Article

Biofertilization alters the composition and interaction of the protistan community in the wheat rhizosphere under field conditions

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Abstract: Biofertilizer, an environment-friendly and renewable plant nutrient source, has been widely applied and studied to reduce dependency on chemical fertilizers. However, most studies focus on the effects of biofertilizer on the bacterial and fungal communities, and we still lack an understanding of biofertilizer on the protistan community. Here, the effects of biofertilizer application on the composition and interaction of the protistan community in the wheat rhizosphere were investigated based on a 4-year field experiment. Biofertilizer application altered soil physicochemical properties and the protistan community composition (ANOSIM, $p < 0.001$), and significantly induced an alpha diversity decline. Random forecast and redundancy analysis demonstrated that nitrogenase activity and available phosphorus were the main drivers. *Trichomonas* classified to the phylum *Metamonada* was enriched by biofertilizer, and was significantly positive connections with soil nitrogenase activity and some function genes involved in nitrogen-fixation and nitrogen-dissimilation. Biofertilization loosely connected biotic interactions, while did not affect the stability of the protistan community. Besides, biofertilizer promoted the connections of protists with fungi, bacteria, and archaea. Combined with the conjunct biotic network (protist, fungi, bacteria, and archaea) and interactions between protists and soil physicochemical properties/function genes, protists may act as keystone taxa potentially driving soil microbiome composition and function.

Keywords: Microbiome; Diazotroph; Nitrogen fixation bacteria; Random Forest; Network; *Trichomona*

1. Introduction

Agroecosystems may provide important regulating and provisioning services [1, 2]. Particularly, the stringently and efficiently managed agroecosystems are expected to mitigate climate change and meet the rising global demand for food production [3]. However, long-term and excessive application of chemical fertilizer has caused many negative effects on ecosystem functioning and services, such as soil compaction, eutrophication, groundwater pollution of nitrate leaching and large hypoxic zones, terrestrial and aquatic acidification as well as eutrophication [4-7]. Biofertilizer, a type of biological products containing living microorganisms like bacteria, algae, or fungi with the potential to improve soil fertility and/or inhibit plant pathogens, is an ecofriendly and renewable source of plant nutrients, as they could reduce dependency on chemical fertilizers [8, 9]. Some biofertilizers have been applied to various agroecosystems and have shown positive effects on soil quality and crop productivity [10-12]. Biofertilizers Ning shield could not only promote plant growth and production yield but also control root-knot nematode disease in the field [13]. Similarly, the application of biofertilizer containing *Bacillus subtilis* could

raise cotton growth and yield by promoting root mobilization as well as nutrients and substances absorption [14].

In addition to the promotion to plant growth and soil quality, biofertilizer could further affect the soil community composition of microorganisms [15, 16] and macroorganisms [17] after the application. For instance, the application of Bacillus subtilis biofertilizer not only increased pakchoi yield, but also mitigated NH_3 volatilization by changing the bacterial community composition and functional gene abundances [18]. Biofertilizer containing biocontrol agent *Bacillus amyloliquefaciens* NJN-6 could increase bacterial abundance, decrease fungal abundance, and change the abundances of some potential taxa (*Sphingobium*, *Dyadobacter*, *Cryptococcus*, *Fusarium*, *Ralstonia*, and *Burkholderia*) involved in suppression of banana *Fusarium wilt* disease [11]. *Paenibacillus triticisoli* BJ-18 could survive and propagate in the plant rhizosphere, root, and shoot, and its application leads to the enrichment of some potential beneficial microorganisms (*Pseudomonas*, *Paenibacillus*, *Klebsiella*, and *Bacillus*), and the decrease of some plant pathogens [15], and thus it was identified as a good candidate as the biofertilizer [12, 19, 20]. However, increasing numbers of studies have focused on bacterial and fungal responses to biofertilization, while soil protistan community response to biofertilizer is rarely investigated, which limits our comprehensive understanding of how biofertilization influences the whole microbial community.

As one of the major taxa of soil microorganisms, protists are taxonomically diverse and functionally versatile [21-23]. Protists are the major predators of bacteria and fungi [21, 24], and their predatory activities are beneficial for the releases of nutrients immobilized in microbial biomass, promoting nutrient turnover and plant nutrient uptake, with consequences for a wide range of ecosystem functions [25-29]. Protists also contribute to nutrient cycling through regulating carbon fixation and organic matter degradation [30, 31]. Thereby, protists play an important role in soil ecological processes and agricultural production. However, limited studies were focused on the effects of chemical fertilizer [32-34], organic fertilizer [35-37], biochar [23, 38] application on protistan communities. Therefore, elucidating the response of protistan community after biofertilizer application is crucial and necessary, which can provide new insights into engineered microorganisms for sustainable agriculture.

