zhang LM (2019) Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. Microbiome 7:1–16. ARTN 33. https://doi.org/10.1186/s40168-019-0647-0