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Title page

Oxytetracycline and ciprofloxacin exposure altered the composition of protistan consumers in an agricultural soil

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ABSTRACT

Protists, an integral component of soil microbiome, are one of the main predators of bacteria. Bacteria can produce toxic secondary metabolites e.g. antibiotics to fight stress under the predation pressure of protists; however, impacts of antibiotics on the profile of protists in soils remain unclear. Here, we constructed a microcosm incubation to investigate the effects of two common antibiotics, oxytetracycline and ciprofloxacin, on the protistan and bacterial communities in arable soils. Rhizaria were the most abundant protist supergroup, followed by Amoebozoa, Stramenopiles and Aveolata. Among trophic functional groups, consumers were predominant within the protistan community. The protistan alpha-diversity was not significantly changed, while the bacterial alpha-diversity was decreased under the pressure of antibiotics. Nevertheless, the antibiotic exposure considerably reduced the relative abundance of protistan lineages in Rhizaria and Amoebozoa, which were the dominant supergroups of protistan consumers, while increased the relative abundance of other consumer and phototrophic protists. Altogether, we provide novel experimental evidence that the bacterivorous consumers, an important functional group of protists, were more sensitive to antibiotics than other functional groups. Our findings have potential implications for the induced alterations of protistan community and their ecological functions under the scenarios of projected increasing global antibiotic usage.

INTRODUCTION

Protists, primarily microscopic eukaryotes, form a focal, but often overlooked, part of the soil food webs.[1] They are highly diverse and ubiquitous in the terrestrial ecosystems (10^4 – 10^8 individuals per gram of soils).[2] Protists can increase the availability of essential nutrients (e.g. carbon, nitrogen, phosphorus, and silicon) for other organisms and plants via photosynthesis[3] or forming symbiosis with bacteria in nutrient cycling.[4-6] More importantly, protists are considered as pivotal regulators on the activities, community structure and evolution of microorganisms mainly via their predation on bacteria and fungi (as main food sources) and other microbial eukaryotes.[1, 7] Under the pressure of predation, bacteria can evolve defense mechanisms to fight against protists, such as by excreting antibiotics (e.g. violacein, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, etc.) as secondary metabolites,[8-11] which is a fundamental biological driver for the antibiotic production and the development of antimicrobial resistance (AMR) in the natural environment.[12]

The overall global antibiotic consumption is predicted to reach a 200% increase by 2030 compared to the period of 2000-2015,[13] with 70% of the overall antibiotics used for growth promotion and disease control in animal husbandry.[14] Due to overuse or misuse of antibiotics, the rapid development and proliferation of AMR have become one of the major global health concerns.[15] The discharge of unmetabolized antibiotics into the environment and their effects on the profile of belowground communities are a great concern.[16] Majority of previous studies examining the effects of antibiotics on soil microorganisms, however, have focused exclusively on bacterial and fungal communities,[17-20] but not soil protists, a key component of the soil microbiome. Previous studies reported growth inhibition, cyst formation and/or death of protists under pressures of antibiotics released by selected bacteria,[8, 9, 21] but these studies were conducted

with several model species of bacteria and protists under *in vitro* conditions. Our knowledge of the impacts of antibiotics on the growth and diversity of protists in the complex soil environment remains limited. This knowledge is critical to an improved mechanistic understanding and prediction of the responses of protists, a pivotal component in soil food webs, to increasing global use of antibiotics.

Therefore, to address this knowledge gap, we selected two commonly-used antibiotics with different action modes, i.e. oxytetracycline (OTC) and ciprofloxacin (CFC), and investigated their effects on the diversity and community composition of protists in a soil microcosm incubation. Both taxonomic composition and trophic functional traits of the protistan community were examined to provide comprehensive insights into the changes in community structure and ecological function traits of protists under the pressure of antibiotics. Notably, we focused on protist consumers due to the dominance of bacterivorous consumers and their integral regulation on bacteria mainly via predation in soil ecosystems. We also compared the responses of protistan communities and bacterial communities to antibiotics during the incubation. We hypothesized that (1) common antibiotics can significantly alter the diversity and composition of soil protist community, as antibiotics may directly act on protist or indirectly through impacting bacterial communities, the food sources for protists; (2) consumer protists are more likely affected by the antibiotics than other functional groups (e.g. phototrophs and parasites), because they directly feed on bacteria that are susceptible to antibiotic exposures.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected from a long-term research vegetable farm at Clyde (38°13'02"S, 145°33'24"E), Victoria, Australia in June 2019. The agricultural field is planted with celery and had been fertilized with chicken manure and inorganic fertilizers for more than ten years. The antibiotic residue in the chicken manure was not measured and chicken is not treated with antibiotics as growth promoters in Australia.[22] This site has a mean annual temperature at 19.4 ℃ and a mean annual precipitation at 819 mm. Surface soils (0–15 cm) were taken by mixing multiple soil cores and transported on ice to the laboratory. Soil samples were ground with a jaw crusher and passed through a 2 mm sieve to remove roots and stones. The soil was characterized as a loamy sand with a pH of 7.6. Soil moisture content was 11.18% measured by the loss of weight after oven drying at 105 °C for 24 h. Total carbon and total nitrogen were 2.12% and 0.24%, respectively, as analyzed using a LECO Trumac CN analyzer (LECO, USA).