This study was conducted based on a 4-year field experiment to evaluate the effects of biofertilizer application on the protistan communities in wheat rhizosphere. High-throughput sequencing was employed to elucidate the influences of biofertilization on i) protistan community diversity and composition, ii) interactions between protistan community and soil physicochemical properties/function genes, and iii) the role of protists in the whole microbial communities (protists, bacteria, fungi, and archaea).

2. Materials and Methods

2.1 Strain and biofertilizer preparation

A novel strain, *P. triticisoli* BJ-18, was isolated from the wheat rhizosphere, having the abilities of N_2-fixing [1043 nmol C2H4 (mg protein h)-1], production of IAA (24.95 mg mL^{-1}), and multiple antagonistic activities against plant pathogens [39, 40]. *P. triticisol*i BJ-18 was inoculated in LB broth and cultured at 30°C and 180 rpm. After centrifugation at 4000 g for 5 min, the suspension was adjusted to 5×10^8 cells mL^{-1} with sterile normal saline. The rice hull powder was mixed with above bacterial suspension (1:1, w/v), and then air-dried in shade as biofertilizer.

2.2 Field experiment design and sample collection

The experiment was conducted at the Wuqiao Experimental Station of China Agricultural University, where winter wheat-summer maize rotation has adopted for many years. All plots were fertilized with 270 kg N ha^{-1} urea (N ≥ 45.4%) and 210 kg N ha^{-1} compound fertilizer (N-K_2O-P2O_2≥ 54%) before sowing. Among them, three plots were

applied with biofertilizer (*P. triticisoli* BJ-18 + rice hull, 60 kg per hectare), whereas the others (control) were applied with the rice hull. Winter wheat was sown in October and harvested in the next June. In addition to biofertilization, other managements were the same for all plots. At maturity, wheat yield was measured from a 1 m^2 area in each plot. The field experiment design and sample collection were detailed in our previous publication [41].

Freshly wheat roots were sampled in May 2018, which was then brought back to the laboratory on ice for subsequent analysis. In order to collect the rhizosphere soil, the roots from each plot were shaken gently, and the soil adhering tightly to the roots were put into the centrifuge tube and rinsed with sterile deionized water. The rhizosphere soil was collected by centrifugation, which was stored at -80°C for molecular and physicochemical analyses.

2.3 Determination of soil physicochemical properties and nitrogenase activity

Soil samples were digested using H_2SO_4 at 370°C, and then total N was determined using a modified Kjeldahl method [42]. Soil available P was extracted with resin and measured according to the modified method [43]. Soil OM was determined according to the description [44]. Acetylene reduction assay was conducted to measure soil nitrogenase activity based on the method [45, 46], with a slight modification.

2.4 DNA extraction and shotgun metagenome sequencing

DNA was extracted using the FastDNA® SPIN Kit (MP Biomedicals, CA, USA). To characterize the microbial communities in the wheat rhizosphere soil, six samples (BIO = 3 and CK = 3) were selected for shotgun metagenomic sequencing at Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China). The average amount of sequencing data per sample was 101 million sequences and 15 GB. The raw reads were quality-filtered with fastp (https://github.com/OpenGene/fastp) to gain clean data. The resulting sequences were assembled in Megahit [47]. Genes (≥ 100 bp) were selected and translated into amino acid sequences using MetaGene [48]. A non-redundant gene set was generated using CD-HIT software (95% identity, 90% coverage) [49]. The non-redundant genes were aligned against RefSeq non-redundant proteins database (https://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/) to assign taxonomic classifications. The obtained raw data have been submitted to the NCBI database (accession no. PRJNA575331) for public use.

2.5 Statistical analysis

The alpha diversity of microbial communities was estimated using the R package MicrobiomSeq [50]. Principal coordinate analysis (PCoA), ANOSIM analysis, and redundancy analysis (RDA) were conducted using the OmicStudio tools (https://www.omicstudio.cn/tool). Statistical analysis was computed by Student's t-test or Wilcoxon rank-sum test in SPSS software, and $p < 0.05$ was considered to be statistically significant. Random forecast (RF) was adopted to forecast correlations between environmental factors and the index of observed species [51]. The ggplot2 was used to visualize the RF model based on the partial dependence plots and two-dimensional interaction plots.

