Received: 11 October 2022 | Accepted: 20 July 2023

DOI: 10.1111/1462-2920.16477

BRIEF REPORT

ENVIRONMENTAL MICROBIOLOGY Applied Microbiology International

Movement of protistan trophic groups in soil–plant continuums

Chenshuo Lin[1,2] | Wen-Jing Li[1,2] | Li-Juan Li[1,2] | Roy Neilson[3] | Xin-Li An[1,2] | Yong-Guan Zhu[1,2,4]

[1]Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China

[2]University of Chinese Academy of Sciences, Beijing, China

[3]Ecological Sciences, The James Hutton Institute, Dundee, Scotland, UK

[4]State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

Correspondence
Yong-Guan Zhu, 1799 Jimei Road, Xiamen 361021, China.
Email: ygzhu@rcees.ac.cn

Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 32061143015, 42090063; Scottish Government Rural and Environment Science and Analytical Services (RESAS)

Abstract

Protists, functionally divided into consumers, phototrophs, and parasites act as integral components and vital regulators of microbiomes in soil–plant continuums. However, the drivers of community structure, assembly mechanisms, co-occurrence patterns, and the associations with human pathogens and different protistan trophic groups remain unknown. Here, we characterized the phyllosphere and soil protistan communities associated with three vegetables under different fertilization treatments (none and organic fertilization) at five growth stages. In this study, consumers were the most diverse soil protist group, had the role of inter-kingdom connector, and were the primary biomarker for rhizosphere soils which were subjected to decreasing deterministic processes during plant growth. In contrast, phototrophs had the greatest niche breadth and formed soil protistan hubs, and were the primary biomarkers for both bulk soils and the phyllosphere. Parasites had minimal input to microbial co-occurrence networks. Organic fertilization increased the relative abundance (RA) of pathogenic protists and the number of pathogen–consumer connections in rhizosphere soils but decreased protistan richness and the number of internal protistan links. This study advances our understanding of the ecological roles and potential links between human pathogens and protistan trophic groups associated with soil–plant continuums, which is fundamental to the regulation of soil–plant microbiomes and maintenance of environmental and human health.

INTRODUCTION

Soil and plant microbiomes are closely interconnected (Brevik et al., 2020). As one of the important sources of plant microorganisms (Edwards et al., 2015), soil microbiomes can not only deliver essential elements for plant growth but also shape the structure, composition, and function of plant microbiomes (Trivedi et al., 2020). The complex microbiomes associated with soil–plant continuums serve as key determinants of multiple ecosystem functions, including nutrient cycling, primary production, plant performance, and pathogen suppression (Delgado-Baquerizo et al., 2016, 2020; Fitzpatrick et al., 2020; Martin et al., 2017; Wall et al., 2015). Thus, microbial communities are crucial for the health of soil–plant continuums (Berg et al., 2020), which is inseparably linked to human health through the one health concept (Banerjee & van der Heijden, 2023). Soil, plant, and human microbiomes are closely interconnected. For example, microbes including opportunistic human pathogens derived from vegetables and soils can integrate with the human gut microbiome by ingestion and thereby affect human health (Hirt, 2020). Therefore, investigating the characteristics of microbiomes associated with soil–plant continuums is important for human and environmental health.

Protists are phylogenetically diverse and functionally important eukaryotes but arguably overlooked components of the soil microbiome (Xiong et al., 2018). Based on life history strategies, protists can be divided into three major trophic groups: phototrophs, consumers, and parasites (Singer et al., 2021). Phototrophs,

including most free-living algae (Santos et al., 2020), are important primary producers, fixing carbon through photosynthesis (Lambert et al., 2022). Approximately one-quarter of the world's photosynthesis is carried out by phototrophs, highlighting their important contribution to soil carbon sequestration (Seppey et al., 2017). Consumers prey on a range of bacteria (including opportunistic human pathogens), fungi, protists (Geisen et al., 2016) as well as nematodes (Mitchell, 2015), with recent studies considering consumers as key determinants of plant health (Triplett et al., 2023) and crop yield (Guo et al., 2021). Consumers can also act as so-called Trojan horses, internalizing, sheltering, and potentially transmitting opportunistic human pathogens such as *Pseudomonas*, *Mycobacterium*, and *Legionella* (Nisar et al., 2022). Parasites account for approximately 15% of known protists (Sun & Luo, 2005). Despite their low abundance, parasites such as *Oomycota*, *Ichtyosporea*, and *Giardia* (Mahé et al., 2018) can cause plant, animal, and human diseases (Bates et al., 2013). These diverse protists interact with bacterial and fungal communities in complex ways, and such inter-kingdom interactions may affect soil microbiome function and the colonization and transmission of potential human pathogens (Duran et al., 2018). However, a detailed understanding of the dynamics of each protistan trophic group associated with soil–plant continuums, their microbial co-occurrence patterns, and associations with the environment and human health is currently unknown.

Protist communities associated with soil–plant continuums are known to respond to numerous environmental factors, including soil properties, biotic factors, and human perturbation (Zhao et al., 2020). For example, bacteria were the key factor driving changes in core soil protist consumers, while core protist phototrophs were governed by climate (Chen et al., 2021). A recent study highlighted that protist consumers and parasites were sensitive to nitrogen fertilization (Fiore-Donno et al., 2020). However, these studies only investigated the influence of single factors on protists. In a soil–plant continuum where multiple environmental factors interact, it is unclear which factors are the main determinants for the different protistan trophic groups and how they respond to various environmental factors during plant growth. Furthermore, deterministic and stochastic processes also influence the protist community assembly in the soil–plant continuum (Muller et al., 2016). Thus, assembly mechanisms for protist communities during plant growth remain poorly understood.

In this study, we used a microcosm experiment with three vegetable species (*Brassica oleracea* var. *capitata*, *Lactuca sativa*, and *Brassica chinensis*) to examine the dynamics of protistan communities associated with the phyllosphere, rhizosphere, and bulk soils at five growth stages under different fertilization treatments (none and organic fertilization). Given the widespread use of fertilizer for vegetable production and the known impact of fertilizer on protists (Ren et al., 2023), it is important to investigate the effects of fertilization on the dynamics of protists associated with the soil–plant continuum. We aim to (1) detail the assembly mechanisms and characteristics of protists with different life strategies (consumers, phototrophs, and parasites) under the effects of agricultural management (with or without organic fertilizer), plant species, compartment niches (phyllosphere and rhizosphere and bulk soils) and plant development stages; and (2) disentangle the role of each protistan group associated with microbial co-occurrence networks and their potential association with human pathogens.

EXPERIMENTAL PROCEDURES

Experimental design and sample collection

We conducted a pot experiment in a greenhouse (25°C) from June to August 2020. Three vegetables, brassica (*B. chinensis*), cabbage (*B. oleracea* var. *capitata*), and lettuce (*L. sativa*) were grown with either an application of organic fertilizer (80 g chicken manure g^{-1} dry soil) or no fertilization. These three vegetables are common green leafy vegetables that consumers can buy in markets in daily life. For each vegetable, three replicates were established for each treatment.

Soil collected from a vegetable field in Xiamen City, Fujian, China (24°64′ N, 118°05′ E) was passed through a 2-mm mesh sieve and pre-incubated for 1 week (25°C). To separate rhizosphere and bulk soils, seeds were sown in nylon mesh bags (20 cm in height; 19.5 cm in diameter), limiting the development of a rhizosphere environment but allowing moisture equilibration (Nuccio et al., 2020). All treatments were kept around 70% water-holding capacity daily with deionized water. All vegetables ripened after 60 days. Rhizosphere and bulk soil samples ($n = 154$) were collected at 0, 14, 28, 42, and 60 days after sowing. Phyllosphere samples were collected on Days 14, 28, 42, and 60, but samples collected at Day 14 were removed from downstream analysis due to an insufficient number of protistan sequences.

