

## Protist communities as indicators of fertilization-induced changes in a species-rich grassland ecosystem

Agriculture, Ecosystems and Environment

Sun, Shuo; Jousset, Alexandre; Geisen, Stefan; Lara, Enrique; Zhang, Pengfei et al

<https://doi.org/10.1016/j.agee.2024.109101>

This publication is made publicly available in the institutional repository of Wageningen University and Research, under the terms of article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne.

Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed using the principles as determined in the Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' project. According to these principles research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and / or copyright owner(s) of this work. Any use of the publication or parts of it other than authorised under article 25fa of the Dutch Copyright act is prohibited. Wageningen University & Research and the author(s) of this publication shall not be held responsible or liable for any damages resulting from your (re)use of this publication.

For questions regarding the public availability of this publication please contact [openaccess.library@wur.nl](mailto:openaccess.library@wur.nl)



## Protist communities as indicators of fertilization-induced changes in a species-rich grassland ecosystem

Shuo Sun<sup>a</sup>, Alexandre Jousset<sup>a</sup>, Stefan Geisen<sup>b</sup>, Enrique Lara<sup>c</sup>, Pengfei Zhang<sup>d,e,\*</sup>, Rong Li<sup>a</sup>, Francisco Dini-Andreote<sup>f,g</sup>, Mohammadhossein Ravanbakhsh<sup>h</sup>, Qirong Shen<sup>a</sup>, George A. Kowalchuk<sup>h</sup>, Wu Xiong<sup>a,\*\*</sup>

<sup>a</sup> Jiangsu Provincial Key Lab for Solid Organic Waste Utilization, Key Lab of Organic-Based Fertilizers of China, Jiangsu Collaborative Innovation Center for Solid Organic Wastes, Educational Ministry Engineering Center of Resource-Saving Fertilizers, Nanjing Agricultural University, Nanjing 210095, People's Republic of China

<sup>b</sup> Laboratory of Nematology, Wageningen University, Wageningen 6700 AA, the Netherlands

<sup>c</sup> Department of Mycology, Real Jardín Botánico-CSIC, Madrid, Spain

<sup>d</sup> State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, College of Ecology, Lanzhou University, Lanzhou, Gansu Province, People's Republic of China

<sup>e</sup> Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN, USA

<sup>f</sup> Department of Plant Science & Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA

<sup>g</sup> The One Health Microbiome Center, Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA

<sup>h</sup> Ecology and Biodiversity Group, Department of Biology, Institute of Environmental Biology, Utrecht University, Padualaan 8, Utrecht 3584 CH, the Netherlands

### ARTICLE INFO

#### Keywords:

Soil biodiversity  
Protists  
Nutrient enrichment  
Above- and below-ground biodiversity  
Qinghai-Xizang Plateau

### ABSTRACT

Anthropogenic activities such as long-term fertilizer application are known to lead to losses in above and belowground biodiversity, thereby negatively impacting ecosystem function. However, our understanding of the relative sensitivity of different soil organisms groups to increasing fertilizer application levels remains largely unknown. To address this knowledge gap, we investigated the impacts of different long-term fertilization regimes, and the associated changes in plant communities, on a broad range of soil organisms, including bacteria, fungi, protists, and nematodes, in the alpine meadow ecosystem of the Qinghai-Xizang Plateau. Results showed that the community composition of protists was the most sensitive to different fertilization regimes as compared to those of bacteria, fungi, and nematodes. Changes in the protist community were also most strongly linked to changes in plant richness and biomass under prolonged fertilization. Nitrogen fertilizer addition leads to more deterministic community assembly processes for protist communities, while phosphorus fertilizer application leads to more stochastic assembly processes. Together, our results suggest that protists may be among the most susceptible soil organisms concerning the impact of human disturbances like fertilizer application, highlighting their potential importance as sensitive ecological indicators of human-induced disturbances in terrestrial ecosystems.

### 1. Introduction

Although fertilizer application has played an important role in increased global food production, it also can have large negative impacts on terrestrial ecosystems (Chen et al., 2017; Zhang et al., 2016). For example, long-term overuse of chemical fertilizers has led to decreases in both above and belowground biodiversity across a range of terrestrial ecosystems (Chalcraft et al., 2008; Storkey et al., 2015; Wang et al.,

2018). Soil biodiversity loss generally has negative effects on ecosystem stability (Loreau and de Mazancourt, 2013) and soil functioning (Bardgett and van der Putten, 2014; Wagg et al., 2014). Soil functions are to a large degree driven by the activities of below-ground microorganisms (Banerjee and van der Heijden, 2023), and changes in soil-borne community structure and function upon disturbance have often been linked to declines in soil functionality (Bissett et al., 2013; Mouillot et al., 2013). Perturbation-induced changes in soil-borne

\* Corresponding author at: State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, College of Ecology, Lanzhou University, Lanzhou, Gansu Province, People's Republic of China.

\*\* Corresponding author.

E-mail addresses: [zhangpengfei@lzu.edu.cn](mailto:zhangpengfei@lzu.edu.cn) (P. Zhang), [wuxiong@njau.edu.cn](mailto:wuxiong@njau.edu.cn) (W. Xiong).

<https://doi.org/10.1016/j.agee.2024.109101>

Received 31 March 2024; Received in revised form 13 May 2024; Accepted 26 May 2024

Available online 5 June 2024

0167-8809/© 2024 Elsevier B.V. All rights reserved, including those for text and data mining, AI training, and similar technologies.

communities can also have important feedback effects on plant species composition, diversity and productivity (Saleem et al., 2019; Wang et al., 2019). Thus, holistic perspectives of soil health should adopt a biological perspective that includes multiple aspects of the below-ground communities (Lehmann et al., 2020), as well as potential links between above- and belowground soil biodiversity (Cappelli et al., 2022; Porazinska et al., 2003).

Responses of soil biodiversity to anthropogenic activities of fertilization have often been centered on changes in bacterial or fungal communities, with less attention given to higher trophic level organisms such as protists and nematodes (Geisen et al., 2018). Such microbial predators can assume a broad range of functional roles within soil food webs (Geisen et al., 2018), and have been shown to be sensitive indicators of ecosystem stability (Geisen et al., 2017; Gong et al., 2024; Xiong et al., 2018). Microbial predators can regulate the diversity and functionality of soil microbiomes, thereby playing important roles in sustaining soil functionality (Gao et al., 2019; Geisen et al., 2018; Hu et al., 2023). For example, phagotrophic protists can promote soil nutrient cycling by preying on bacteria and releasing nutrients (Gao et al., 2019), making them available to plants. Therefore, to advance our understanding of the anthropogenic impacts of fertilization regimes on natural and agroecosystems, it is important to adopt a more holistic approach to include multiple groups of soil organisms and explore their potential links with associated changes in aboveground species richness. However, few studies to date have examined these relationships in the light of different fertilization gradient experiments, especially in alpine meadow ecosystems.

The "trophic sensitivity hypothesis" suggests that predators are less tolerant to increasing environmental stress as compared to their prey (Vasseur and McCann, 2005; Voigt et al., 2003), but we are generally lacking data to examine the validity of this hypothesis as related to belowground microbial communities (Petchey et al., 1999; Thakur et al., 2021). Evidence from large spatial scales suggests that the community assembly of smaller microorganisms, such as bacteria, are governed mainly by stochastic processes, primarily due to the plasticity of their metabolic pathway, making them less sensitive to environmental changes (Farjalla et al., 2012; Wu et al., 2018). Compared to microbial prey, relatively larger organisms, such as protists and nematodes seem to be driven to a greater extent by deterministic, selection-based processes, potentially making them more susceptible to environmental influences (Luan et al., 2020). In this study, we hypothesized that higher trophic level organisms within the soil biota, e.g. protists and nematodes, would be more sensitive to anthropogenic fertilization regimes as compared to bacteria and fungi. To examine this hypothesis, we conducted a seven-year-long field experiment in the Qinghai-Xizang Plateau to evaluate the influence of a range of fertilization practices across the breadth of the soil organisms, including bacteria, fungi, protists and nematodes, as well as their potential feedback on plant diversity and biomass. We also examined the community assembly processes predominating across the different groups of soil organisms based upon normalized stochasticity ratio (NST) analysis (Jiao et al., 2020; Ning et al., 2019) high-throughput amplicon sequencing, in order to relate community assembly to relative sensitivity to fertilization disturbance.