Laboratory microcosm incubation

Two commonly used synthetic antibiotics in livestock production and human therapy,[16] oxytetracycline (OTC; $C_{22}H_{24}N_2O_9$ $2H_2O$; purity: 90.9%, CAS Number: 6153-64-6, Sigma-Aldrich, Australia) and ciprofloxacin (CFC; $C_{17}H_{18}FN_3O_3$; purity: ⩾ 98%, CAS Number: 85721-33-1, Sigma-Aldrich, Australia), were selected for the microcosm incubation. The two antibiotics have distinct action modes, with OTC and CFC functioning as bacteriostatic and bactericidal antibiotics to disrupt protein synthesis and DNA replication of bacterial cells, respectively.[23, 24] The environmental concentrations of OTC and CFC normally range from micrograms (μg) to

81 milligrams (mg) per kg soil with a typical level of above 1 mg kg^{-1} in agricultural soils.[18, 25, 26]
82 Therefore, we used two environment-relevant concentrations (1 mg kg^{-1} dry soil and 5 mg kg^{-1} dry
83 soil) of OTC and CFC in this incubation study.

84 For soil microcosms, twenty grams of soil (oven dry-weight equivalent) were added into
85 each 250 ml vial. There were five treatments with four replicates (shown in **Figure. S1**), namely
86 control soils without antibiotics, and soils treated with OTC and CFC solutions to achieve two
87 final concentrations (1 mg kg^{-1} dry soil and 5 mg kg^{-1} dry soil). Soils were thoroughly mixed and
88 soil moisture content was maintained as the first-day condition (11.18%) using autoclaved
89 deionized water. The incubation vials were loosely covered to maintain aerobic conditions and
90 incubated in the dark at 25 °C. Autoclaved deionized water was supplemented every three days to
91 maintain the water content. Samples were destructively collected at days 1, 7, 14, 21 and 28, and
92 stored at − 20 °C before molecular analyses.

93 **Characterizing the community compositions of bacteria and protists**

94 Soil DNA was extracted from 0.25 g of soil samples using the Powersoil® DNA Isolation
95 Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The
96 quantity and purity of extracted DNA were evaluated using the NanoDrop spectrophotometer
97 (ND2000c, NanoDrop Technologies, Wilmington, DE, USA).

98 To determine the composition and diversity of bacterial and protistan communities in soils,
99 the V4 region of the bacterial 16S rRNA gene and the eukaryotic 18S rRNA gene were amplified
100 with the primer sets 515F/806R[27] and TAReuk454FWD1/TAReukREV3[28], respectively. The
101 amplicons were purified, quantified, pooled in equimolar and then sequenced on an Illumina
102 MiSeq PE 300 × 2 Sequencer (Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China).
103 Sequences were demultiplexed and trimmed, and the sequences shorter than 75 bp were removed

from the dataset. To guarantee the quality of downstream analyses, we filtered raw pair-end reads to discard those reads containing three or more ambiguous nucleotides and reads with a low average quality score ($Q < 20$). The generated high-quality sequences were processed and analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) 1.91 pipeline.[29] UPARSE was used for chimera removal, and operational taxonomic units (OTUs) were clustered at 3% sequence dissimilarity.[30] A representative sequence of each OTU was randomly selected and used for taxonomic assignments. The taxonomic classifications of bacterial OTUs were assigned by blasting against the SILVA database (V_138),[31] while the Protist Ribosomal Reference (PR2) database (V_4.5)[32] was used to assign taxonomic classification to protists.

Functional assignment for the protistan community

To estimate trophic functional traits of the protistan community, we assigned protistan taxonomic genera into four main functional groups: consumers, parasites, phototrophs and undetermined.[33, 34] Consumers (or predators) represented a group of protists feeding on a wide range of bacteria and fungi (as their favorite foods) as well as preying on other soil eukaryotes (algae, nematodes and other protists).[1] Bacterivorous predators that prey on bacteria are the most abundant group of consumers.[35] Parasites are a group of protists parasitizing on various hosts (animals, plants or other organisms), while phototrophs are protist species obtaining energy via photosynthesis. Unknown taxa of protists were classified as "undetermined". Protistan taxa at the genus level are considered to belong to the same trophic functional group.[34] We adopted the classifications of Dumack et al. (2019) for protist taxa of the supergroup Rhizaria,[36] and Adl et al. (2019)[34] and Voss et al. (2019)[37] for protists in other supergroups **(Table S1)**.

125 **Statistical analyses**

126 Basic mathematical calculations were performed in Microsoft Excel 2016 (Microsoft, USA).
127 Analysis of variance (ANOVA) and Spearman's correlation analyses were used to analyze the
128 differences in diversity, taxonomic compositions and abundance among different treatments over
129 time in SPSS 25 (IBM, USA). *P* value < 0.05 was considered as a significant difference. Principal
130 coordinate analysis (PCoA) based on the Bray-Curtis distance and permutational multivariate
131 analysis of variance (PERMANOVA) with the Adonis function were performed to evaluate the
132 profiles of microbial communities using the 'vegan' package [38] in R. Response ratios based on the
133 mean relative abundance of protists at the class level were calculated by natural log of the ratio of
134 impacts [39, 40].