The interactions were visualized by a co-occurrence network [52]. A connected link denotes a high and significant ($0.8 < |r| < 1$, $p < 0.05$) Spearman's correlation. The size of each node is proportional to the number of links (connections, i.e., degree), and the thickness of each link is proportional to the absolute value of Spearman's correlation coefficient. The resulting networks were modeled using the Gephi [53].

3. Results

3.1. Wheat yield and soil physicochemical properties

The biofertilizer was applied as base fertilizer to the soil where winter wheat-summer maize was rotated for four years (from 2015 to 2018). The wheat yield and soil physicochemical properties were analyzed, and the detailed information can be found in our previous publication [41]. Biofertilizer application led to the increase of wheat yield (**Table S1**). Compared to the control, biofertilizer application significantly increased soil nitrogenase activity, as well as the contents of organic matter, total N, and available P ($p < 0.05$, hereafter) (**Fig. S1**).

3.2 Soil protistan community diversity

Compared to the control, biofertilizer application significantly increased the alpha diversities (ACE, Fisher, and Observed species) of protistan community (**Fig. 1A**). The RF was applied to further evaluate the effects of soil physicochemical properties on alpha diversities of the protistan community (**Fig. 1B**). Observed species values produced the best results, where more than 84.9% of variations in the protistan community could be explained by above environmental variables. Among the soil physicochemical parameters, nitrogenase activity, available P, and total N had the greatest influence on alpha diversities of the protistan community.

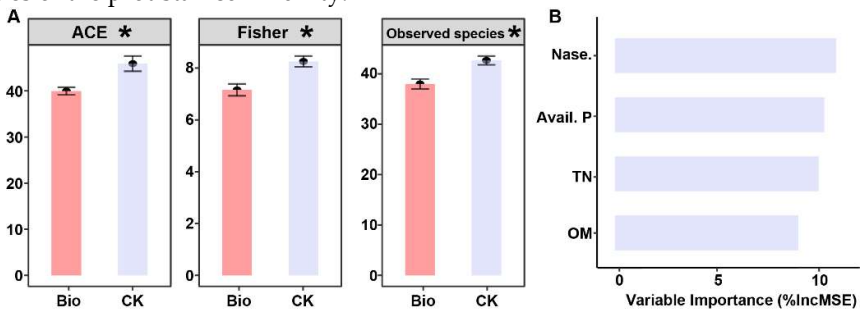

Figure 1: Comparison of the diversity indices (A) and random forest prediction of the relationships between individual physicochemical properties and the Observed species indices (B).* and ** indicate significant differences between biofertilizer application and control treatments at $p < 0.05$ and $p < 0.01$, respectively. Bio: biofertilizer application treatment, CK: control treatment.

The differences of protistan community between biofertilizer application and control groups were evaluated by PCoA based on the Bray-Curtis similarity (**Fig. 2**). The first two principal coordinates respectively explained 64.28% (PCoA1) and 10.79% (PCoA2) of the variation in the protistan community. The biofertilizer samples were clearly separated from control samples along the PCoA1 (ANOSIM, $p < 0.001$), indicating that biofertilizer application strongly affected the composition of the protistan community.

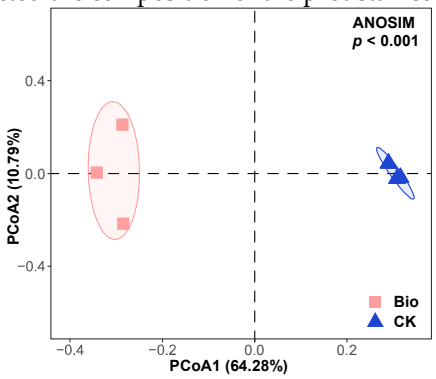

Figure 2: The PCoA analysis based on the Bray-Curtis distance of the protistan communities. Bio: biofertilizer application treatment, CK: control treatment.