## 2. Materials and methods

### 2.1. Site description and experimental procedures

The long-term experiment was performed at the Gansu Gannan Grassland Ecosystem National Observation and Research Station (Azi Substation) of Lanzhou University (101°51'E, 33°40'N), as previously described (Liu et al., 2024; Zhang et al., 2015). The experimental site, situated on the eastern Qinghai-Xizang Plateau at an average altitude of 3,500 m, experiences an average annual temperature of 1.2°C and receives approximately 2,580 hours of cloud-free solar radiation annually (Li et al., 2011; Ma et al., 2011; Ren et al., 2010). Dominant vegetation

includes *Kobresia* spp. (Cyperaceae), *Agrostis* spp., *Festuca ovina*, *Anemone rivularis* (Ranunculaceae), and *Saussurea* spp. (Asteraceae) (Luo et al., 2006).

A total of sixty 200 m<sup>2</sup> plots were initially established in 2011. Three fertilizer treatments (N-nitrogen, P-phosphorous, and N+P) were applied to six plots, accompanied by six control plots. Each plot was separated by a 1 m walkway, and the entire experimental area was enclosed with wire mesh to minimize potential external disturbances, such as grazing by herbivores. The three fertilizer treatments represent varying levels of nutrient addition: N addition (N5, N10, N15 corresponding to 5 g, 10 g, and 15 g N m<sup>-2</sup> year<sup>-1</sup>, respectively); P addition (P2, P4, P8 corresponding to 2 g, 4 g, and 8 g P m<sup>-2</sup> year<sup>-1</sup>, respectively); and N+P addition (NP2, NP4, NP8 corresponding to 2 g, 4 g, and 8 g P m<sup>-2</sup> year<sup>-1</sup>, with 10 g N m<sup>-2</sup> year<sup>-1</sup>). The experiment employed a randomized complete block design. Plots were established in areas that did not differ in above-ground observed species diversity, community biomass, and community composition before the start of the experiment (Zhang et al., 2015; Zhou et al., 2019). Nitrogen, in the form of ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), and phosphorus, as calcium dihydrogen phosphate (Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>), were applied annually in May. After 7 years of experimental treatments, soil (0–15 cm) and plant samples were collected from each plot in August 2017. Vegetation harvest was conducted from a 0.5×0.5 m plot, with quadrat locations selected randomly. Upon collection, plants were sorted by species and weighed after drying at 80°C for 48 hours. Species were identified and recorded. The soil was sieved through a 2 mm mesh to remove plant roots. Soil samples were transported to the laboratory on dry ice and stored at -80 degrees Celsius for DNA extraction. Total soil DNA was extracted using the DNeasy PowerMax Soil Kit (Qiagen, Germany), following the manufacturer's instructions using 10 g soils.

### 2.2. Profiling soil belowground communities, bioinformatics and statistical analyses

Bacterial communities were profiled by targeting the V4 region of the 16 S rRNA gene using the primer set 520 F (5-AYTGGGYD-TAAAGNG-3) and 802 R (5-TACNVGGGTATCTAATCC-3) (Claessen et al., 2009). Fungal analyses focused on the ITS1 region with the primer set ITS1F (5-GGAAGTAAAAGTCGTAACAAGG-3) and ITS2 (5-GCTGCGTTCTTCATCGATGC-3) (White et al., 1990). For protists and nematodes, the V4 region of the 18 S rRNA gene was targeted using the primer set V4\_1f (5-CCAGCASCYCGCGTAATWCC-3) and TAREukREV3 (5- ACTTTCGTTCTTGATYRA-3) (Bass et al., 2016). The obtained raw sequence data for each of these amplicons were analyzed following previous protocols (Xiong et al., 2020). Briefly, sequences were paired, and those with expected errors greater than 1.0 were removed. High-quality sequences were then clustered into operational taxonomic units (OTUs) at a 97 % nucleotide similarity threshold. Chimeric sequences were removed using UCHIME (Edgar et al., 2011). OTUs with less than two sequences were also eliminated, and one representative sequence from each OTU was used for taxonomic assignments. Bacterial sequences were taxonomically assigned using the RDP database (version 18) (Cole et al., 2009; Wang et al., 2007); for fungal sequences, we used the UNITE database (Kõljalg et al., 2013), and eukaryotic sequences using the PR2 database (Protists Ribosomal Reference Database) (Guilou et al., 2013). Protistan operational taxonomic units (OTUs) were classified into distinct functional groups based on their anticipated feeding modes, encompassing phagotrophs, parasites, phototrophs, plant pathogens, and saprotrophs, following established protocols (Adl et al., 2019; Dumack et al., 2020; Xiong et al., 2019). All our analyses were based on relative abundances, including taxonomy data and protistan functional groups.

We evaluated the impact of distinct fertilization regimes on the community composition of soil organisms (including bacteria, fungi, protists, and nematodes) using the Bray-Curtis dissimilarity metric between the treatment and control groups, following methods outlined in a

previous study (Zhang et al., 2011). Additionally, we performed Wilcoxon tests to assess the impacts of distinct fertilization treatments on the composition of specific soil organisms (Gehan, 1965). Furthermore, we used Random Forest (RF) analysis to examine the explanatory power of different groups of soil organisms at the phylum or supergroup levels in relation to plant richness and biomass (Archer, 2022). To gauge the significance of characteristic variables, we calculated the percentage increases in Mean Squared Error (MSE), which reflects the extent of decline in the predictive performance of the random forest model (Archer, 2022). The statistical significance of the model was evaluated using the "A3" package in R, employing 1,000 permutations of the response variable (Fortmann-Roe, 2015). Data handling, visualization, and statistical analyses were carried out using the R platform. We evaluated microbial community assembly processes by using the "NST" package in R to calculate the taxonomic normalized stochasticity ratio (tNST) using default settings to investigate the relative contributions of deterministic versus stochastic processes to microbiome community assembly (Ning et al., 2019). We used values of tNST > 0.5 to denote a dominance of the deterministic process (e.g., selection), while values of tNST < 0.5 denotes stochastic processes (e.g., random dispersal, ecological drift) (Zhu et al., 2024).