135 **RESULTS**

136 **Composition of the eukaryotic community**

137 Sequencing and quality filtering resulted in a total of 5,881,949 eukaryotic 18S rRNA gene
138 sequences across all samples, and these sequences were clustered into 4,614 OTUs. After removal
139 of fungal, metazoan, plant (Streptophyta) and unclassified sequences, 2,439,519 high-quality
140 sequences were clustered into 2,573 protist OTUs (55.77% of total OTUs) from all samples with
141 an average of 24,395 sequences per sample. Protists were the most dominant eukaryote (41.47%
142 of the total eukaryotic sequences), followed by fungi (25.08%), metazoa (20.68%) and other
143 unknown eukaryotes (11.62%) **(Figure. 1A)**. Plants, represented by Streptophyta, accounted for
144 approximately 1.15 % of the total sequences.

145 Rhizaria, mostly comprised of the phylum Cercozoa, were the most abundant supergroup of
146 protists (33.73% of the total protist sequences) across all soil samples, followed by other

supergroups including Amoebozoa (28.21%), Stramenopiles (23.21%) and Alveolata (7.49%) (Figure. 1B). Opisthokonta and Archaeplastida had similar relative abundances around 3.01% and 3.41%, respectively. Three rare supergroups of soil protists were also detected: Hacrobia (0.69%), Excavata (0.20%) and Apusozoa (0.06%).

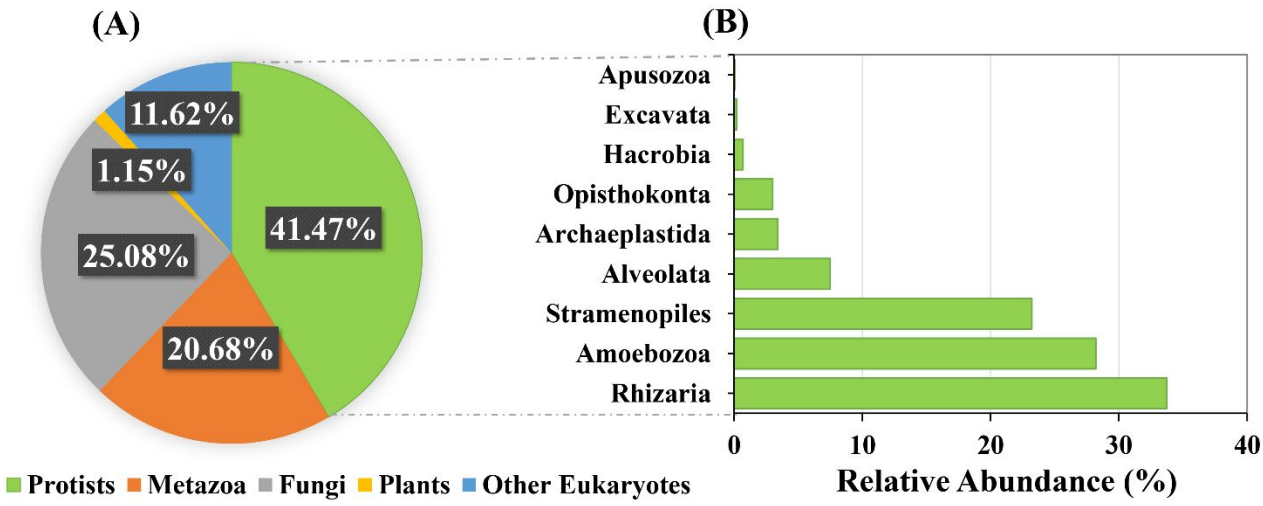

Figure 1. The community compositions of eukaryotes across all soil samples (A) and the relative abundance of protist supergroups (B).

Effects of antibiotics on the protistan and bacterial diversity

The application of OTC or CFC had no significant impacts on the alpha diversity of protists ($F = 0.06$, $P = 0.132$), but significantly decreased the bacterial diversity ($F = 2.77$, $P = 0.033$), as revealed by the Shannon index (Figure. S2, Table 1). Incubation time significantly altered the alpha diversity of protists ($F = 5.94$, $P < 0.001$) (Table 1), which significantly decreased over time during the incubation. Antibiotics, sampling time and their interactions significantly affected the beta diversity of both protists and bacteria, and stronger effects were observed in the diversity of bacterial community (P values < 0.001) (Table 1).

Principal coordinate analysis (PCoA) based on the Bray-Curtis distance differentiated the impacts of OTC and CFC on the protistan community composition at different time points **(Figure. 2)**. Significant effects of OTC addition on the community compositions of soil protists were observed at Day 14 (Adonis test, $P < 0.05$) and Day 21 ($P < 0.01$), while CFC addition significantly changed the community compositions of soil protists at Day 7 ($P < 0.05$) and Day 14 ($P < 0.001$). No significant changes in the profile of protists were observed at the beginning or end of the incubation either for the OTC or CFC treatments ($P > 0.05$).

Table 1. Two-way ANOVA analyses to examine the effects of antibiotics, sampling time and their interactive effects on the bacterial and protistan communities.