All samples for molecular analyses were frozen immediately at −20°C. Soil samples were sieved <2 mm before analyses of soil physicochemical properties including, pH, moisture, dissolved organic carbon (DOC), dissolved total nitrogen (DTN), total nitrogen (TN), and total carbon (TC). Briefly, soil pH was measured with a soil-to-water ratio of 1:2.5 (w/v) using a pH metre (IS126C, Insmark, Shanghai, China). DOC and DTN were extracted at a soil-to-water ratio of 1:5 (w/v) on a TOC analyser (TOC-LCPH, Shimadzu, Japan). TC and TN were measured using a combustion method using an element analyser (Vario MAX C/N, Germany).

DNA extraction and amplicon sequencing

Soil microbial DNA was extracted from 0.5 g of soil. For phyllosphere DNA extraction, approximately 10 g of leaves were placed into individual conical flasks (250 mL) containing 100 mL 0.01 M sterile buffer solution (120 mg NaCl, 4 mg $MgSO_4{\cdot}7H_2O$, 4 mg $CaCl_2{\cdot}2H_2O$, 359 mg $Na_2HPO_4{\cdot}12H_2O$, and 130 mg KH_2PO_4 in 1 L of deionized water) and shaken (200 rpm) at 25°C for 2 h. Phyllosphere samples were sonicated for 10 min and filtered through a 0.22-μm cellulose membrane to collect the microbial communities. The membranes with adhering phyllosphere microbes were cut into small pieces with sterilized scissors. Both soil and phyllosphere DNA was extracted using a FastDNA Spin Kit for Soil (MP Bio, USA) according to the manufacturer's instructions. Extracted DNA was quantified using a NanoDrop Spectrophotometer (Nanodrop 2000, Thermo Scientific, USA) and visualized on a 1.0% agarose gel electrophoresis.

Primer sets 799F/1193R (Beckers et al., 2017), ITS1F/ITS2R (Bodenhausen et al., 2013), and TAReukFWD1F/TAReukREV3R (Stoeck et al., 2010) that excluded chloroplasts, amplified the V5–V7 region of the bacterial 16S rRNA genes, the fungal ITS1 region, and the V4 region of the eukaryotic 18S rRNA gene. Sequencing was performed using an Illumina MiSeq PE300 sequencing platform (Majorbio, Shanghai, China). Bacterial 16S rRNA was quantified using a Roche 480 (Roche Inc., USA) following an SYBR Green approach as described previously (Chen et al., 2017).

Sequencing

Amplicon sequences were processed using the QIIME2 pipeline (version 2018.11, https://qiime2.org) (Bolyen et al., 2019). Amplicon sequence variants (ASVs) were identified using the DADA2 plugin (Callahan et al., 2015) for error correction, chimera identification, quality filtering, and doubleton removal. Protistan sequences were taxonomically assigned using the Protist Ribosomal Reference (PR2) database version 4.14.0 (https://github.com/pr2database/pr2database). Bacterial and fungal taxonomic identity was determined using the Silva (silva_132_16S.97) (Quast et al., 2013) and UNITE (Nilsson et al., 2019) databases, respectively. Chloroplast and mitochondrial sequences were removed from the bacterial, protistan, and fungal ASV tables. Potential bacterial pathogen ASVs were identified by comparison with a reference database of bacterial pathogen 16S rRNA sequences as previously described with an *E*-value $<1 \times 10^{-5}$ and a strict similarity threshold (>99%) (Yang et al., 2020). For protistan ASV, any ASVs with a taxonomic assignment of 'Fungi, Metazoa, Rhodophyta, Streptophyta, or Embryophycaea' were removed. Community matrices were rarefied to 1050 reads per sample for protists; 41,379 reads for bacteria; and 11,035 reads for fungi. We defined core protists and core soil protistan as protistan ASVs present in either all samples or all soil samples, respectively. Protistan trophic groups were assigned as consumers (predators of bacteria, other protists, and micrometazoa) (Geisen, Mitchell, Adl, Bonkowski, Dunthorn, Ekelund, Fernández, et al., 2018), parasites (parasites of plants, protists, and metazoa), phototrophs (photoautotrophic algae act as primary producers), or undetermined (Adl et al., 2019). We also identified parasites associated with human and animal gut microbes using a microeukaryotic gut parasite database derived from wastewater treatment plants (Freudenthal et al., 2022). Raw sequences have been deposited into the GenBank Sequence Read Archive with accession number PRJNA849277.

HT-qPCR assays

Marker genes for human pathogens in soil samples collected at Day 60 (harvest time) were quantified by a TaqMan probe-based HT-qPCR method (WaferGen SmartChip Real-Time PCR system platform; WaferGen Inc., USA) as previously described (An et al., 2020). HT-qPCR assays were performed in triplicate using a TaqMan® Gene Expression Master Mix kit. Primer and pathogen information is detailed in Table S1.

Statistical analysis

To determine the distribution of protistan communities in the phyllosphere and soils with different treatments, principal coordinates analysis (PCoA) was performed based on Bray–Curtis dissimilarity measures. Permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was used to determine the effect of factors on community dissimilarity using the 'vegan' R package (v3.6.2) (Oksanen et al., 2018). Protist alpha diversity was determined using the number of observed ASVs after subsampling from the rarefied QIIME2 ASV table. Linear mixed models (LMMs) were used to assess the effects of experimental treatments on protistan diversity using the R package 'lme4' (Bates et al., 2017). More detailed information about LMMs was shown in the Supporting Information. Nonparametric Mann–Whitney tests or Kruskal–Wallis tests (SPSS, IBM, USA) were applied to compare microbial abundances, diversity, or soil properties parameters. SourceTracker analysis was used to identify the sources of protistan communities in the soil–plant continuum (Knights et al., 2011). Indicator protists were identified by linear discriminant analysis (LDA) and effect size (LEfSe) using the suite of 'Omicstuido'

online tools (https://www.omicstudio.cn/tool/) (Segata et al., 2011). The modified stochasticity ratio (MST) quantified the relative importance of stochastic and deterministic processes for protistan community composition, with 50% set as the threshold for determining whether deterministic or stochastic processes were drivers (Ning et al., 2019). Niche breadth was calculated according to Levin's niche breadth using the R package spaa (Zhou & Ning, 2017). A wide niche breadth indicates that an ASV occurs widely and evenly among samples (Mo et al., 2020).

To explore the potential co-occurrence pattern of protistan, bacterial (including identified potential pathogens), and fungal taxa associated with the soil–plant continuum, networks of different compartment niches were built with microbial ASVs occurring in >30% of samples and visualized by Gephi 0.9.2. A pairwise Spearman correlation with a coefficient of (ρ) >0.5 (or <−0.5) and an associated p-value <0.01 was selected for the network analysis using the 'psych' R package (Spearman; *P*. adjust method: FDR) (Revelle, 2020). Nodes were assigned their network roles based on their within-module connectivity (Zi) and among-module connectivity (Pi) (Guimera & Nunes Amaral, 2005). Nodes were classified as peripheral (Zi < 2.5 and Pi < 0.62), module hub (Zi > 2.5 and Pi < 0.62), network hub (Zi > 2.5 and Pi > 0.62), and connector (Zi < 2.5 and Pi > 0.62) (Shi et al., 2020). The number of each taxon as connectors and module hubs, the number of pathogen–protist connections, and the proportion of intra-kingdom and inter-kingdom edges were also determined.

Structural equation models (SEM) were used to analyse the effects of organic fertilizer, plant growth stage, soil properties (pH, DOC, TN, TC, water content, and DTN), compartment niche, bacterial, fungal, and protistan communities of trophic groups within protistan communities associated with soil–plant continuums. All model indicators satisfied the following requirements: chi-square (p > 0.05), high goodness of fit index (GFI > 0.9), and the root mean square error of approximation (RMSEA < 0.05) (Schermelleh-Engel et al., 2003). SEM used Amos Graphics v22 (IBM Corp., Armonk, NY, USA).