### 3. Results and discussion

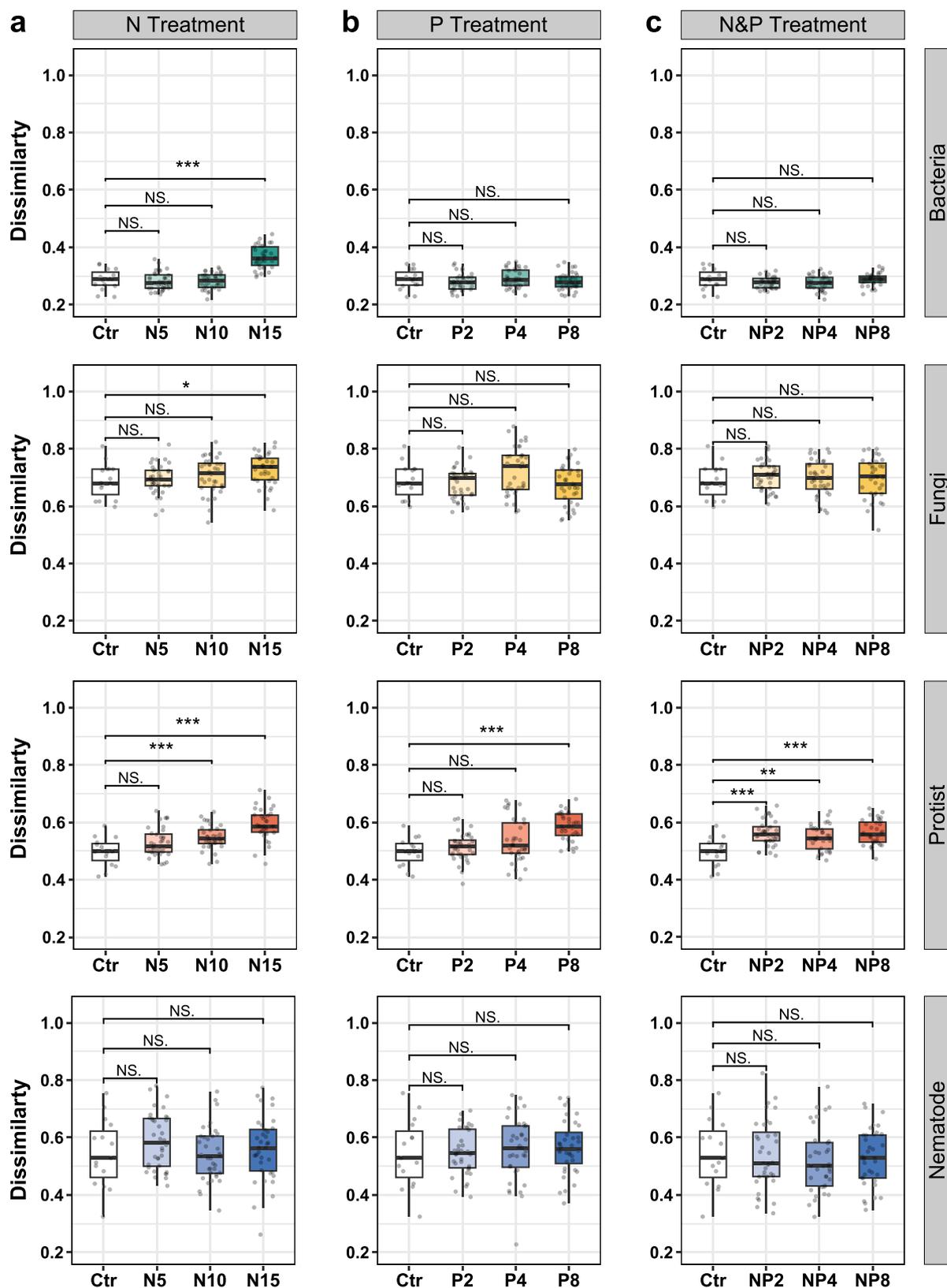
Here, we reported protist communities represent the most sensitive group of below-ground organisms to long-term fertilizer application as compared to other soil-borne organisms in grassland ecosystems. Therefore, we highlight the potential use of protists as bioindicators of human-induced disturbances such as the application of chemical fertilizers in terrestrial ecosystems. Our results presented that the dissimilarity within protist communities notably increased ( $p < 0.001$ ) at a median nitrogen concentration ( $10 \text{ g m}^{-2} \text{ year}^{-1}$ ) compared to the control treatment (Fig. 1a), while the dissimilarity of bacterial ( $p < 0.001$ ) and fungal ( $p < 0.05$ ) communities only revealed significant variations at the highest level of nitrogen addition ( $15 \text{ g m}^{-2} \text{ year}^{-1}$ ). Additionally, the dissimilarity within the protist community exhibited significant differences upon the introduction of higher phosphorus levels ( $p < 0.001$ ) compared to other soil organisms (Fig. 1b). Moreover, the protist communities exhibited distinct dissimilarities ( $p < 0.001$ ) even at the lowest phosphorus concentrations in the context of phosphorus gradient experiments conducted alongside high nitrogen treatment (Fig. 1c). In contrast, the bacterial, fungal, and nematode communities did not display significant changes. However, we did not observe a significant change in nematode community structure across various gradient fertilization regimes. Previous research has indicated significant differences in nematode community diversity at higher levels of fertilizer addition (Wang et al., 2022). The distinct results may be attributed to the modest intensity of fertilization in our study, which did not reach the necessary threshold for eliciting changes in the nematode community.

The high susceptibility of protist communities to exogenous nutrient addition indicates that this often overlooked soil-borne group may serve as a potential bioindicator of soil perturbation. Numerous studies have already demonstrated changes in the composition of bacterial and fungal communities due to alterations in soil abiotic factors resulting from fertilizer application (Ai et al., 2018; Sun et al., 2015; Zhou et al., 2016). Our study delves more deeply into the sensitivity of protist communities, revealing them to be the most responsive group to human disturbance via fertilizer addition, while bacterial and fungal communities are only impacted by intensive environmental disturbance. Our observations also support the idea that protists may have a narrower ecological niche as compared to other microorganisms, as they show less tolerance to environmental changes (Payne, 2013). Previous research has also found that protist communities sensitively respond to nitrogen fertilizer application in agricultural soils (Zhao et al., 2019), with microcosm experiments revealing a high sensitivity of protist communities to organic pollution pressure (Wu et al., 2022). These findings