	Treatments		**Sampling time**		**Treatment× Sampling time**	
	F	*P*-values	*F*	*P*-values	*F*	*P*-values
1) Bacteria						
Alpha diversity	**2.77**	0.033	**14.38**	<0.001	**4.55**	<0.001
Beta diversity	**22.37**	<0.001	**48.38**	<0.001	**20.38**	<0.001
2) Protists						
Alpha diversity	0.06	0.132	**5.94**	<0.001	1.62	0.08
Beta diversity	**7.10**	<0.001	**22.0**	<0.001	**5.90**	<0.001

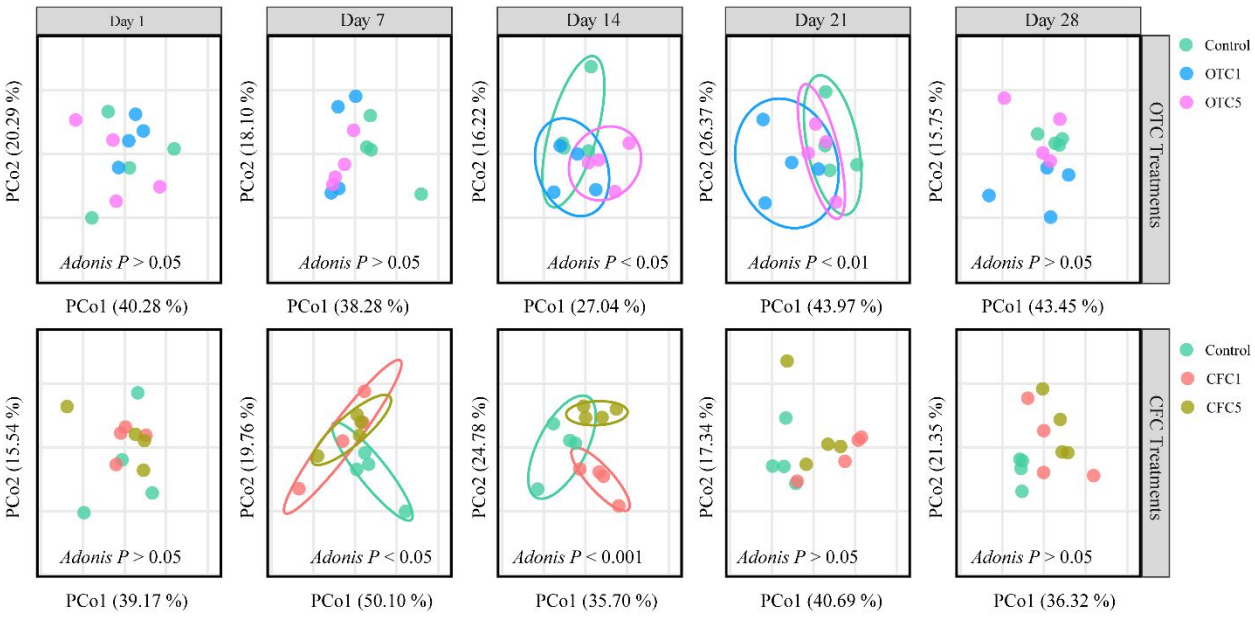

Figure 2. Principal coordinate analysis (PCoA) based on the Bray-Curtis distance showing the effects of antibiotics on the changes in the protistan community compositions over time. Adonis test estimates significant differences in the profile of protists among different treatment. OTC, oxytetracycline; CFC, ciprofloxacin.

Effects of antibiotics on the community composition of protists

The addition of antibiotics had no significant effects on the total relative abundance of protist supergroups ($P > 0.05$), except Archaeplastida **(Figures. 3 and S3)**. However, we identified that some specific protist taxa at the class level in several supergroups including Rhizaria, Amoebozoa, Stramenopiles, Archaeplastida and Apusozoa were sensitive to OTC and CFC. In Rhizaria, OTC5 significantly reduced the relative abundance of Endomyxa ($P < 0.05$), while a higher abundance of Filosa-Sarcomonadea was found in all CFC-treated soils ($P < 0.05$).

Among Amoebozoa**,** most of common amoebozoan lineages at the class level were not affected by antibiotics. However, the relative abundance of a rare taxon Discosea-Flabellinia (< 1% of total supergroup) was significantly decreased by the CFC addition, as revealed by the log-

based response ratios ($P < 0.05$, **Figure. 4**). Likewise, in Stramenopiles, CFC addition had significant effects on less abundant taxa, with an increase of Labyrinthulea in the CFC1 and CFC5 treatments and a decline of Bacillariophyta in the CFC5 treatment ($P < 0.05$; **Figure. 3**). Notably, Archaeplastida, entirely composed of green algae Chlorophyta, was the only supergroup being significantly increased by antibiotic addition, with more abundant Chlorophyceae and Trebouxiophyceae at the class level observed in the OTC and CFC1 treatments compared to control soils **(Figures. 3 and 4)**. Similarly, Apusomonadidae_Group-2 of a rare supergroup Apusozoa considerably increased their abundance in the CFC5 treatment ($P < 0.01$).