RESULTS

Dynamics of soil and phyllosphere protists

Phototroph PASV1 (Archaeplastida; *Desmodesmus komarekii*) was the only core protist (Figure S1) present in almost all soil and phyllosphere samples. We also detected 37 core soil protists but no core phyllosphere protists (Figure S1).

Archaeplastida (28.7%), Rhizaria (23.8%), and Amoebozoa (16.4%) were the most abundant soil protist phylum (Figure 1A). Driven by consumers, the most diverse protists (Figure 1B; p < 0.05), the diversity of soil protists was significantly greater than phyllosphere protists (nearly seven-fold; Figure 1B; p < 0.001). In terms of relative abundance (RA), consumers with an average RA of 54.5% decreased until 42 days and thereafter slightly increased, whereas phototrophs with an average RA of 41.4% had the opposite trend, except for those associated with soil and planted with Brassica (Figure 1A). The diversity of soil consumers had a similar dynamic pattern to that of RA (Figure 1C). Consumers had a greater RA in the rhizosphere and fertilized soils than in bulk and control soils (Figure 1A; p < 0.05), and were also the main protistan biomarkers in the rhizosphere and fertilized soils (Figure S2A,B). In contrast, phototrophs were the main protistan biomarkers in bulk and control soils (Figure S2A,B) and had a greater RA than in rhizosphere (Figure S3; p < 0.001) and fertilized soils (Figure 1A; p < 0.05). Soil parasites had the lowest diversity and RA (~2.6%) (Figure 1A,B) with *Oomycota* (often plant pathogens) being the most abundant soil parasite accounting for about 1.6% of total protists (Figure S4). Fertilized bulk soil harboured more plant, animal, and human parasites such as *Oomycota*, *Anurofeca*, and *Cryptosporidiidae*, especially at Day 0 (Figure S4; p < 0.05). Additionally, significant negative correlations were found between the absolute abundance of *Legionella* spp. *23S rRNA* (the most frequently detected pathogen marker gene) and the diversity of consumers and phototrophs in soil (Figure 1D; p < 0.05). The RA of consumers and phototrophs, respectively positively and negatively correlated with *Legionella* spp. *23S rRNA* (Figure 1D; p < 0.001). Parasites were not correlated with human pathogen marker genes.

The taxonomic composition and diversity of phyllosphere protists differed significantly from soil (Figure 1E; $p = 0.001$; Figure 1B; p < 0.001; Table S2; PERMANOVA, $R^2 = 22.9\%$, p < 0.001; Table S3; LMM $F^2 = 629.4$, p < 0.001). The most abundant phyllosphere protist phyla were Stramenopiles (63.3%) and Archaeplastida (21.8%; Figure 1A). Phototrophs dominated the phyllosphere protist communities with the greatest RA (84.2%; Figure 1A; p < 0.05) and diversity (p < 0.001; Figure 1B); however, their RA decreased with time. Although 66% of phyllosphere protistan ASVs (accounting for 86.2% of RA of phyllosphere protists) were present in soil (Figure S5A), external sources were the largest contributor (72.1%) to phyllosphere protist communities (Figure S5B).

Drivers of protist variation

To assess the contribution of potential drivers of protist communities along soil–plant continuums, we used

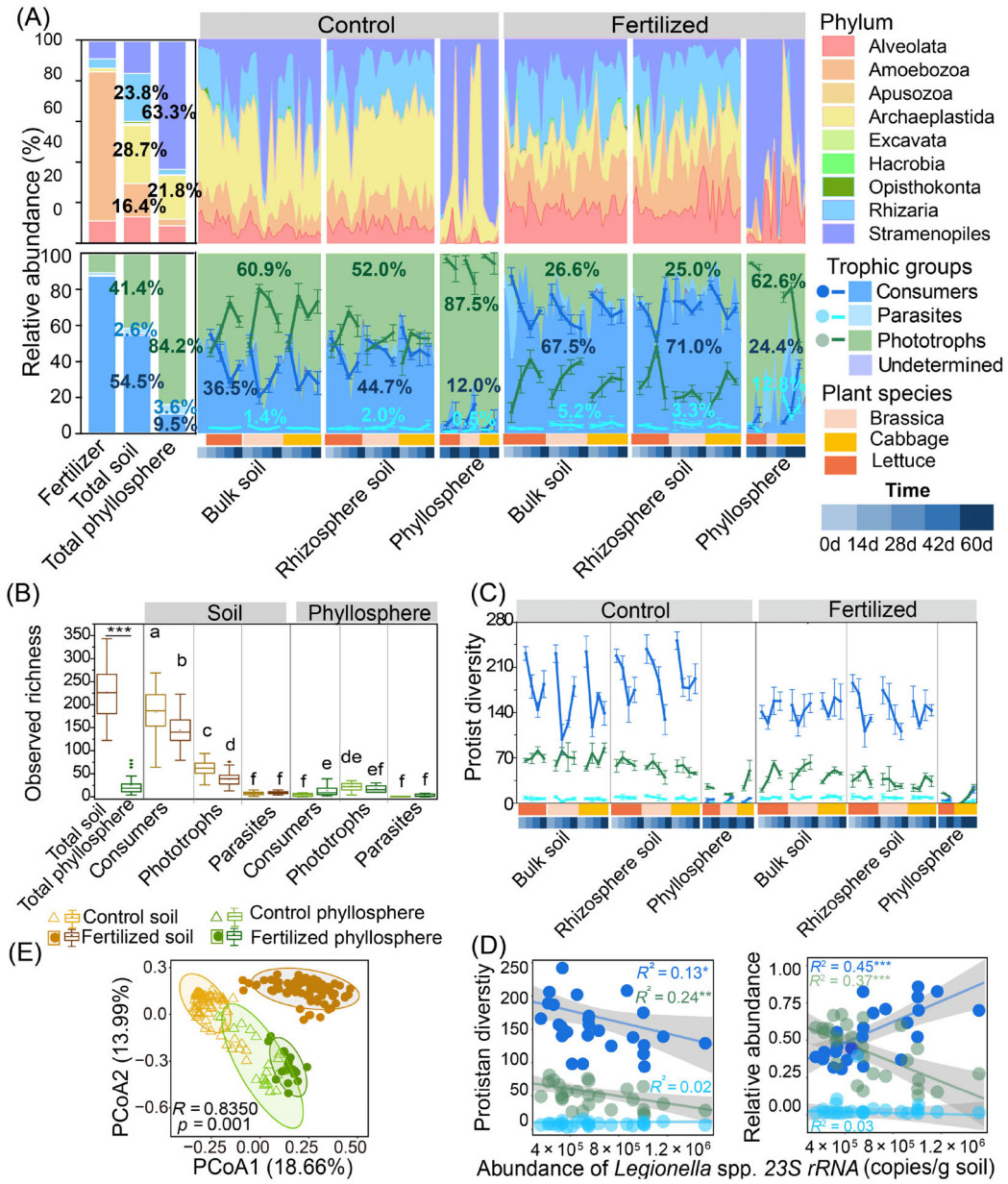

FIGURE 1 (A) Protistan community composition at the phylum level and the dynamic relative abundance (RA) of different protistan trophic groups. Numbers and trendlines in dark green, blue, and dark blue represent the average relative abundance of phototrophs, parasites, and consumers and their temporal variations, respectively. (B) Observed the richness of each trophic protist group in the soil and phyllosphere. Different letters above the boxes indicate a significant difference determined by the nonparametric Kruskal–Wallis test. Significant differences between total protists diversity and total phyllosphere diversity are indicated with asterisks (sign test, ***$p < 0.001$). (C) The dynamic richness of each protistan trophic group in soil–plant systems with different vegetable species during plant growth. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (D) The linear dependences are depicted by Pearson correlation 'R^2,' © Linear regression analysis of the absolute abundance of *Legionella* spp. *23S rRNA* and the diversity and RA of soil protists. (E) Principal coordinate analysis (PCoA) based on Bray–Curtis distances indicates the distinct protistan communities in the soil–plant continuum with different fertilizer treatments.