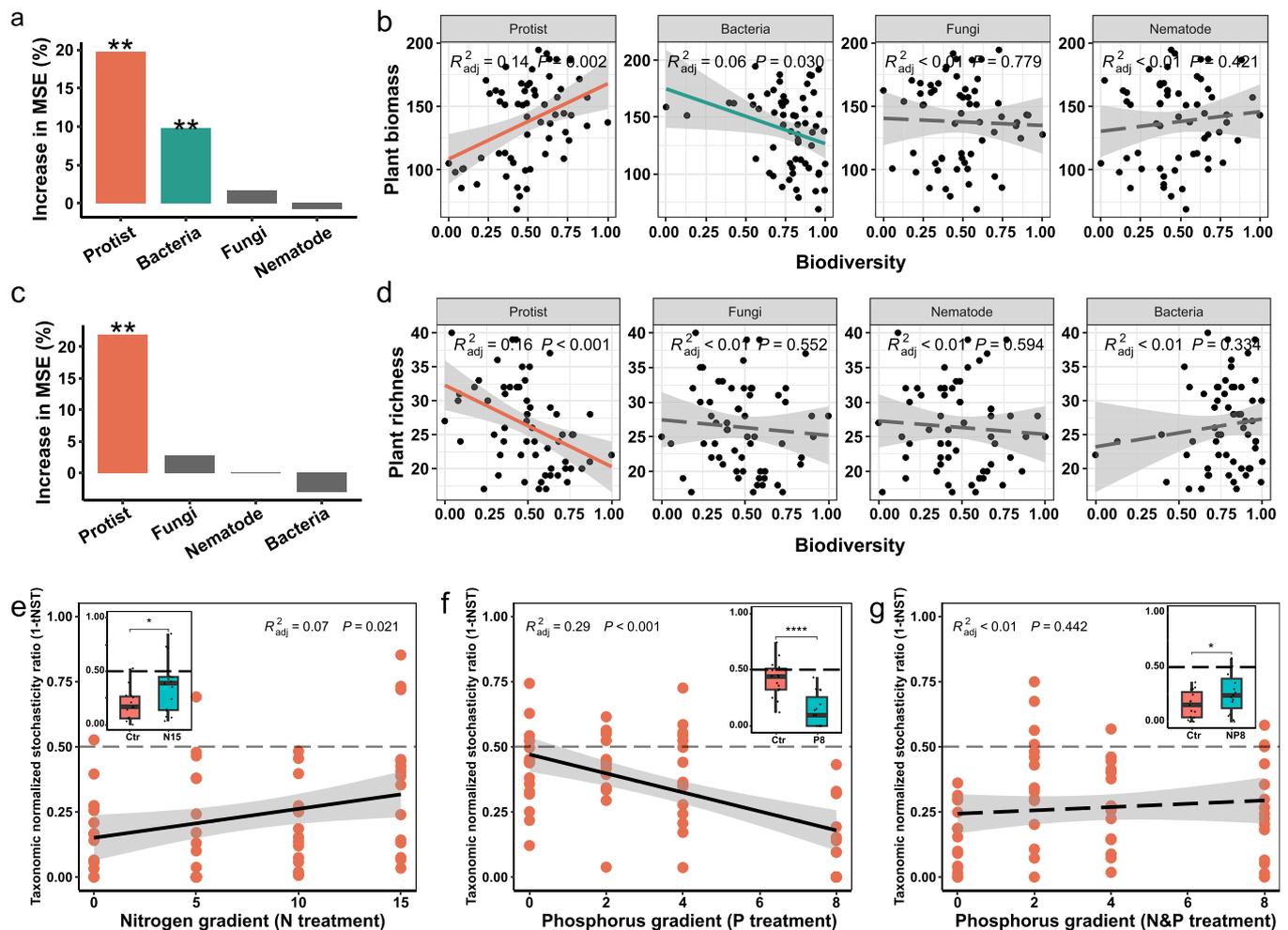
collectively suggest that the protist community exhibits reduced resilience to increasing environmental pressures, confirming the nutrient sensitivity hypothesis (Vasseur and McCann, 2005; Voigt et al., 2003). We propose that exogenous environmental changes exert top-down effects on soil-borne organism communities, directly impacting protist diversity, and subsequently cascading down to affect lower trophic levels (Hu et al., 2024; Thakur and Geisen, 2019). Our findings confirmed that long-term fertilizer application can influence the composition of protist communities, even at extremely low application levels in natural ecosystems. These changed belowground communities caused by human activities such as nutrient enrichment, may subsequently trigger changes in soil ecosystem function and stability (Ma et al., 2021). Our results propose to include soil protists to examine the impacts of global environmental changes and human activity on the functionality of the soil food web (Bates et al., 2013; Mukherjee et al., 2018; Stefan et al., 2014).

Protists were the soil-borne organisms that were most associated with changes in the aboveground plant communities. We used random forest modeling to explore which belowground soil organisms, including bacteria, fungi, protists, and nematodes, could be used to predict plant biomass (Fig. 2a) and richness (Fig. 2c) under nutrient enrichment. Results revealed that plant biomass was best predicted by protist diversity (Fig. 2a), with a positive relationship (Fig. 2b). This could be attributed to the predation function by protists, which can enhance nutrient turnover by releasing nutrients from bacterial biomass in support of plant performance (Gao et al., 2019; Glücksmann et al., 2010). This partly explains the positive correlation between plant biomass and protist richness, and the negative correlation with bacterial richness (Fig. 2b) (Krome et al., 2009). Increased protist diversity has been reported to support diverse soil functions (Geisen et al., 2020; Saleem et al., 2013). For example, photosynthetic protists contribute nutrients to the soil, while parasitoids lyse their hosts, thereby enriching the nutrient content (Jassey et al., 2022; Mahé et al., 2017). Additionally, the random forest model indicated that protist diversity has the highest explanatory power on plant richness (Fig. 2c), with a negative correlation (Fig. 2d). We also examined taxonomic compositions of the different groups of soil organisms in predicting vegetation parameters. Results identified the top ten most important bioindicators, which collectively explained 34 % of the variation in observed plant biomass (Figure S2a). Results showed that Opisthokonta from the protist community are the most important predictor of plant biomass (Figure S1a), exhibiting a positive trend with plant biomass (Figure S2b). Additionally, the top ten bioindicators collectively explained 39 % of the observed variation in plant richness (Figure S2a). A positive linear relationship was observed between plant richness and Amoebozoa (Figure S2b), which was in line with previous findings (Romeralo et al., 2011) supporting a potential role of amoebae in the silica cycle, a crucial element for enhancing plant resilience (Creevy et al., 2016; Wilkinson and Mitchell, 2010). However, the specific mechanisms driving this correlation require further investigation and experimental support. The observed connections between protists and plant communities support that soil protists may serve as useful targets for improving plant diversity and productivity, and contributing to a positive impact on ecosystem functionality (Gao et al., 2019). In addition to protists, the fungal phyla Basidiomycota and Mortierellomycota exhibited detectable links to plant species richness, followed by the influence of Rhizaria (Figure S2a).

Our research further discovered that the fertilization regime also affects the assembly patterns of protist communities. The contribution of the deterministic processes in the assembly of protist communities increased along the nitrogen fertilizer gradient (Fig. 2e), whereas the addition of phosphorus fertilizer promoted more stochastic processes in protist community assembly (Fig. 2f). However, overall, the community assembly of protists was predominantly determined by stochastic processes under fertilization (value of  $1 - \text{tNST} < 0.5$ ), which is consistent with recent research (Hu et al., 2024; Kang et al., 2022). This may be



**Fig. 1.** Effects of nutrient enrichment on dissimilarity in community composition of different soil organisms under nitrogen (a), phosphorus (b), and nitrogen + phosphorus (c) application. Dissimilarity in soil species composition between nutrient-enriched treatment groups and control groups was assessed through the utilization of the Bray-Curtis distance metric. *P* values were indicated by asterisks: \**p* < 0.05, \*\**p* < 0.01, and \*\*\**p* < 0.001. The three types of fertilizer treatments correspond to three levels of nutrient addition: N addition (N5 = 5 g N m<sup>-2</sup> year<sup>-1</sup>; N10 = 10 g N m<sup>-2</sup> year<sup>-1</sup>; N15 = 15 g N m<sup>-2</sup> year<sup>-1</sup>); P addition (P2 = 2 g P m<sup>-2</sup> year<sup>-1</sup>; P4 = 4 g P m<sup>-2</sup> year<sup>-1</sup>; P8 = 8 g P m<sup>-2</sup> year<sup>-1</sup>); NP addition (N10 & P2 = 2 g P m<sup>-2</sup> year<sup>-1</sup>; N10 & P4 = 4 g P m<sup>-2</sup> year<sup>-1</sup>; N10 & P8 = 8 g P m<sup>-2</sup> year<sup>-1</sup>, with 10 g N m<sup>-2</sup> year<sup>-1</sup>), respectively.