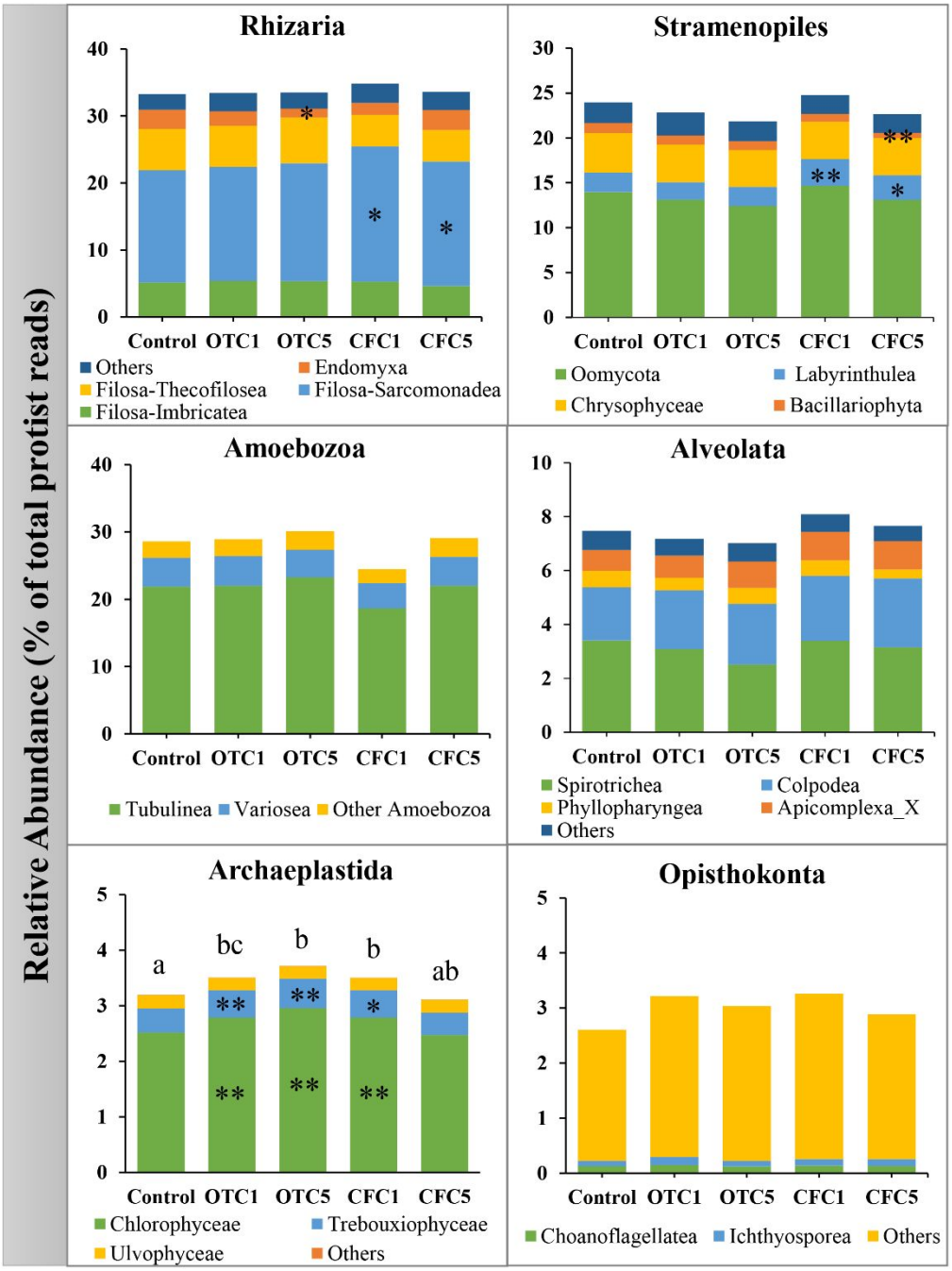

Figure 3. Taxonomic profiles of soil protists at the class level for all dominant supergroups. Asterisks or letters indicate significant difference in protistan supergroups in treated samples compared to controls under exposure to oxytetracycline (OTC) and ciprofloxacin (CFC) (ANOVA, $*P < 0.05$ and $**P < 0.01$).