3.3 Soil protistan community composition

The composition of the protistan community was summarized at the phylum and genus levels (**Fig. 3**). At the phylum level, *Amoebozoa* was the most abundant in all samples, accounting for over 29.6%–32.4% of the whole protistan community. *Choanozoa* ranked the second in abundance, followed by *Apicomplexa*, *Sulcozoa*, *Euglenozoa*, *Metamonada*, *Ciliophora*, *Foraminifera*, *Cercozoa*, *Perkinsozoa*, *Loukozoa*, and *Euglenozoa*. Compared to the control, biofertilizer application significantly increased the relative abundance of *Metamonada*, whereas it significantly decreased the relative abundances of *Choanozoa* and *Cercozoa* (**Fig. 3A**). Some protists at the genus level, including *Salpingoeca*, *Plasmodiophora*, and *Entamoeba*, were significantly enriched in the control group (**Fig. 3B**).The relative abundance of *Trichomonas* was higher in the biofertilization group than in the control group.

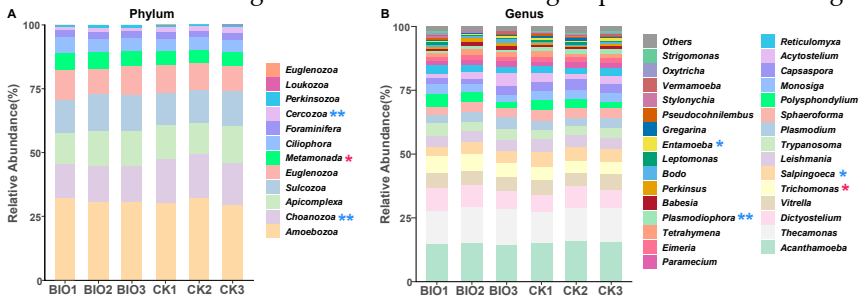

Figure 3: The relative abundances at the phylum (A) and genus (B) levels. * and ** indicate significant differences between biofertilizer application and control treatments at $p < 0.05$ and $p < 0.01$, respectively. The red * indicates that biofertilizer increases the relative abundance of microorganisms, and the blue * indicates that biofertilizer decreases the relative abundance of microorganisms. Bio: biofertilizer application treatment, CK: control treatment.

3.4 Interactions between protistan community and soil physicochemical properties/functional genes

RDA was applied to explore the influences of environmental factors on the protistan community composition (**Fig. 4**). Soil nitrogenase activity was identified as the most important factor, followed by available P and total N, which was consistent with the result of RF model. Spearman's correlation coefficient was used to evaluate relationships between the top twenty genera and soil physicochemical properties (**Fig. 5**). *Trichomonas* was the only genus, whose relative abundance was significantly positively correlated with the physicochemical properties (nitrogenase activity). The relative abundance of *Paratrimastix* was significantly negatively correlated with all the physicochemical properties, while the relative abundances of other genera (e.g., *Entamoeba*, *Plasmodiophora*, *Gregarina*, *Salpingoeca*, *Euglena*, and *Strigomonas*) were only negatively correlated with one or more physicochemical properties.

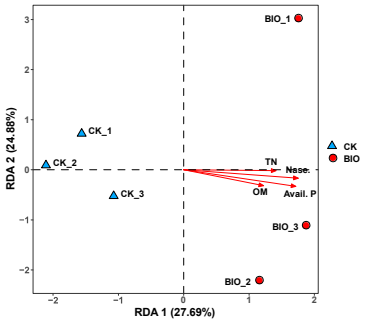

Figure 4: Redundancy analysis (RDA) demonstrating the impact of environmental factors on the protistan community compositions. Bio: biofertilizer application treatment, CK: control treatment.

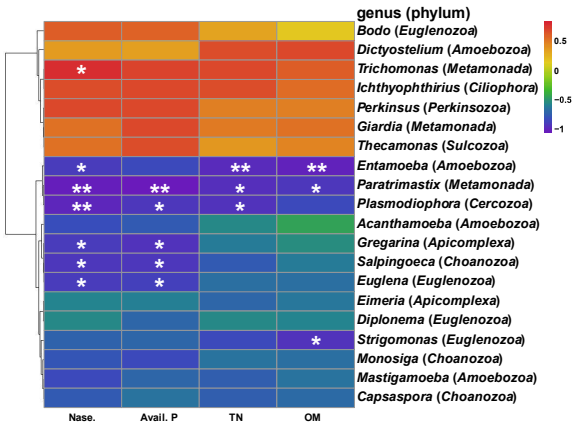

Figure 5: Heatmap visualization of the Spearman correlations between protists and soil physico-chemical properties. * and ** indicate significant correlation at $p < 0.05$ and $p < 0.01$.