**Fig. 2.** The relative importance of soil-borne groups in explaining plant richness and biomass, as well as the assembly processes of protist communities under different fertilization treatments. Relative importance (% increase in the mean square error, MSE) of different groups of soil organisms (including bacteria, fungi, protists and nematodes) in explaining plant biomass (a) and plant richness (c) across all the samples. The linear relationships between the species richness of different soil organisms and plant biomass (b) and richness (d). The taxonomic normalized stochasticity ratio (1-tNST) of protist community under nitrogen (e), phosphorus (f), nitrogen + phosphorus (g) application.  $P$  values are indicated by asterisks as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ .

attributed to the relatively low fertilizer application levels in our experiment site. Soil microbial communities on the Qinghai-Xizang Plateau may predominantly adopt a dormant lifestyle to cope with environmental stress, consequently, weakening deterministic processes as they activate under different micro-environmental conditions (Nemergut et al., 2013; Velasco-González et al., 2020). These results suggest that the diverse patterns in soil protist community assembly may be attributed to distinct selection strategies in response to external nutrient pressures. The varied response patterns in protist community assembly may also be related to changes in soil bacterial prey induced by fertilizer addition. Recent studies have shown the relative importance of deterministic processes of protist communities increased in the plant rhizosphere, possibly due to the plant selection of bacterial communities (Yue et al., 2023). The addition of nitrogen fertilizer, in particular, often leads to soil acidification, and soil pH is a predominant factor driving the structure of bacterial communities (Hu et al., 2024). In addition, we found that predatory protists positively correlated with nitrogen enrichment, while phototrophic protists positively linked to phosphorus addition (Fig. 3). Nitrogen addition promotes plant growth, which can enrich bacterial density that promotes phagotrophic protists (Gao et al., 2019; Kowalchuk et al., 2002; Yue et al., 2023). Phototrophic protists may have a relatively high demand for phosphorus, given the extra phosphorus required in the energy transfer processes required for photosynthesis (Neupane et al., 2019).

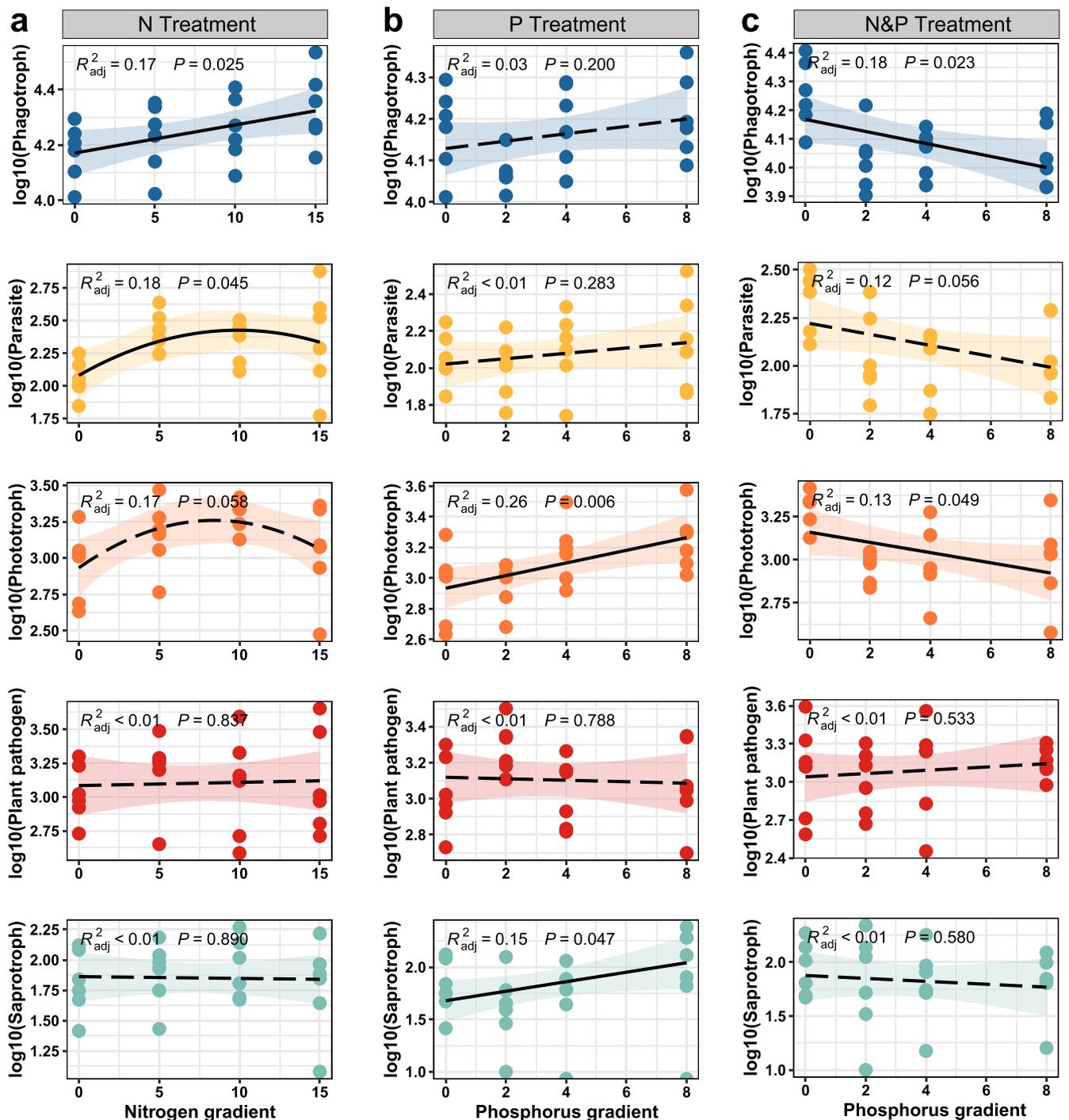
In summary, our findings highlighted that the protist communities are more sensitive to anthropogenic fertilizer additions as compared to bacterial, fungal, and nematode communities in an alpine meadow ecosystem, and they were highly correlated with plant richness and biomass. We emphasize that protist communities may serve as important bioindicators of ecosystem perturbation and plant biodiversity under anthropogenic interference in less disturbed natural ecosystems.

#### CRediT authorship contribution statement

**Pengfei Zhang:** Writing – review & editing, Project administration, Investigation. **Enrique Lara:** Writing – review & editing. **Stefan Geisen:** Writing – review & editing. **Alexandre Jousset:** Writing – review & editing, Methodology. **Qirong Shen:** Writing – review & editing. **Wu Xiong:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis. **Mohammadhossein Ravanbakhsh:** Writing – review & editing. **Francisco Dini Andreote:** Writing – review & editing. **George A. Kowalchuk:** Writing – review & editing, Supervision. **Rong Li:** Writing – review & editing. **Shuo Sun:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis.

#### Declaration of Competing Interest

The authors declare no conflicts of interest.



**Fig. 3.** The relationship between the relative abundance of different protists functional groups and nitrogen (a), phosphorus (b), and nitrogen + phosphorus (c) application. Colored lines show the regression plots of the different soil organism groups. Variables are categorized into different nutrient addition types. The numbers on the X-axis represent the annual fertilizer addition rates per square meter. Values are mean effect sizes with bootstrapped 95 % confidence intervals (CI). Solid lines indicate statistical significance for the relationships, while the dashed lines indicate no statistical significance for the relationships.

#### Data availability

Data will be made available on request.

#### Acknowledgements

This project received fundings from the National Natural Science Foundation of China (42107141 and 42377296), National Key Research and Development Program of China (2023YFD1901402 and

2023YFD1901105), and the Fundamental Research Funds for the Central Universities (YDZX2023023 and XUEKEN2023039).

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2024.109101](https://doi.org/10.1016/j.agee.2024.109101).