SEM analysis in combination with PCoA, PERMANOVA, and LMM analyses. For soil protists, organic fertilization, directly and indirectly, has an impact on the community and diversity of phototrophs and consumers. (Figure 2A,B; Table S2; PERMANOVA $R^2 = 22.9\%$, $p < 0.001$; Table S3; LMM $F_2 = 11.6$, $p < 0.01$). Organic fertilization reduced consumer and phototroph richness (Figure 1B; $p < 0.001$; Figure 2A), whereas it increased consumer RA, and reduced phototroph RA (Figure 1A; $p < 0.05$). Growth time directly influenced the community and diversity of phototrophs and consumers (Figure 2A,B) and separated the distribution of protists (Figure S6). Although plant species had a limited influence on soil protists (Tables S2 and S3; Figure S7A,B), it significantly influenced phototroph diversity (Figure 2A). Phototrophs

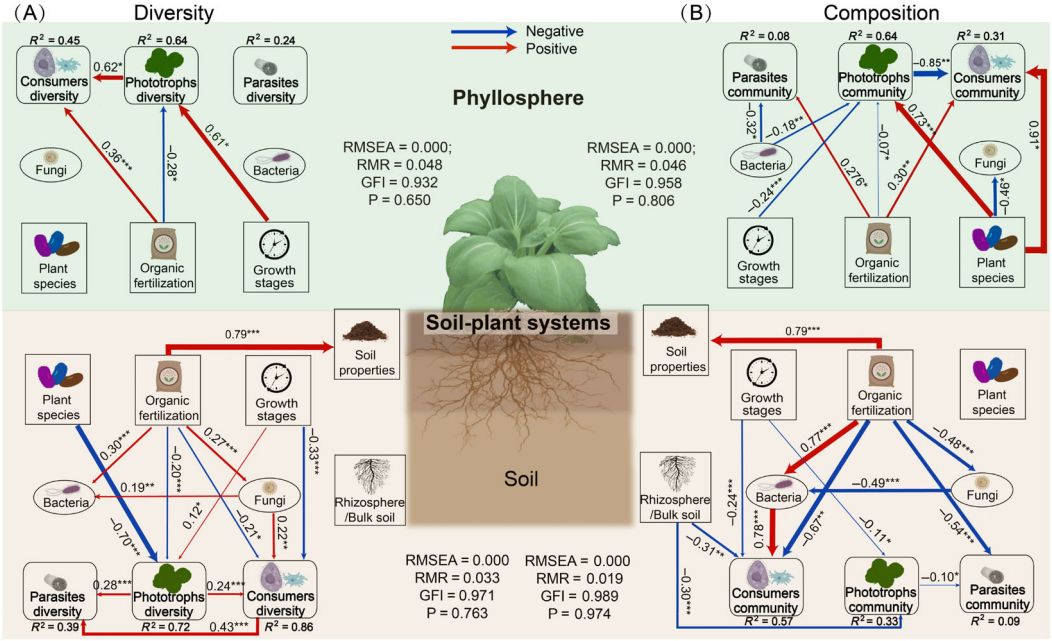

FIGURE 2 Structural equation models showing the effects of biotic and abiotic factors on the (A) diversity and (B) community structure of consumers, phototrophs, and parasites in the soil–plant systems. *p < 0.05, **p < 0.01, ***p < 0.001. R^2 suggests the proportion of variance explained. The non-significant contributions are not shown in this figure.

were also a biotic driver of consumer diversity and composition from both soils and the phyllosphere (Figure 2A,B).

Plant species had a stronger influence on the phyllosphere than soil protist communities (Table S2). Phyllosphere protistan communities were separated by plant species, especially driven by Brassica (Figure S8A). At the genus level, *Navicula*, *Raphid-pennateX*, and *Hrysophyceae CladeC* were enriched in cabbage, while *Hantzschia* and *Sellaphora* were enriched in lettuce (Figure S8B; p < 0.05). Organic fertilization directly and positively affected the RA of phyllosphere consumers and parasites (Figures 1A and 2B; p < 0.05) and consumer richness (Figures 1B and 2A; p < 0.05).

Community assembly of protistan trophic groups

Protists in bulk and rhizosphere soils fitted the neutral model better than phyllosphere protists (Figure 3A). Mean MST of soil protists was generally greater than the 0.5 threshold whereas MST values for phyllosphere protists were mostly below, indicating that stochastic processes had more effect on soil than phyllosphere protists (Figure 3A). Stochastic processes had more influence on all protistan trophic groups in fertilized bulk soils than in control bulk soils (Figure 3A; p < 0.05). The influence of stochastic or deterministic processes on each protist trophic group and their respective niche breadths were not significantly different between bulk and rhizosphere soils (Figure 3A,B).

Phototrophs had the broadest niche breadth among all protist trophic groups in both soils and the phyllosphere (Figure 3B; p < 0.05). The effects of stochastic processes on phototrophs in rhizosphere soil decreased over time (Figure 3C). Soil consumers were more affected by deterministic processes than other protist trophic groups except in fertilized rhizosphere soils. In the control soil, deterministic processes had a greater relative contribution to consumers during early growth (Day 14). However, the contribution of stochastic processes to consumers gradually increased over time and became dominant at Day 60 (Figure 3C).

Co-occurrence networks among protists, bacteria, and fungi

Protists had the second-greatest number of nodes in all soil networks and formed the largest and second-largest modules in control and fertilized soil networks, respectively (Figure 4A). Approximately one-third of core soil protists in the control soil networks were modular hubs, whereas, in fertilized soil, they had multiple roles (Table S4). In contrast, phyllosphere networks were characterized by a few protistan nodes (only 11) with low average degrees (Figure 4A,C) but stronger links (all >0.7).

Consumers were the dominant protistan connectors (79.4%–84.5%) in all soil networks (Figure 4B).