Furthermore, Spearman's correlation coefficient between the protistan community and soil functional genes was calculated (**Fig. 6**). The relative abundance of *Trichomonas* showed a significantly positive correlation with the genes involved in N-fixation and N-dissimilation. The relative abundances of *Plasmodiophora*, *Paratrimastix*, *Salpingoeca*, and *Acanthamoeba* were significantly positively correlated with genes related to N-assimilation and P-uptake/internal cycling, but negatively correlated with other genes. In addition, *Bodo*, *Mastigamoeba*, *Gregarina*, and *Euglena* also showed a significant correlation with one or some functional genes.

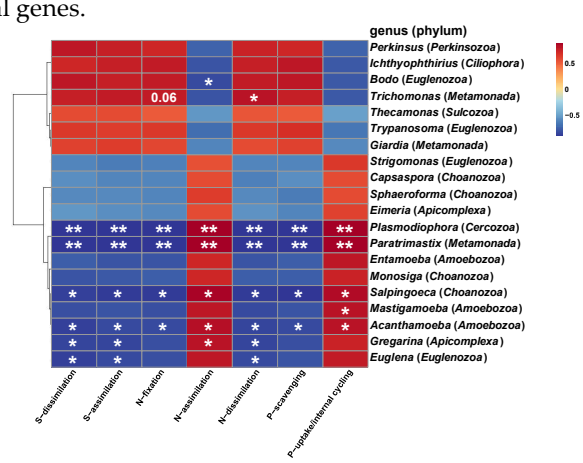

Figure 6: Heatmap visualization of the Spearman correlations between protists and soil function genes involving in nitrogen, phosphorus, and sulfate. * and ** indicate significant correlation at $p < 0.05$ and $p < 0.01$.

3.5 Interaction networks

The interaction networks of protistan community (Spearman > |0.8| and $p < 0.05$) of biofertilizer application group and control group were generated (**Fig. 7A,B**). The results revealed that there were remarkable differences in the topological parameters of the networks. The network of the biofertilizer application group had 46 nodes, 518 strong and significant links, and 1 036 degrees (**Fig. 7C, Tables S2,S3**). In the control group, the interactions were more complicated, showing 50 nodes, 746 links, and 1 492 degrees (**Fig. 7C, Tables S4,S5**). The results demonstrated that biofertilizer application suppressed the potential interactions within protistan community. Additionally, the ratio of positive link number to negative link numbers in each network was calculated, which showed ~50% in both networks of biofertilizer application and control groups (**Fig. 7D, Tables S3,S5**).