## References

- Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A., Agatha, S., Berney, C., Brown, M.W., Burki, F., Cárdenas, P., Cepička, I., Chistyakova, L., del Campo, J., Dunthorn, M., Edvardsen, B., Eglit, Y., Guillaou, L., Hampl, V., Heiss, A.A., Hoppenrath, M., James, T.Y., Karnkowska, A., Karpov, S., Kim, E., Kolisko, M., Kudryavtsev, A., Lahr, D.J.G., Lara, E., Le Gall, L., Lynn, D.H., Mann, D.G., Massana, R., Mitchell, E.A.D., Morrow, C., Park, J.S., Pawłowski, J.W., Powell, M.J., Richter, D.J., Rueckert, S., Shadwick, L., Shimano, S., Spiegel, F.W., Torruella, G., Youssef, N., Zlatogursky, V., Zhang, Q., 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. *J. Eukaryot. Microbiol.* 66, 4–119. <https://doi.org/10.1111/jeu.12691>.
- Ai, C., Zhang, S., Zhang, X., Guo, D., Zhou, W., Huang, S., 2018. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. *Geoderma* 319, 156–166. <https://doi.org/10.1016/j.geoderma.2018.01.010>.
- Archer, E., 2022. rfPermute: Estimate Permutation p-Values for Random Forest Importance Metrics.
- Banerjee, S., van der Heijden, M.G.A., 2023. Soil microbiomes and one health. *Nat. Rev. Microbiol.* 21, 6–20. <https://doi.org/10.1038/s41579-022-00779-w>.
- Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511. <https://doi.org/10.1038/nature13855>.
- Bass, D., Silberman, J.D., Brown, M.W., Pearce, R.A., Tice, A.K., Jousset, A., Geisen, S., Hartikainen, H., 2016. Coprophilic amoebae and flagellates, including *Guttulinopsis*, *Rosculus* and *Helkesimastix*, characterise a divergent and diverse rhizarian radiation and contribute to a large diversity of faecal-associated protists. *Environ. Microbiol.* 18, 1604–1619. <https://doi.org/10.1111/1462-2920.13235>.
- Bates, S.T., Clemente, J.C., Flores, G.E., Walters, W.A., Parfrey, L.W., Knight, R., Fierer, N., 2013. Global biogeography of highly diverse protistan communities in soil. *ISME J.* 7, 652–659. <https://doi.org/10.1038/ismej.2012.147>.
- Bissett, A., Brown, M.V., Siciliano, S.D., Thrall, P.H., 2013. Microbial community responses to anthropogenically induced environmental change: towards a systems approach. *Ecol. Lett.* 16, 128–139. <https://doi.org/10.1111/ele.12109>.
- Cappelli, S.L., Domeignoz-Horta, L.A., Loaiza, V., Laine, A.-L., 2022. Plant biodiversity promotes sustainable agriculture directly and via belowground effects. *Trends Plant Sci.* 27, 674–687. <https://doi.org/10.1016/j.tplants.2022.02.003>.
- Chalcraft, D.R., Cox, S.B., Clark, C., Cleland, E.E., Suding, K.N., Weiher, E., Pennington, D., 2008. Scale-dependent responses of plant biodiversity to nitrogen enrichment. *Ecology* 89, 2165–2171. <https://doi.org/10.1890/07-0971.1>.
- Chen, D., Yuan, L., Liu, Y., Ji, J., Hou, H., 2017. Long-term application of manures plus chemical fertilizers sustained high rice yield and improved soil chemical and bacterial properties. *Eur. J. Agron.* 90, 34–42. <https://doi.org/10.1016/j.eja.2017.07.007>.
- Claesson, M.J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J.R., Smidt, H., Vos, W. M., de Ross, R.P., O'Toole, P.W., 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLoS One* 4, e6669. <https://doi.org/10.1371/journal.pone.0006669>.
- Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., Tiedje, J.M., 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* 37, D141–D145. <https://doi.org/10.1093/nar/gkn879>.
- Creevy, A.L., Fisher, J., Puppe, D., Wilkinson, D.M., 2016. Protist diversity on a nature reserve in NW England—with particular reference to their role in soil biogenic silicon pools. *Pedobiology* 59, 51–59. <https://doi.org/10.1016/j.pedobi.2016.02.001>.
- Dumack, K., Fiore-Donno, A.M., Bass, D., Bonkowski, M., 2020. Making sense of environmental sequencing data: ecologically important functional traits of the protistan groups Cercozoa and Endomyxa (Rhizaria). *Mol. Ecol. Resour.* 20, 398–403. <https://doi.org/10.1111/1755-0998.13112>.
- Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194–2200. <https://doi.org/10.1093/bioinformatics/btr381>.
- Farjalla, V.F., Srivastava, D.S., Marino, N.A.C., Azevedo, F.D., Dib, V., Lopes, P.M., Rosado, A.S., Bozelli, R.L., Esteves, F.A., 2012. Ecological determinism increases with organism size. *Ecology* 93, 1752–1759. <https://doi.org/10.1890/11-1144.1>.
- Fortmann-Roe, S., 2015. Consistent and Clear Reporting of Results from Diverse Modeling Techniques: The A3 Method. *J. Stat. Softw.* 66, 1–23. <https://doi.org/10.18637/jss.v066.i07>.
- Gao, Z., Karlsson, I., Geisen, S., Kowalchuk, G., Jousset, A., 2019. Protists: Puppet Masters of the Rhizosphere Microbiome. *Trends Plant Sci.* 24, 165–176. <https://doi.org/10.1016/j.tplants.2018.10.011>.
- Gehan, E.A., 1965. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. *Biometrika* 52, 203–224. <https://doi.org/10.1093/biomet/52.1-2.203>.
- Geisen, S., Mitchell, E.A.D., Adl, S., Bonkowski, M., Dunthorn, M., Ekelund, F., Fernández, L.D., Jousset, A., Krashevskaya, V., Singer, D., Spiegel, F.W., Walochnik, J., Lara, E., 2018. Soil protists: a fertile frontier in soil biology research. *FEMS Microbiol. Rev.* 42, 293–323. <https://doi.org/10.1093/femsre/fuy006>.
- Geisen, S., Mitchell, E.A.D., Wilkinson, D.M., Adl, S., Bonkowski, M., Brown, M.W., Fiore-Donno, A.M., Heger, T.J., Jassey, V.E.J., Krashevskaya, V., Lahr, D.J.G., Marcisz, K., Mulot, M., Payne, R., Singer, D., Anderson, O.R., Charman, D.J., Ekelund, F., Griffiths, B.S., Rønn, R., Smirnov, A., Bass, D., Belbahri, L., Berney, C., Blandinier, Q., Chatzinotas, A., Clarholm, M., Dunthorn, M., Feest, A., Fernández, L. D., Foissner, W., Fournier, B., Gentekaki, E., Hájek, M., Helder, J., Jousset, A., Koller, R., Kumar, S., La Terza, A., Lamentowicz, M., Mazzei, Y., Santos, S.S., Seppely, C.V.W., Spiegel, F.W., Walochnik, J., Winding, A., Lara, E., 2017. Soil protistology rebooted: 30 fundamental questions to start with. *Soil Biol. Biochem.* 111, 94–103. <https://doi.org/10.1016/j.soilbio.2017.04.001>.