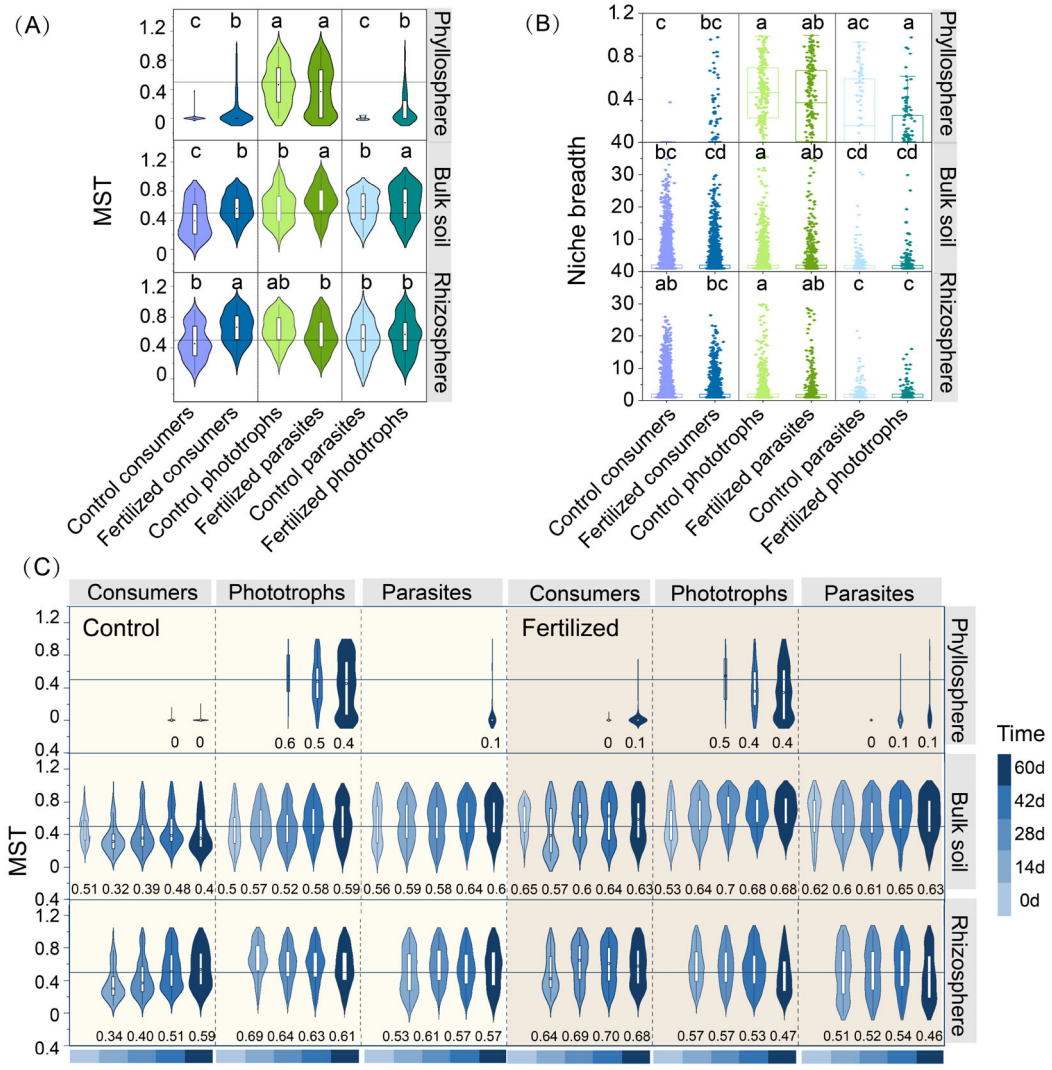

FIGURE 3 The modified stochasticity ratio (MST) values (A) and niche breadth (B) of each protistan trophic group in the phyllosphere, bulk, and rhizosphere soils of two treatments. Different letters above the boxes indicate a significant difference determined by the nonparametric Kruskal–Wallis test. The dynamic MST values of each protistan trophic group in the phyllosphere, bulk, and rhizosphere soils of two treatments during plant growth (C).

Consumer–bacteria links were the main inter-kingdom connectivity in soil networks and were greater in rhizosphere soils (Figure 4C,D). For example, consumers tightly connected with *Candidatus koribacter* in control rhizosphere soils and were closely linked to *Candidatus solibacter*, *Rhodoplanes*, and *Nitrospira* in fertilized rhizosphere soils (Figure S9). Soil phototrophs with the greatest average degree (Figure 4C) were the dominant protistan module hubs (75.0%–85.7%), especially in control bulk soils (Figure 4B). Phototrophs, linked mostly with consumers and other phototrophs (Figure 4D), and occupied central positions in some protistan modules, such as Module2 (Figure 4A). Phototrophs were also the dominant phyllosphere protist accounting for 91% of phyllosphere protistan nodes. Parasites were rare and mainly peripheral in soil–plant networks.

Compared to the unfertilized group, organic fertilization promoted inter-kingdom connections linking (about 6.6 times) consumers and bacteria as well as fungi and consumers, of which 14.7%–24.1% were negative connections, particularly in rhizosphere soil (Figure 4A,C,D). By contrast, the proportion of protistan intra-kingdom links was fewer in fertilized soils (Figure 4D) and protistan cluster module2 reduced under fertilization (Figure 4A). Moreover, potential human pathogens and protists (mainly consumers) were more closely associated in the fertilized soils, especially the rhizosphere (Figure 4D). This included protists from the Amoeba, Cercozoa, and Chlorophyta, which were linked to potential pathogens including *Mycobacterium gilvum*, *Bacillus megaterium*, *Bacillus cytotoxicus*, and *Mycobacterium* sp. (Table S5).

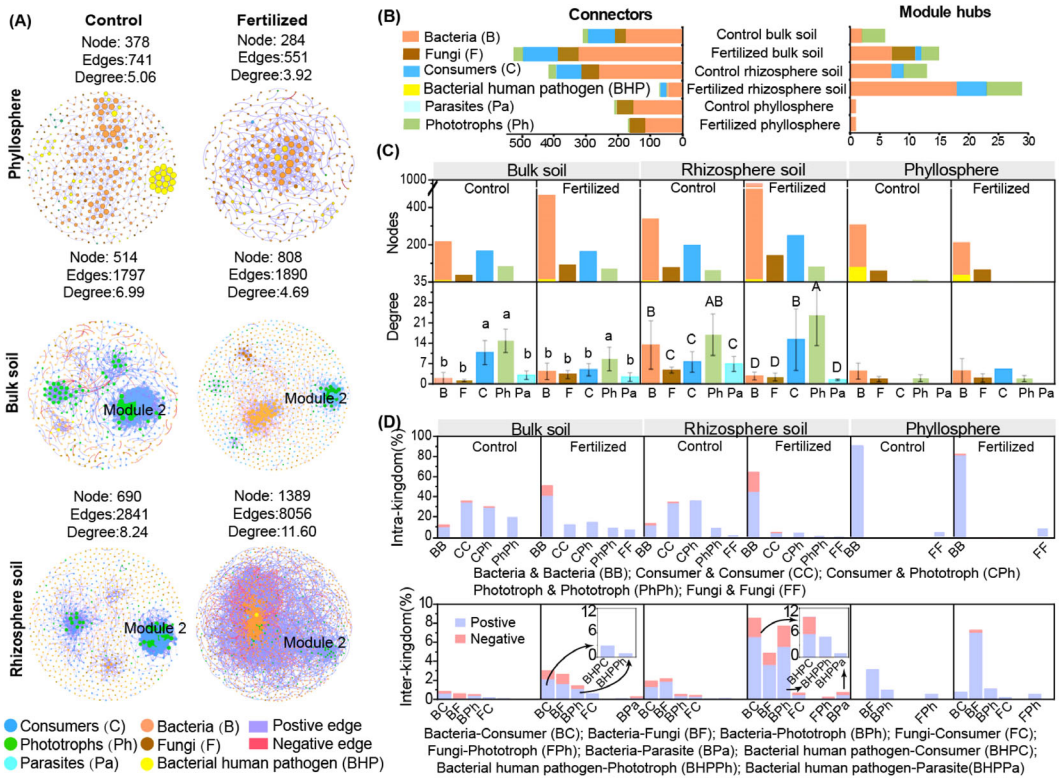

FIGURE 4 Microbial network of the soil–plant continuum. (A) Co-occurrence networks of microbial amplicon sequence variants showing microbial inter-kingdom network patterns differed among compartment niches (phyllosphere, rhizosphere soil, and bulk soil) and fertilized treatments. Edges between nodes correspond to either positive (pale blue) or negative (red) correlations using the Spearman method. (B) The number of each taxon node as connectors and module hubs. (C) Comparison of topological features (numbers of nodes and average degree) among bacterial, fungal, consumers, phototrophs, and parasites taxa at different compartment niches. Different letters above the boxes indicate a significant difference determined by the nonparametric Kruskal–Wallis test. (D) The proportion of intra-kingdom and inter-kingdom edges showing positive (pale blue) or negative (red) correlations in the microbial networks.