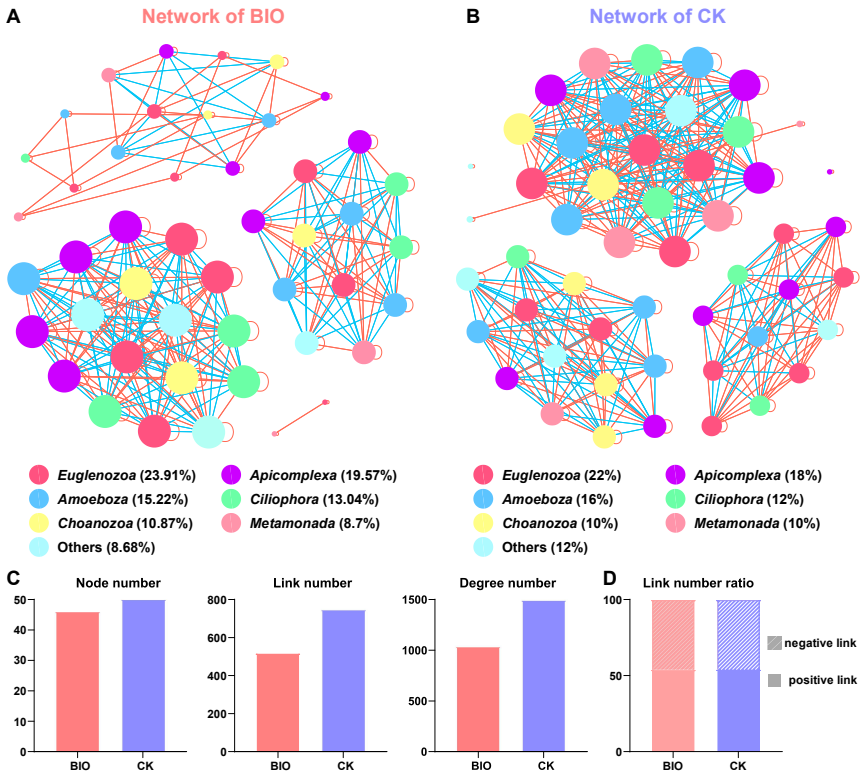

Figure 7: The co-occurrence network of the protist-protist of biofertilizer application (A) and control treatment (B). The topological characterizations including node number, link number, and degree number (C). The ratio of negative link to positive link (D). The connection stands for the Spearman correlation with significance ($p < 0.05$) and high significance ($0.8 < |r| < 1$). Bio: biofertilizer application treatment, CK: control treatment.

Furthermore, the interactions within the whole microbial communities (including protists, fungi, bacteria, and archaea) were analyzed to access the ecological roles of protists in soil microbial communities (**Fig. 8A,B**). The interaction network of the biofertilizer application group revealed 157 nodes with 8 031 connections (links) and 16 060 degrees (**Fig. 8C, Tables S6,S7**). The interactions in the control were more complicated. The 241 nodes corresponded to 19 779 significant and strong links and 39 556 degrees (**Fig. 8C, Tables S8,S9**). Compared to the control (54.4%), biofertilizer application increased the ratio of positive correlation (60.5%) (**Fig. 8D, Tables S7,S9**). The connections of protists-fungi, protists-bacteria, and protists-archaea accounted for 8.9%, 3.2%, and 2.6% of the entire network in biofertilization group and 7.3%, 2.7%, and 1.9% of the entire network in control (**Tables S7,S9**), which suggested that biofertilizer application enhanced the interactions of protist with fungi, bacteria, and archaea. In both biofertilizer application and control groups, higher link ratio of protists-fungi indicated that the protists were more tightly linked to fungi than bacteria and archaea, and the result was also reflected in the networks of protists-fungi, protists-bacteria, and protists-archaea (**Fig. S2, Tables 10,11,12**).

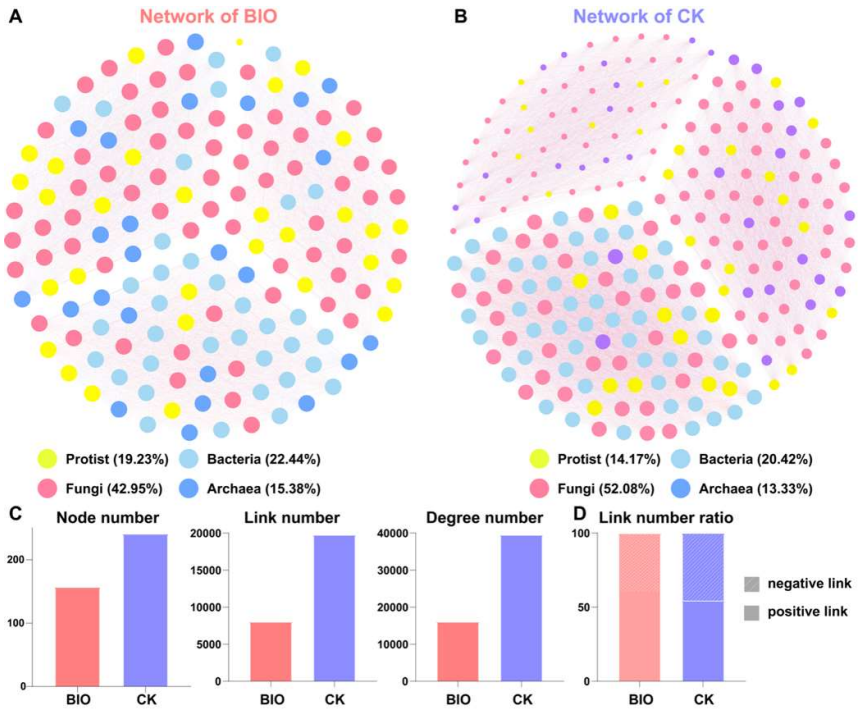

Figure 8: The co-occurrence network of the whole microbial communities in biofertilizer group (A) and in control (B). The topological characterizations including node number, link number, and degree number (C). The ratio of negative link to positive link (D). The connection stands for the Spearman correlation with significance ($p < 0.05$) and high significance ($0.8 < |r| < 1$). Bio: biofertilizer application treatment, CK: control treatment.