- Geisen, S.A., Lara, E., Mitchell, E. A. D., Völcker, E., Krashevskaya, V., 2020. Soil protist life matters! *Soil Org.* 92, 189–196. <https://doi.org/10.25674/so92iss3pp189>.
- Glücksman, E., Bell, T., Griffiths, R.I., Bass, D., 2010. Closely related protist strains have different grazing impacts on natural bacterial communities. *Environ. Microbiol.* 12, 3105–3113. <https://doi.org/10.1111/j.1462-2920.2010.02283.x>.
- Gong, X., Qiao, Z., Yao, H., Zhao, D., Eisenhauer, N., Scheu, S., Liang, C., Liu, M., Zhu, Y.-G., Sun, X., 2024. Urbanization simplifies soil nematode communities and coincides with decreased ecosystem stability. *Soil Biol. Biochem.* 190, 109297. <https://doi.org/10.1016/j.soilbio.2023.109297>.
- Guillaou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W.H.C.F., Lara, E., Le Bescot, N., Logares, R., Mahé, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawłowski, J., Probert, I., Sauvadet, A.-L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., Christen, R., 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Res.* 41, D597–D604. <https://doi.org/10.1093/nar/gks1160>.
- Hu, S., Li, G., Berlines de Gea, A., Teunissen, J., Geisen, S., Wilschut, R.A., Schwelm, A., Wang, Y., 2023. Microbiome predators in changing soils. *Environ. Microbiol.* 25, 2057–2067. <https://doi.org/10.1111/1462-2920.16461>.
- Hu, X., Gu, H., Liu, J., Wei, D., Zhu, P., Cui, X., Zhou, B., Chen, X., Jin, J., Wang, G., 2024. Different long-term fertilization regimes affect soil protists and their top-down control on bacterial and fungal communities in Mollisols. *Sci. Total Environ.* 908, 168049. <https://doi.org/10.1016/j.scitotenv.2023.168049>.
- Jassey, V.E.J., Walcker, R., Kardol, P., Geisen, S., Heger, T., Lamentowicz, M., Hamard, S., Lara, E., 2022. Contribution of soil algae to the global carbon cycle. *New Phytol.* 234, 64–76. <https://doi.org/10.1111/nph.17950>.
- Jiao, S., Yang, Y., Xu, Y., Zhang, J., Lu, Y., 2020. Balance between community assembly processes mediates species coexistence in agricultural soil microbiomes across eastern China. *ISME J.* 14, 202–216. <https://doi.org/10.1038/s41396-019-0522-9>.
- Kang, L., Chen, L., Zhang, D., Peng, Y., Song, Y., Kou, D., Deng, Y., Yang, Y., 2022. Stochastic processes regulate belowground community assembly in alpine grasslands on the Tibetan Plateau. *Environ. Microbiol.* 24, 179–194. <https://doi.org/10.1111/1462-2920.15827>.
- Köljal, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhun, T., Eberhardt, U., Duena, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martin, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Pöldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* 22, 5271–5277. <https://doi.org/10.1111/mec.12481>.
- Kowalchuk, GeorgeA., Buma, D.S., de Boer, W., Klinkhamer, P.G.L., van Veen, J.A., 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Antonie Van Leeuwenhoek* 81, 509–520. <https://doi.org/10.1023/A:1020565523615>.
- Krome, K., Rosenberg, K., Bonkowski, M., Scheu, S., 2009. Grazing of protozoa on rhizosphere bacteria alters growth and reproduction of *Arabidopsis thaliana*. *Soil Biol. Biochem.* 41, 1866–1873. <https://doi.org/10.1016/j.soilbio.2009.06.008>.
- Lehmann, J., Bossio, D.A., Kögel-Knabner, I., Rillig, M.C., 2020. The concept and future prospects of soil health. *Nat. Rev. Earth Environ.* 1, 544–553. <https://doi.org/10.1038/s43017-020-0080-8>.
- Li, W., Wen, S., Hu, W., Du, G., 2011. Root-shoot competition interactions cause diversity loss after fertilization: a field experiment in an alpine meadow on the Tibetan Plateau. *J. Plant Ecol.* 4, 138–146. <https://doi.org/10.1093/jpe/rtq031>.
- Liu, L., Ren, Y., Sun, S., Liu, C., Ding, K., Li, R., Zhang, P., Shen, B., Ravanbakhsh, M., Xiong, W., Shen, Q., 2024. Unraveling fertilization effects on the dynamics of arbuscular mycorrhizal fungal community in the Qinghai-Tibet Alpine Meadow. *Soil Ecol. Lett.* 6, 240248. <https://doi.org/10.1007/s42832-024-0248-0>.
- Loreau, M., de Mazancourt, C., 2013. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. *Ecol. Lett.* 16, 106–115. <https://doi.org/10.1111/ele.12073>.
- Luan, L., Jiang, Y., Cheng, M., Dini-Andreote, F., Sui, Y., Xu, Q., Geisen, S., Sun, B., 2020. Organism body size structures the soil microbial and nematode community assembly at a continental and global scale. *Nat. Commun.* 11, 6406. <https://doi.org/10.1038/s41467-020-20271-4>.
- Luo, Y., Qin, G., Du, G., 2006. Importance of assemblage-level thinning: A field experiment in an alpine meadow on the Tibet plateau. *J. Veg. Sci.* 17, 417–424. <https://doi.org/10.1111/j.1654-1103.2006.tb02462.x>.
- Ma, M., Zhou, X., Du, G., 2011. Soil seed bank dynamics in alpine wetland succession on the Tibetan Plateau. *Plant Soil* 346, 19–28. <https://doi.org/10.1007/s11104-011-0790-2>.
- Ma, X., Geng, Q., Zhang, H., Bian, C., Chen, H.Y.H., Jiang, D., Xu, X., 2021. Global negative effects of nutrient enrichment on arbuscular mycorrhizal fungi, plant diversity and ecosystem multifunctionality. *New Phytol.* 229, 2957–2969. <https://doi.org/10.1111/nph.17077>.
- Mahé, F., de Vargas, C., Bass, D., Czech, L., Stamatakis, A., Lara, E., Singer, D., Mayor, J., Bunge, J., Sernaker, S., Siemensmeyer, T., Trautmann, I., Romac, S., Berney, C., Kozlov, A., Mitchell, E.A.D., Seppely, C.V.W., Egge, E., Lentendu, G., Wirth, R., Trueba, G., Dunthorn, M., 2017. Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. *Nat. Ecol. Evol.* 1, 1–8. <https://doi.org/10.1038/s41559-017-0091>.