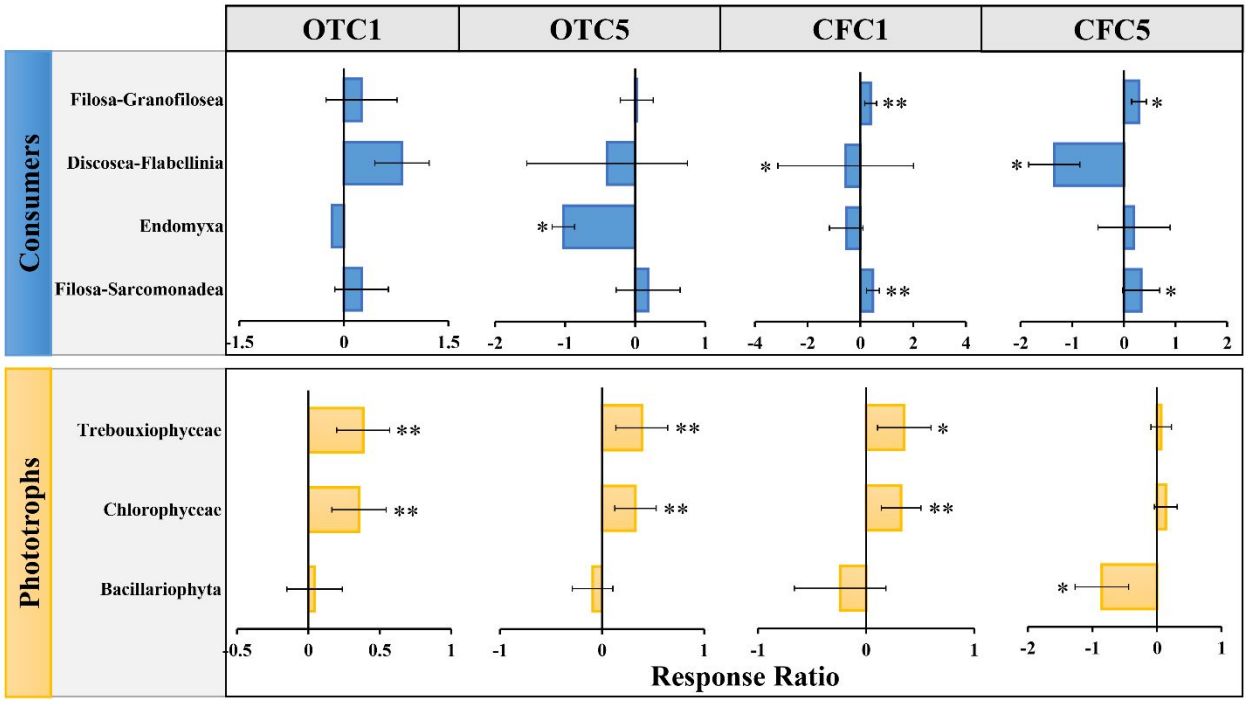

Figure 4. Response ratios of the relative abundances of functional protist lineages to the addition of antibiotics. Only the protist lineages (at the class level) of consumer and phototrophic groups with significant differences compared to controls (*$P < 0.05$ and **$P < 0.01$, ANOVA) are plotted on a log base 2 scale. Error bars indicate standard errors (n = 4). OTC, oxytetracycline; CFC, ciprofloxacin.

Effects of antibiotics on functional groups of protists

Functional groups of protists encompassed consumers (70.08% of protists), parasites (11.75%), phototrophs (5.19%) and undetermined (12.98%) (**Figure. 5A**). The composition of consumers spanned a wide range of dominant supergroups: Rhizaria (31.68%), Amoebozoa (28.26%), Alveolata (7.20%) and Stramenopiles (1.93%) **(Figure. 5B)**. Within consumers, a significant decrease in the relative abundance of Endomyxa (Rhizaria) and Discosea-Flabellinia (Amoebozoa) at the class level was observed in the OTC5 and CFC treatments, respectively (**Figure. 4**, ANOVA, $P < 0.05$). In contrast, consumers Filosa-Granofilosea and Filosa-

Sarcomonadea (Rhizaria) were more abundant in the CFC-treated soils than control **(Figures. 4 and 5C)**. Parasites and undetermined groups showed a similar pattern, with no significant effects of antibiotics in their relative abundances observed among treatments ($P > 0.05$) **(Figure. S4)**. In addition, the relative abundance of phototrophic protists was significantly increased in the OTC1 treatment ($P < 0.05$) **(Figure. S4)**. Among them, the relative abundance of Chlorophyceae and Trebouxiophyceae (Archaeplastida) increased in the OTC and CFC1 treatments, while the relative abundance of Bacillariophyta (Stramenopiles) decreased in the CFC5 treatment **(Figure. 4)**.

PCoA analyses illustrated that both OTC and CFC altered the overall profile of consumer community over time **(Figure. S5)**. The OTC addition only changed the community composition of protistan consumers at Day 14 (Adonis test, $P < 0.01$), and the consumer community composition was significantly affected by CFC at Day 7 ($P < 0.05$) and Day 14 ($P < 0.01$).