4. Discussion

As a microbiome-based product, biofertilizer is ecofriendly and inexpensive, which can provide essential nutrients for plants and increase soil fertility after application. With the development of high-throughput sequencing, more and more researches focus on the impact of biofertilizer application on the crop microbiome [15, 16]. Protists are a ubiquitous group of microscopic eukaryotes, , however, they are taxonomically ill-defined and usually ignored in most previous studies of the crop microbial community [54]. Therefore, a better understanding of this important microbial taxon will help to comprehensively and systematically explore the ecological effects of biofertilization on the plants/crops, which is conducive to optimizing the microbe-based fertilizer products extending our previous study on biofertilization-induced taxonomic changes of bacteria (N_2-fixing bacteria) and fungi [41].

4.1 Diversity and composition of the protistan community

In this study, biofertilizer application significantly increased wheat yield and improved some rhizosphere physicochemical properties (including nitrogenase activity, total N, organic matter, and available P) of rhizosphere soil. The increased soil nitrogenase activity and total N can be explained by the fact that the biofertilizer applied in this study contained N_2-fixing bacteria (*P. triticisoli* BJ-18), and N_2-fixing bacteria carried nitrogenase genes [15, 20]. Although the phosphate-solubilizing capacity of *P. triticisoli* BJ-18 was very low, the soil available P still increased, which may be due to the other enriched indigenous phosphate-solubilizing microorganisms under the induction of *P. triticisoli* BJ-18 [15, 40].

Biofertilization practices significantly decreased the alpha diversity of the protistan community (Fig. 1), while other agricultural managements such as the addition of chemical fertilizer or antibiotics in soil showed no influence [32, 55], which suggested that biotic factor may impose a higher pressure on the protistan diversity than the abiotic factor. RF

model (Fig. 2B) and RDA (Fig. 4) indicated that nitrogenase activity was the main factor influencing the alpha diversity and composition of the protistan community, which may explain the ammonia content accumulation during biological N fixation. Ammonia accumulation may have toxic effects on protists because it can readily diffuse through cell membranes and cause cell rupture [34, 56, 57]. Additionally, some protists are able to fix atmospheric N through their symbiotic interactions with N_2-fixing bacteria [58, 59]. For example, the uncultured bacterium *Bacteroidales* can live specifically within the cells of protist *Pseudotrichonympha grassii*, by which it can not only fix atmospheric N but also recycle the putative N waste products of parabasalid protist *P. grassii* [58].

At the genus level, biofertilizer application significantly increased the relative abundance of *Trichomonas* (phylum: *Metamonada*), while decreased the relative abundances of *Salpingoeca* (*Choanozoa*), *Plasmodiophora* (*Cercozoa*), *Entamoeba* (*Amoebozoa*) (Fig. 3). *Trichomonas* is known for having some parasite causing trichomoniasis, which is a prevalent non-viral sexually transmitted disease, and a significant amount of new cases are identified each year globally [60]. Therefore, the increased *Trichomonas* by biofertilizer in this study need to be further studied, which is crucial for agricultural ecological health and food security. The CBL–CIPK network, mainly involving in plant abiotic stresses (e.g., salt, cold, and drought), was also found within the protist's cells of *Trichomonas vaginalis* and *Naegleria gruberi* [61, 62], which indicated that some strains *Trichomonas* might be beneficial for the plant. The relative abundance of *Trichomonas* showed significantly positive interactions with soil nitrogenase activity and some functional genes involving in N fixation and dissimilation (Figs. 5,6). Whether this strain is directly involved in these biological processes is still unknown and therefore more research is needed on the genomic level of protists, especially soil protists. Besides, both genera *Pseudotrichonympha* and *Trichomonas* belonged to the phylum *Metamonada*, *Pseudotrichonympha* could show N_2 fixation capability by symbiosis with N_2 fixation bacteria [58]. Therefore, we deduced that some strains of *Trichomonas* may also have a similar symbiosis with N_2 fixation bacteria.