DISCUSSION

Different protistan trophic groups have different roles in soil–plant continuums

In this study, consumers which are known to be dominant soil protists (Oliverio et al., 2020), were important inter-kingdom protistan connectors, especially in rhizosphere soils. With a range of dietary sources and occupying different trophic niches (Geisen, Mitchell, Adl, Bonkowski, Dunthorn, Ekelund, Fernandez, et al., 2018), consumers are considered top-down regulators of soil microbiome composition and function (Shu et al., 2021) and are referred to as so-called 'puppet masters' of the rhizosphere (Gao et al., 2019). Here, some consumers connected tightly to multifunctional bacteria in rhizosphere soils, for example, *C. koribacter*, *C. solibacter*, and *Rhodoplanes*, all of which have previously been reported as key-stone phylotypes involved in redox reactions and elemental cycling of carbon, nitrogen, phosphorus, and sulfur (Fan et al., 2021). Thus, consumers may influence plant production (Guo et al., 2021) and soil function (Xiong et al., 2020). Consumers had a greater RA in rhizosphere soils and were influenced by strong deterministic processes until Day 14. This may be due to plant roots selectively recruiting consumers at an early stage for building healthy plant-associated microbial groups through root exudation (Chase & Myers, 2011; Guo et al., 2018). The decline in rhizosphere consumer diversity and RA over time may be due to a weakening of the influence of selective recruitment through declining root exudation (Ceja-Navarro et al., 2021) and the subsequent stability of protistan communities.

Soil phototrophs, which had the greatest average degree, were the main protistan module hubs in soil networks and mostly had intra-kingdom edges. A total of 18 soil consumers including *Vamprellda*, *Englyphida*, and *Rhogo* known as algivores (Seppey et al., 2017) were closely connected ($r > 0.7$) to phototrophs (algae). This concurred with a previous study that suggested widespread algivory by consumers in croplands (Seppey et al., 2017) and implied possible bottom-up control of consumers by phototrophs (Thakur & Geisen, 2019). Phototrophs may also influence other protists (Bashir et al., 2022) by regulating nutrient

cycling as they provide a carbon input to soils and contribute to nitrogen mobilization (Schmidt et al., 2016). The wider niche breadth of phototrophs indicated greater environmental adaptability (Comte et al., 2014) and their ability to obtain carbon through photosynthesis (Leliaert et al., 2012), supported survival leading to phototrophs predominating in oligotrophic phyllosphere environments (Sun et al., 2021).

In the studied soil–plant continuum, parasitic protists were a minority component and had minor roles in microbial co-occurrence networks. Notably, *Oomycota*, which can infect plant roots (Thines, 2018) and reduce plant growth (Bagchi et al., 2014) was the most abundant soil parasitic protist class and had significantly increased abundance in fertilized soil. This agrees with that previously reported for soil with multiple fertilization regimes (Sun et al., 2021). We also found that *Oomycota* RA and total RA of genera associated with human and animal gut microbes was greatest in recently fertilized soils, suggesting an increased potential risk to plant and human health associated with soil immediately after fertilization.

Fertilizers increased parasitic and consumer–pathogen connections

In this study, organic fertilization stimulated consumer–bacteria connections, especially in fertilized rhizosphere soils, which were hotspots for inter-kingdom connections (Schneider et al., 2015). Potentially, organic fertilizer may have stimulated bottom-up regulation by providing nutrients for bacterial growth (Figure S10) (Li et al., 2022). Overgrowth of bacterial biomass and increase in pH (Figure S11) after fertilization are known key factors for the soil consumer community (Figure S12) (Dupont et al., 2016; Mazel et al., 2021; Moerman et al., 2020; Murase et al., 2015), leading to a possible activation of bacterivorous consumers while inhibiting algivorous consumers (Sanders, 2022). Additionally, a reduction in algivory, combined with a reduction of protistan diversity may lead to fewer internal linkages within protist communities associated with fertilized soils resulting in the inhibition of protistan module hubs (phototrophs).

The RA of human (Cryptosporidiidae) (Morgan-Ryan et al., 2002) and plant (*Oomycotes*) parasites was significantly increased by organic fertilization, suggesting an increased potential risk for plant and human pathogenicity. More consumer–pathogen connections were also found in fertilized rhizosphere soils, suggesting that more consumers preyed upon human pathogens. However, some human pathogens (Folkins et al., 2020; Hoque et al., 2022) such as *Legionella* spp. can resist digestion after predation and can replicate and evolve within predatory amoebae before being expelled into the environment (Park et al., 2020), which may explain the positive correlation between consumers RA and *Legionella* in this study.

CONCLUSIONS

In this study, consumers were essential soil intra-kingdom connectors deterministically selected by the rhizosphere during the early stages of plant growth. Phototrophs were the dominant phyllosphere protists and were the main soil network hubs closely associated with other protists, especially consumers. Organic fertilizer promoted pathogenic protists and consumer–pathogen associations in rhizosphere soils, thus, posing a potential but small risk to human health. However, organic fertilizer also suppressed the internal network linking of soil protists and the diversity of consumers and phototrophs. These findings significantly advance our current understanding of protistan community composition and highlight the importance of protists associated with soil–plant continuums.

AUTHOR CONTRIBUTIONS

Chenshuo Lin: Conceptualization (equal); data curation (lead); investigation (equal); methodology (equal); writing – original draft (lead). **Wen-Jing Li:** Data curation (equal); investigation (equal); methodology (equal). **Li-Juan Li:** Data curation (equal); investigation (equal); methodology (equal). **Roy Neilson:** Writing – review and editing (equal). **Xin-Li An:** Conceptualization (equal); writing – review and editing (equal). **Yong-Guan Zhu:** Conceptualization (lead); funding acquisition (lead); project administration (lead); supervision (equal); validation (equal); writing – review and editing (equal).

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (32061143015 and 42090063). The James Hutton Institute receives financial support from Scottish Government Rural and Environment Science and Analytical Services (RESAS).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw sequences have been deposited into the GenBank Sequence Read Archive with accession number PRJNA849277.

ORCID

Yong-Guan Zhu https://orcid.org/0000-0003-3861-8482

REFERENCES

Adl, S.M., Bass, D., Lane, C.E., Lukes, J., Schoch, C.L., Smirnov, A. et al. (2019) Revisions to the classification, nomenclature, and diversity of eukaryotes. *The Journal of Eukaryotic Microbiology*, 66(1), 4–119.

An, X.L., Wang, J.Y., Pu, Q., Li, H., Pan, T., Li, H.Q. et al. (2020) High-throughput diagnosis of human pathogens and fecal contamination in marine recreational water. *Environmental Research*, 190, 109982.

Bagchi, R., Gallery, R.E., Gripenberg, S., Gurr, S.J., Narayan, L., Addis, C.E. et al. (2014) Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature*, 506, 85–88.

Banerjee, S. & van der Heijden, M.G.A. (2023) Soil microbiomes and one health. *Nature Reviews Microbiology*, 21(1), 6–20.

Bashir, I., War, A.F., Rafiq, I., Reshi, Z.A., Rashid, I. & Shouche, Y.S. (2022) Phyllosphere microbiome: diversity and functions. *Microbiological Research*, 254, 126888.

Bates, D.M., Maechler, M., Bolker, B.M. & Walker, S.C. (2017) Package lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-13. *Methods in Ecology and Evolution*, 4, 133–142.

Bates, S.T., Clemente, J.C., Flores, G.E., Walters, W.A., Parfrey, L.W., Knight, R. et al. (2013) Global biogeography of highly diverse protistan communities in soil. *The ISME Journal*, 7(3), 652–659.

Beckers, B., Beeck, M.O.D., Weyens, N., Boerjan, W. & Vangronsveld, J. (2017) Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome*, 5, 25.

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Verges, M.C., Charles, T. et al. (2020) Microbiome definition re-visited: old concepts and new challenges. *Microbiome*, 8(1), 1–22.

Bodenhausen, N., Horton, M.W. & Joy, B. (2013) Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. *PLoS One*, 8(2), e56329.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A. et al. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857.