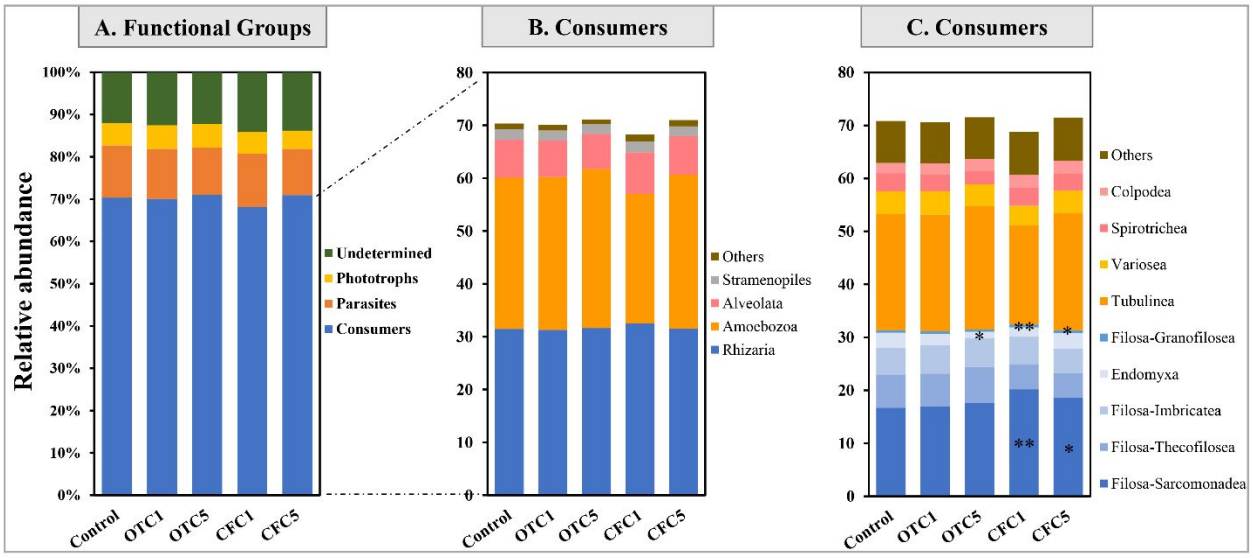

Figure 5. The relative abundances of functional groups of protists (A), and major protistan consumers at the supergroup level (B) and the class level (C) across the five treatments. Significant differences compared to controls under exposure to oxytetracycline (OTC) and ciprofloxacin (CFC) are indicated by asterisks: *$P < 0.05$ and **$P < 0.01$ (ANOVA, LSD test).

DISCUSSION

In our study, in contrast to the hypothesis 1, the addition of antibiotics did not change the alpha diversity of soil protists but significantly decreased the bacterial diversity **(Table 1)**. Antibiotics imposed a weaker pressure on the protistan diversity than the bacterial diversity, which indicated that protists may have a higher tolerance to antibiotic perturbation or only a small proportion of protistan taxa are sensitive to antibiotics. Nevertheless, the antibiotics caused significant changes in the community composition of soil protists and consumers during the middle of the incubation but no difference among treatments observed at the end of the incubation **(Figures 2 and S5)**, which could be possibly explained by (i) the rapid degradation of antibiotics in soil microcosms; (ii) the recovery of soil protists at the late stage under the reduced pressure of antibiotics; and (iii) the recovery of bacterial biomass at the late stage under the reduced antibiotic pressure, resulting in more food sources for protistan consumers. Within the trophic functional groups of protists, we also found that the relative abundance of some protist lineages of the consumers was substantially influenced by the antibiotic disturbance **(Figure 4)**. Protistan consumers primarily function as bacterivores (feeding on bacteria), eukaryvores (feeding on algae, fungi, protists and other eukaryotes) and omnivores (feeding on eukaryotes and bacteria).[41, 42] In agreement with the hypothesis 2, protistan consumers were more affected by antibiotics than other functional groups of protists. The reduction in the abundance of bacterivorous consumers Discosea-Flabellinia (Amoebozoa) were found in response to CFC, whereas the higher concentration of OTC5 caused a negative influence on the abundance of the Endomyxa. In contrast, the relative abundance of bacterivorous consumers Filosa-Granofilosea and Filosa-Sarcomonadea increased in the CFC-treated soils. Probably, under the antibiotic pressure, Filosa-

Sarcomonadea and Filosa-Granofilosea might take advantage of antibiotic-susceptible bacteria as food source to maintain and promote their growth.

In this microcosm incubation, the different responses of protistan consumers to antibiotic exposure could be due to direct and indirect effects of antibiotics. Firstly, we proposed that the negative influence of antibiotics on protistan consumers might be directly caused by toxicity of antibiotics which adversely impact the cellular processes of protist population (including growth restriction, encystation, cyst reactivation, paralysis and cell lysis)[9-11] and consequently result in the fatality of sensitive protists. This is supported by the insignificant correlations between the relative abundance of protistan consumers (Endomyxa and Discosea-Flabellinia) and the bacterial taxa (showing significant changes in their abundances during incubation) under the OTC and CFC exposure **(Table S2)**. These results indicate that the decreasing abundance of the bacterivorous consumers Endomyxa and Discosea-Flabellinia might be due to the direct toxicity of antibiotics OTC and CFC, rather than the alteration of their food sources (bacteria). In particular, the bactericide CFC imposed a stronger stress on the protistan community than the bacteriostatic OTC, and more significant impacts were rendered by the higher concentrations (5 mg kg^{-1}) of both antibiotics **(Figures. 3, 4, 5C and S3)**. The bactericidal CFC might be more toxic to the protistan community than bacteriostatic OTC, probably owing to their different action modes: CFC is a bactericide to cause cellular death by targeting DNA replication, while OTC functions as a bacteriostat with inhibitory effects on protein synthesis.[24, 43] However, molecular mechanisms of antibiotic actions on protists need to be deciphered in future studies. Secondly, antibiotics could have indirect effects on the protistan community by impacting their main preys – bacteria. Antibiotics are renowned as a strong selection pressure for the development and evolution of the antibiotic resistance of bacterial community,[44] and thus the food source of some protist consumers

may be considerably shifted by the increasing population of antibiotic resistant bacteria (ARB) and death of the susceptible bacterial population. In this work, the OTC and CFC treatments dramatically altered the relative abundances of some bacterial taxa (e.g., Rokubacteria, Latescibacteria and Hydrogenedentes), which had significantly negative associations with the increasing abundance of the protistan consumers Filosa-Granofilosea and Filosa-Sarcomonadea **(Table S2)**. The antibiotic pressures might increase the availability of antibiotic-susceptible bacteria and non-antibiotic-producing ARB, which could be a food source to maintain and facilitate the growth and development of the protistan consumers. Therefore, it is not surprising to observe the decrease in some protistan taxa but increase in other taxa of consumers under the pressure of antibiotics in our incubation study. Together, our results highlight that consumers, an important functional group of protists that mainly prey on bacteria, are susceptible to antibiotics in the complex soil matrix, and further support the previous perceptions that predation-resistant bacteria can produce antibiotics to fight against the protist predation in natural environments.