4.2 Microbial interaction networks

In ecosystem studies, the co-occurrence network analysis has become an increasingly critical tool for exploring the symbiosis patterns of microbial communities [63], and the biotic interactions are the most important factors of the microbial community structure and function [64]. Biofertilizer application inhibited the potential interactions within the protistan community (Fig. 7), which was consistent with the result that inoculation with N_2-fixing bacteria suppressed bacterial interactions in the maize rhizosphere [15]. The reduction of alpha diversity profoundly contributed to microbial connection changes [33, 65]. Besides, in both biofertilizer and control treatments, the ratios among positive and negative link numbers were ~50% in this study. Communities, which have a large proportion of members interacting with positive links, were unstable. In contrast, negative links may stabilize co-oscillation and promote community stability [66]. Therefore, our study indicated that biofertilization suppressed network potential interactions, while did not affect the network stability in the protistan community.

This comprehensive knowledge on microbial community functioning is only achieved when the entire microbial communities and not only individual parts are studied [33]. Therefore, investigations on potential soil microbiome interactions (e.g., protists, fungi, bacteria, and archaea) are urgently needed, which could profile complex soil food webs [67, 68]. The increased proportion of positive connections was observed in the biofertilizer treatment group (Fig. 8). The stress gradient hypothesis predicts that connections will shift from negative to positive with increasing stress in ecological communities [69]. Therefore, the foreign microorganism introduced by biofertilizer put pressure on native microbial communities.

Besides, in this study, biofertilizer application loosened the biotic interactions of the wheat rhizosphere microbial communities by the topological parameters (Fig. 8). This might be explained by increasing the protist proportion (5.1%) in biofertilizer treatment,

as top-down driving forces of protists on the microbial community including fungi, bacteria, and archaea [70, 71]. Three main modules emerged in both networks of biofertilizer and control treatments, with protists presented in all modules. In the control network, one module mainly contained protists, bacteria, and fungi, and another two mainly contained protists, fungi, and archaea; while each module contained protists, fungi, bacteria, and archaea in the network of biofertilizer treatment, which further demonstrated that biofertilizer application increased the importance of protists on shaping soil microbial communities. Each module appeared to be generally associated with a specific range of functions (Zhou et al., 2011), suggesting that biofertilizer application promoted tightly interactions between similarly functioning protists, fungi, bacteria, and archaea. Combined with the significant connections between protists and soil physicochemical properties and function genes (Fig. 5,6), this result further demonstrated that biofertilizer application drove protistan community structure, and thus protists as keystone taxa potentially drove soil microbiome structure and function.

5. Conclusions

In conclusion, we provide novel insights and evidence that biofertilization significantly changed the composition, induced an alpha diversity decline, and loosely interacted biotic interaction of the protistan community in the wheat rhizosphere based on a 4-year field experiment. The above changes about the protistan communities could be attributed to the interactions of soil physicochemical properties, function genes, and other microorganisms (fungi, bacteria, and archaea), and in turn, protists as keystone taxa may drive the entire soil microbiome in a top-down behaviour. Overall, these results suggest that the biofertilizer is a driver of the soil protistan community, contributing to soil ecosystem functioning. It must be noted that our results and conclusions are only based on one biofertilizers and one host plant. We advise that the potential effect of different biofertilizer on the composition and interaction of the protistan community in the various host plant should be further investigated.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Nitrogenase activity and physicochemical properties of soil samples collected in 2018. The detail information could be found in our previous publication (Li, et al. 2021). * and ** indicate significant differences between biofertilizer application and control treatments at $p < 0.05$ and $p < 0.01$, respectively. 1: nmol ethylene g^{-1} h^{-1}, Figure S2: The co-occurrence network of the protist-bacteria (A), protist-fungi (B), and protist-archaea(C). The connection stands for the Spearman correlation with significance ($p < 0.05$) and high significance ($0.8 < |r| < 1$). Table S1: Wheat yield (kg/ha), Table S2: Node table for biotic interaction in biofertilizer group, Table S3. Link table for biotic interaction in biofertilizer group, Table S4: Node table for biotic interaction in control group, Table S5: Link table for biotic interaction in control group, Table S6: Node table for biotic interaction of the whole microbial communities in biofertilizer group, Table S7: Link table for biotic interaction of the whole microbial communities in biofertilizer group, Table S8: Node table for biotic interaction of the whole microbial communities in control group, Table S9: Link table for biotic interaction of the whole microbial communities in control group, Table S10: The biotic interaction of protista-bacteria, Table S11: The biotic interaction of protista-fungi, Table S12: The biotic interaction of protista-archeae.

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Conflicts of Interest: The authors declare no conflicts of interest.

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