Brevik, E.C., Slaughter, L., Singh, B.R., Steffan, J.J., Collier, D., Barnhart, P. et al. (2020) Soil and human health: current status and future needs. *Air, Soil and Water Research*, 13, 1–23.

Callahan, B.J., Mcmurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A. & Holmes, S.P. (2015) DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581–583.

Ceja-Navarro, J.A., Wang, Y., Ning, D., Arellano, A., Ramanculova, L., Yuan, M.M. et al. (2021) Protist diversity and community complexity in the rhizosphere of switchgrass are dynamic as plants develop. *Microbiome*, 9(1), 96.

Chase, J.M. & Myers, J.A. (2011) Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society B*, 366(1576), 2351–2363.

Chen, B.B., Xiong, W., Qi, J.J., Pan, H.B., Chen, S., Peng, Z.H. et al. (2021) Trophic interrelationships drive the biogeography of protistan community in agricultural ecosystems. *Soil Biology and Biochemistry*, 163, 108445.

Chen, Q.L., An, X.L., Li, H., Zhu, Y.G., Su, J.Q. & Cui, L. (2017) Do manure-borne or indigenous soil microorganisms influence the spread of antibiotic resistance genes in manured soil? *Soil Biology and Biochemistry*, 114, 229–237.

Comte, J., Lindstrm, E.S., Eiler, A. & Langenheder, S. (2014) Can marine bacteria be recruited from freshwater sources and the air? *The ISME Journal*, 8(12), 2423–2430.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D. et al. (2016) Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*, 7, 10541.

Delgado-Baquerizo, M., Reich, P.B., Trivedi, C., Eldridge, D.J., Abades, S., Alfaro, F.D. et al. (2020) Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nature Ecology & Evolution*, 4(2), 210–220.

Dupont, A.O., Griffiths, R.I., Bell, T. & Bass, D. (2016) Differences in soil micro-eukaryotic communities over soil pH gradients are strongly driven by parasites and saprotrophs. *Environmental Microbiology*, 18(6), 2010–2024.

Duran, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P. et al. (2018) Microbial interkingdom interactions in roots promote arabidopsis survival. *Cell*, 175(4), 973–983.e14.

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E. & Sundaresan, V. (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences of the United States of America*, 112(8), E911–E920.

Fan, K.K., Delgado-Baquerizo, M., Guo, X.S., Wang, D.Z., Zhu, Y.G. & Chu, H.Y. (2021) Biodiversity of key-stone phylotypes determines crop production in a 4-decade fertilization experiment. *The ISME Journal*, 15(2), 550–561.

Fiore-Donno, A.M., Richter-Heitmann, T. & Bonkowski, M. (2020) Contrasting responses of protistan plant parasites and phagotrophs to ecosystems, land management and soil properties. *Frontiers in Microbiology*, 11, 1823.

Fitzpatrick, C.R., Salas-Gonzalez, I., Conway, J.M., Finkel, O.M., Gilbert, S., Russ, D. et al. (2020) The plant microbiome: from ecology to reductionism and beyond. *Annual Review of Microbiology*, 74, 81–100.

Folkins, M.A., Dey, R. & Ashbolt, N.J. (2020) Interactions between human reovirus and free-living amoebae: implications for enteric virus disinfection and aquatic persistence. *Environmental Science & Technology*, 54(16), 10201–10206.

Freudenthal, J., Ju, F., Burgmann, H. & Dumack, K. (2022) Microeukaryotic gut parasites in wastewater treatment plants: diversity, activity, and removal. *Microbiome*, 10(1), 27.

Gao, Z., Karlsson, I., Geisen, S., Kowalchuk, G. & Jousset, A. (2019) Protists: puppet masters of the rhizosphere microbiome. *Trends in Plant Science*, 24(2), 165–176.

Geisen, S., Koller, R., Hünninghaus, M., Dumack, K., Urich, T. & Bonkowski, M. (2016) The soil food web revisited: diverse and widespread mycophagous soil protists. *Soil Biology and Biochemistry*, 94, 10–18.

Geisen, S., Mitchell, E.A.D., Adl, S., Bonkowski, M., Dunthorn, M., Ekelund, F. et al. (2018) Soil protists: a fertile frontier in soil biology research. *FEMS Microbiology Reviews*, 42(3), 293–323.

Geisen, S., Mitchell, E.A.D., Adl, S., Bonkowski, M., Dunthorn, M., Ekelund, F. et al. (2018) Soil protists: a fertile frontier in soil biology research. *FEMS Microbiology Letters*, 42(3), 293–323.

Guimera, R. & Nunes Amaral, L.A. (2005) Functional cartography of complex metabolic networks. *Nature*, 433, 895–900.

Guo, S., Xiong, W., Hang, X., Gao, Z., Jiao, Z., Liu, H. et al. (2021) Protists as main indicators and determinants of plant performance. *Microbiome*, 9(1), 64.

Guo, S., Xiong, W., Xu, H., Hang, X., Liu, H., Xun, W. et al. (2018) Continuous application of different fertilizers induces distinct bulk and rhizosphere soil protist communities. *European Journal of Soil Biology*, 88, 8–14.

Hirt, H. (2020) Healthy soils for healthy plants for healthy humans: how beneficial microbes in the soil, food and gut are interconnected and how agriculture can contribute to human health. *EMBO Reports*, 21(8), e51069.

Hoque, M.M., Noorian, P., Espinoza-Vergara, G., Manuneedhi Cholan, P., Kim, M., Rahman, M.H. et al. (2022) Adaptation to an amoeba host drives selection of virulence-associated traits in *Vibrio cholerae*. *The ISME Journal*, 16(3), 856–867.

Knights, D., Kuczynski, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G. et al. (2011) Bayesian community-wide culture-independent microbial source tracking. *Nature Methods*, 8(9), 761–763.

Lambert, B.S., Groussman, R.D., Schatz, M.J., Coesel, S.N., Durham, B.P., Alverson, A.J. et al. (2022) The dynamic trophic architecture of open-ocean protist communities revealed through machine-guided metatranscriptomics. *Proceedings of the National Academy of Sciences of the United States of America*, 119(7), e2100916119.

Leliaert, F., Smith, D.R., Moreau, H., Herron, M.D., Verbruggen, H., Delwiche, C.F. et al. (2012) Phylogeny and molecular evolution of the green algae. *Critical Reviews in Plant Sciences*, 30(1), 1–46.

Li, W.J., Li, H.Z., An, X.L., Lin, C.S., Li, L.J. & Zhu, Y.G. (2022) Effects of manure fertilization on human pathogens in endosphere of three vegetable plants. *Environmental Pollution*, 314, 120344.

Mahé, F., de Vargas, C., Bass, D., Czech, L., Stamatakis, A., Lara, E. et al. (2018) Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. *Nature Ecology & Evolution*, 1(4), 0091.

Martin, F.M., Uroz, S. & Barker, D.G. (2017) Ancestral alliances: plant mutualistic symbioses with fungi and bacteria. *Science*, 356(6340), eaad4501.

Mazel, F., Malard, L., Niculita-Hirzel, H., Yashiro, E., Mod, H.K., Mitchell, E.A.D. et al. (2021) Soil protist function varies with elevation in the Swiss Alps. *Environmental Microbiology*, 24(4), 1689–1702.

Mitchell, E.A.D. (2015) Pack hunting by a common soil amoeba on nematodes. *Environmental Microbiology*, 17(11), 4538–4546.

Mo, Y., Zhang, W., Wilkinson, D.M., Yu, Z., Xiao, P. & Yang, J. (2020) Biogeography and co-occurrence patterns of bacterial generalists and specialists in three subtropical marine bays. *Limnology and Oceanography*, 66(3), 793–806.

Moerman, F., Arquint, A., Merkli, S., Wagner, A., Altermatt, F. & Fronhofer, A.E. (2020) Evolution under pH stress and high population densities leads to increased density-dependent fitness in the protist *Tetrahymena thermophila*. *Evolution*, 74(3), 573–586.