We found the increasing abundance of the phototrophic group and two phototrophic taxa Trebouxiophyceae and Chlorophyceae (Archaeplastida, Chlorophyta), in opposition to an insignificant difference in parasitic protists during the incubation with antibiotics **(Figures. 4 and S4)**. The protistan consumers including eukaryvores and omnivores can prey on phototrophic protists (e.g., algae). The addition of antibiotics in the soil microcosms might impose a pressure on the growth of antibiotic-susceptible bacteria, and these susceptible bacteria might be a major energy source for soil protist consumers. Hence, there might probably be less predatory activities of consumers on algae and other phototrophic protists, and this could maintain and increase the abundance of phototrophic groups and taxa. The well-adaptation and viability of phototrophic protists were also reported after the addition of fertilizers[45] and in a severe desert condition[46]. For

parasites, the stability of this trophic group in our investigation may be explained by that action modes of antibiotics do not target on main hosts (soil metazoa) of parasitic protists while parasites are mainly associated and driven by their hosts (plants and other soil organisms).[47]

In conclusion, we provide novel evidence that short-term addition of common synthetic antibiotics significantly influenced the community composition of soil protists and the relative abundances of specific protist lineages in the laboratory microcosm incubation. Our results highlight that consumers, an important functional group of protists that mainly prey on bacteria, are sensitive to both of the antibiotics. Our findings support the previous perceptions that bacteria can produce antibiotics to fight against the protist predation in natural environments, and further caution the potentially substantial impacts of synthetic antibiotics on the protistan community and their ecological functions under the scenarios of projected increasing global antibiotic usage.

ASSOCIATED CONTENT

Supporting Information

The experimental design of the laboratory microcosm study; alpha diversity of the protist and bacterial communities; changes in the relative abundance of protistan taxa at the class level in rare protist supergroups; the relative abundances of functional groups of protists at the supergroup and class levels; the effects of antibiotics on the distribution patterns of protistan consumers; taxonomic classifications and functional trait assignments of protists; significant correlations (Spearman) between the abundance of bacterial and consumer taxa with significant changes within and after the incubation with OTC and CFC.

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Notes

The authors declare no competing financial interest.

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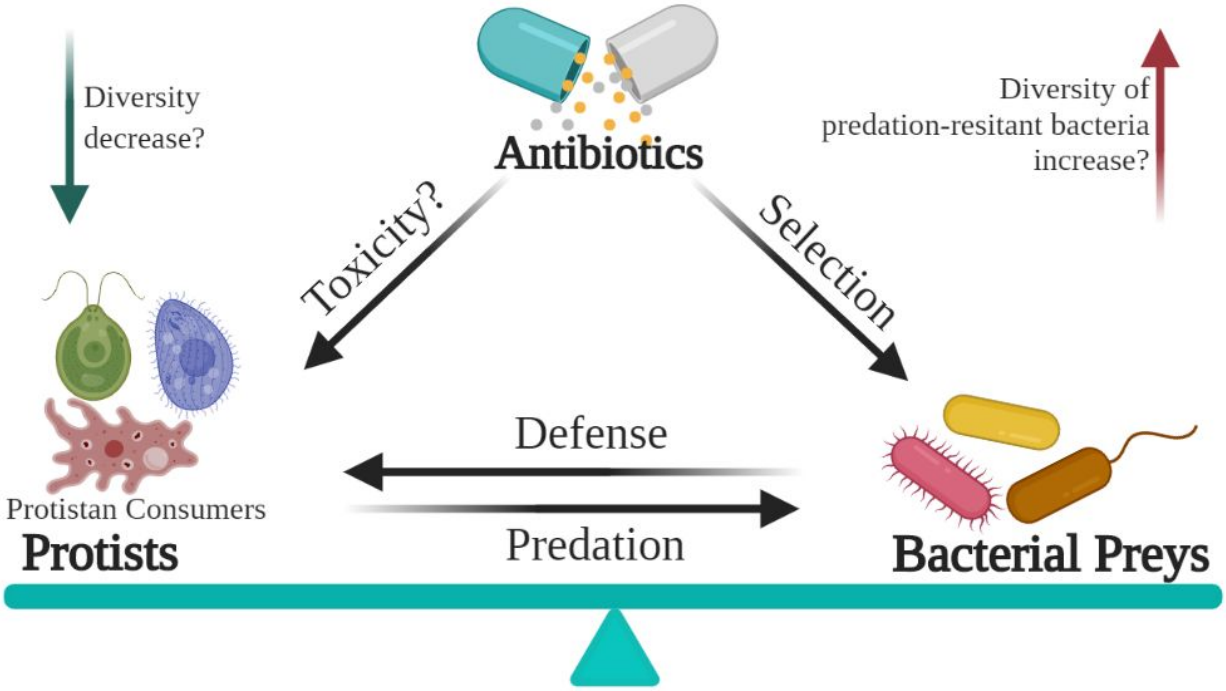
472 **Abstract Art**



473