Morgan-Ryan, U.M., Fall, A., Ward, L.A., Hijjawi, N., Sulaiman, I., Fayer, R. et al. (2002) *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*. *The Journal of Eukaryotic Microbiology*, 49(6), 433–440.

Muller, D.B., Vogel, C., Bai, Y. & Vorholt, J.A. (2016) The plant microbiota: systems-level insights and perspectives. *Annual Review of Genetics*, 50, 211–234.

Murase, J., Hida, A., Ogawa, K., Nonoyama, T., Yoshikawa, N. & Imai, K. (2015) Impact of long-term fertilizer treatment on the microeukaryotic community structure of a rice field soil. *Soil Biology and Biochemistry*, 80, 237–243.

Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D. et al. (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, 47(D1), D259–D264.

Ning, D., Deng, Y., Tiedje, J.M. & Zhou, J. (2019) A general framework for quantitatively assessing ecological stochasticity. *Proceedings of the National Academy of Sciences of the United States of America*, 116(34), 16892–16898.

Nisar, M.A., Ross, K.E., Brown, M.H., Bentham, R., Hinds, J. & Whiley, H. (2022) Molecular screening and characterization of *Legionella pneumophila* associated free-living amoebae in domestic and hospital water systems. *Water Research*, 226, 119238.

Nuccio, E.E., Starr, E., Karaoz, U., Brodie, E.L., Zhou, J., Tringe, S.G. et al. (2020) Niche differentiation is spatially and temporally regulated in the rhizosphere. *The ISME Journal*, 14(4), 999–1014.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Minchin, P.R. et al. (2018) R package 'vegan' version 2.5-1. Community Ecol Package, Vers. 2, 1–295.

Oliverio, A.M., Geisen, S., Delgado-Baquerizo, M., Maestre, F.T., Turner, B.L. & Fierer, N. (2020) The global-scale distributions of soil protists and their contributions to belowground systems. *Science Advances*, 6(4), eaax8787.

Park, J.M., Ghosh, S. & O'Connor, T.J. (2020) Combinatorial selection in amoebal hosts drives the evolution of the human pathogen *Legionella pneumophila*. *Nature Microbiology*, 5(4), 599–609.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41, D590–D596.

Ren, P., Sun, A., Jiao, X., Shen, J.P., Yu, D.T., Li, F. et al. (2023) Predatory protists play predominant roles in suppressing soil-borne fungal pathogens under organic fertilization regimes. *Science of the Total Environment*, 863, 160986.

Revelle, W.R. (2020) psych: procedures for psychological, psychometric, and personality research.

Sanders, R.W. (2022) Protists: flagellates and amoebae. In: Mehner, T. & Tockner, K. (Eds.) *Encyclopedia of inland waters*, 2nd edition. Oxford: Elsevier, pp. 630–638.

Santos, S.S., Schöler, A., Nielsen, T.K., Hansen, L.H., Schloter, M. & Winding, A. (2020) Land use as a driver for protist community structure in soils under agricultural use across Europe. *Science of the Total Environment*, 717, 137228.

Schermelleh-Engel, K., Moosbrugger, H. & Müller, H. (2003) Evaluating the fit of structural equation models: tests of significance and descriptive goodness-of-fit measures. *MPR Online*, 8(8), 23–74.

Schmidt, O., Dyckmans, J. & Schrader, S. (2016) Photoautotrophic microorganisms as a carbon source for temperate soil invertebrates. *Biology Letters*, 12(1), 20150646.

Schneider, D., Engelhaupt, M., Allen, K., Kurniawan, S., Krashevska, V., Heinemann, M. et al. (2015) Impact of lowland rainforest transformation on diversity and composition of soil prokaryotic communities in Sumatra (Indonesia). *Frontiers in Microbiology*, 6, 01339.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S. et al. (2011) Metagenomic biomarker discovery and explanation. *Genome Biology*, 12(6), R60.

Seppey, C.V.W., Singer, D., Dumack, K., Fournier, B., Belbahri, L., Mitchell, E.A.D. et al. (2017) Distribution patterns of soil microbial eukaryotes suggests widespread algivory by phagotrophic protists as an alternative pathway for nutrient cycling. *Soil Biology and Biochemistry*, 112, 68–76.

Shi, Y., Delgado-Baquerizo, M., Li, Y., Yang, Y., Zhu, Y.G., Penuelas, J. et al. (2020) Abundance of kinless hubs within soil microbial networks are associated with high functional potential in agricultural ecosystems. *Environment International*, 142, 105869.

Shu, L.F., He, Z.Z., Guan, X.T., Yang, X.Q., Tian, Y.H., Zhang, S.Y. et al. (2021) A dormant amoeba species can selectively sense and predate on different soil bacteria. *Functional Ecology*, 35(8), 1708–1721.

Singer, D., Seppey, C.V.W., Lentendu, G., Dunthorn, M., Bass, D., Belbahri, L. et al. (2021) Protist taxonomic and functional diversity in soil, freshwater and marine ecosystems. *Environment International*, 146, 106262.

Stoeck, T., Bass, D., Nebel, M., Christen, R. & Richards, T.A. (2010) Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Molecular Ecology*, 19(1), 21–31.

Sun, A., Jiao, X.Y., Chen, Q., Trivedi, P., Li, Z., Li, F. et al. (2021) Fertilization alters protistan consumers and parasites in crop-associated microbiomes. *Environmental Microbiology*, 23(4), 2169–2183.

Sun, Y.H. & Luo, Y.M. (2005) Environmental and health risks of pathogens in sewage sludge and their abating approaches. *The Soil*, 37(5), 474–481.

Thakur, M.P. & Geisen, S. (2019) Trophic regulations of the soil microbiome. *Trends in Microbiology*, 27(9), 771–780.
Thines, M. (2018) Oomycetes. *Current Biology*, 28(15), R812–R813.
Triplett, L.R., Taerum, S.J. & Patel, R.R. (2023) Protists at the plant-bacterial interface: impacts and prospective applications. *Physiological and Molecular Plant Pathology*, 125, 102011.
Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T. & Singh, B.K. (2020) Plant-microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology*, 18(11), 607–621.
Wall, D.H., Nielsen, U.N. & Six, J. (2015) Soil biodiversity and human health. *Nature*, 528(7580), 69–76.
Xiong, W., Jousset, A., Guo, S., Karlsson, I., Zhao, Q., Wu, H. et al. (2018) Soil protist communities form a dynamic hub in the soil microbiome. *The ISME Journal*, 12(2), 634–638.
Xiong, W., Song, Y., Yang, K., Gu, Y., Wei, Z., Kowalchuk, G.A. et al. (2020) Rhizosphere protists are key determinants of plant health. *Microbiome*, 8(1), 27.
Yang, K., Chen, Q.L., Chen, M.L., Li, H.Z., Liao, H., Pu, Q. et al. (2020) Temporal dynamics of antibiotic resistome in the plastisphere during microbial colonization. *Environmental Science & Technology*, 54(18), 11322–11332.
Zhao, Z.B., He, J.Z., Quan, Z., Wu, C.F. & Geisen, S. (2020) Fertilization changes soil microbiome functioning, especially phagotrophic protists. *Soil Biology and Biochemistry*, 148, 107863.
Zhou, J.Z. & Ning, D.L. (2017) Stochastic community assembly: does it matter in microbial ecology? *Microbiology and Molecular Biology Reviews*, 81(4), e00002–e00017.

SUPPORTING INFORMATION

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

How to cite this article: Lin, C., Li, W.-J., Li, L.-J., Neilson, R., An, X.-L. & Zhu, Y.-G. (2023) Movement of protistan trophic groups in soil–plant continuums. *Environmental Microbiology*, 1–12. Available from: https://doi.org/10.1111/1462-2920.16477