- Mouillot, D., Graham, N.A.J., Villéger, S., Mason, N.W.H., Bellwood, D.R., 2013. A functional approach reveals community responses to disturbances. *Trends Ecol. Evol.* 28, 167–177. <https://doi.org/10.1016/j.tree.2012.10.004>.
- Mukherjee, S., Mishra, A., Trenberth, K.E., 2018. Climate change and drought: a perspective on drought indices. *Curr. Clim. Change Rep.* 4, 145–163. <https://doi.org/10.1007/s40641-018-0098-x>.
- Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and processes of microbial community assembly. *Microbiol. Mol. Biol. Rev.* 77, 342–356. <https://doi.org/10.1128/MMBR.00051-12>.
- Neupane, P., Bhujju, S., Thapa, N., Bhattarai, H.K., 2019. ATP synthase: structure, function and inhibition. *Biomol. Concepts* 10, 1–10. <https://doi.org/10.1515/bmc-2019-0001>.
- Ning, D., Deng, Y., Tiedje, J.M., Zhou, J., 2019. A general framework for quantitatively assessing ecological stochasticity. *Proc. Natl. Acad. Sci. USA* 116, 16892–16898. <https://doi.org/10.1073/pnas.1904623116>.
- Payne, R.J., 2013. Seven reasons why protists make useful bioindicators. *Acta Protozool.* 52, 105–113. <https://doi.org/10.4467/16890027AP.13.0011.1108>.
- Petchey, O.L., McPhearson, P.T., Casey, T.M., Morin, P.J., 1999. Environmental warming alters food-web structure and ecosystem function. *Nature* 402, 69–72. <https://doi.org/10.1038/47023>.
- Porazinska, D.L., Bardgett, R.D., Blaauw, M.B., Hunt, H.W., Parsons, A.N., Seastedt, T.R., Wall, D.H., 2003. Relationships at the aboveground–belowground interface: plants, soil biota, and soil processes. *Ecol. Monogr.* 73, 377–395. [https://doi.org/10.1890/0012-9615\(2003\)073\[0377:RATAIP\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2003)073[0377:RATAIP]2.0.CO;2).
- Ren, Z., Li, Q., Chu, C., Zhao, L., Zhang, J., Dexiecuo Ai, Yang, Y., Wang, G., 2010. Effects of resource additions on species richness and ANPP in an alpine meadow community. *J. Plant Ecol.* 3, 25–31. <https://doi.org/10.1093/jpe/rtp034>.
- Romeralo, M., Moya-Laraño, J., Lado, C., 2011. Social Amoebae: environmental factors influencing their distribution and diversity across South-Western Europe. *Micro Ecol.* 61, 154–165. <https://doi.org/10.1007/s00248-010-9715-5>.
- Saleem, M., Fetzer, I., Harms, H., Chatzinotas, A., 2013. Diversity of protists and bacteria determines predation performance and stability. *ISME J.* 7, 1912–1921. <https://doi.org/10.1038/ismej.2013.95>.
- Saleem, M., Hu, J., Jousset, A., 2019. More than the sum of its parts: microbiome biodiversity as a driver of plant growth and soil health. *Annu. Rev. Ecol. Syst.* 50, 145–168. <https://doi.org/10.1146/annurev-ecolsys-110617-062605>.
- Stefan, G., Cornelia, B., Jörg, R., Michael, B., 2014. Soil water availability strongly alters the community composition of soil protists. *Pedobiologia* 57, 205–213. <https://doi.org/10.1016/j.pedobi.2014.10.001>.
- Storkey, J., Macdonald, A.J., Poulton, P.R., Scott, T., Köhler, I.H., Schnyder, H., Goulding, K.W.T., Crawley, M.J., 2015. Grassland biodiversity bounces back from long-term nitrogen addition. *Nature* 528, 401–404. <https://doi.org/10.1038/nature16444>.
- Sun, R., Zhang, X.-X., Guo, X., Wang, D., Chu, H., 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biol. Biochem.* 88, 9–18. <https://doi.org/10.1016/j.soilbio.2015.05.007>.
- Thakur, M.P., Geisen, S., 2019. Trophic regulations of the soil microbiome. *Trends Microbiol.* 27, 771–780. <https://doi.org/10.1016/j.tim.2019.04.008>.
- Thakur, M.P., van der Putten, W.H., Apon, F., Angelini, E., Vreš, B., Geisen, S., 2021. Resilience of rhizosphere microbial predators and their prey communities after an extreme heat event. *Funct. Ecol.* 35, 216–225. <https://doi.org/10.1111/1365-2435.13696>.
- Vasseur, D.A., McCann, K.S., 2005. A mechanistic approach for modeling temperature-dependent consumer-resource dynamics. *Am. Nat.* 166, 184–198. <https://doi.org/10.1086/431285>.
- Velasco-González, I., Sanchez-Jimenez, A., Singer, D., Murciano, A., Díez-Hermano, S., Lara, E., Martín-Cereceda, M., 2020. Rain-fed granite rock basins accumulate a high diversity of dormant microbial eukaryotes. *Micro Ecol.* 79, 882–897. <https://doi.org/10.1007/s00248-019-01463-y>.
- Voigt, W., Perner, J., Davis, A.J., Eggers, T., Schumacher, J., Bährmann, R., Fabian, B., Heinrich, W., Köhler, G., Lichter, D., Marstaller, R., Sander, F.W., 2003. Trophic levels are differentially sensitive to climate. *Ecology* 84, 2444–2453. <https://doi.org/10.1890/02-0266>.
- Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proc. Natl. Acad. Sci.* 111, 5266–5270. <https://doi.org/10.1073/pnas.1320054111>.
- Wang, C., Liu, D., Bai, E., 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biol. Biochem.* 120, 126–133. <https://doi.org/10.1016/j.soilbio.2018.02.003>.
- Wang, G., Schultz, P., Tipton, A., Zhang, J., Zhang, F., Bever, J.D., 2019. Soil microbiome mediates positive plant diversity-productivity relationships in late successional grassland species. *Ecol. Lett.* 22, 1221–1232. <https://doi.org/10.1111/ele.13273>.
- Wang, P., Yang, F., Chen, X., Li, J., Zhou, X., Guo, H., 2022. Long-term fertilization effects on soil biotic communities are mediated by plant diversity in a Tibetan alpine meadow. *Plant Soil* 474, 525–540. <https://doi.org/10.1007/s11104-022-05356-x>.
- Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73, 5261–5267. <https://doi.org/10.1128/AEM.00062-07>.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In: *PCR Protocols*. Elsevier, pp. 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>.
- Wilkinson, D.M., Mitchell, E.A.D., 2010. Testate amoebae and nutrient cycling with particular reference to soils. *Geomicrobiol. J.* 27, 520–533. <https://doi.org/10.1080/01490451003702925>.
- Wu, S., Dong, Y., Deng, Y., Cui, L., Zhuang, X., 2022. Protistan consumers and phototrophs are more sensitive than bacteria and fungi to pyrene exposure in soil. *Sci. Total Environ.* 822, 153539. <https://doi.org/10.1016/j.scitotenv.2022.153539>.
- Wu, W., Lu, H.-P., Sastri, A., Yeh, Y.-C., Gong, G.-C., Chou, W.-C., Hsieh, C.-H., 2018. Contrasting the relative importance of species sorting and dispersal limitation in shaping marine bacterial versus protist communities. *ISME J.* 12, 485–494. <https://doi.org/10.1038/ismej.2017.183>.
- Xiong, W., Jousset, A., Guo, S., 2018. Soil protist communities form a dynamic hub in the soil microbiome. *ISME J.* 12, 634–638. <https://doi.org/10.1038/ismej.2017.171>.
- Xiong, W., Li, R., Guo, S., Karlsson, I., Jiao, Z., Xun, W., Kowalchuk, G.A., Shen, Q., Geisen, S., 2019. Microbial amendments alter protist communities within the soil microbiome. *Soil Biol. Biochem.* 135, 379–382. <https://doi.org/10.1016/j.soilbio.2019.05.025>.
- Xiong, W., Song, Y., Yang, K., Gu, Y., Wei, Z., Kowalchuk, G.A., Xu, Y., Jousset, A., Shen, Q., Geisen, S., 2020. Rhizosphere protists are key determinants of plant health. *Microbiome* 8, 27. <https://doi.org/10.1186/s40168-020-00799-9>.
- Yue, Y., Liu, C., Xu, B., Wang, Y., Lv, Q., Zhou, Z., Li, R., Kowalchuk, G.A., Jousset, A., Shen, Q., Xiong, W., 2023. Rhizosphere shapes the associations between protistan predators and bacteria within microbiomes through the deterministic selection on bacterial communities. *Environ. Microbiol.* 25, 3623–3629. <https://doi.org/10.1111/1462-2920.16528>.
- Zhang, Liu, W., Bai, Y., Zhang, G., Han, X., 2011. Nitrogen deposition mediates the effects and importance of change in changing biodiversity. *Mol. Ecol.* 20, 429–438. <https://doi.org/10.1111/j.1365-294X.2010.04933.x>.
- Zhang, P., Zhou, X., Li, J., Guo, Z., Du, G., 2015. Space resource utilisation: a novel indicator to quantify species competitive ability for light. *Sci. Rep.* 5, 16832. <https://doi.org/10.1038/srep16832>.
- Zhang, Y., Loreau, M., Lü, X., He, N., Zhang, G., Han, X., 2016. Nitrogen enrichment weakens ecosystem stability through decreased species asynchrony and population stability in a temperate grassland. *Glob. Change Biol.* 22, 1445–1455. <https://doi.org/10.1111/gcb.13140>.
- Zhao, Z.-B., He, J.-Z., Geisen, S., Han, L.-L., Wang, J.-T., Shen, J.-P., Wei, W.-X., Fang, Y.-T., Li, P.-P., Zhang, L.-M., 2019. Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. *Microbiome* 7, 33. <https://doi.org/10.1186/s40168-019-0647-0>.
- Zhou, J., Jiang, X., Zhou, B., Zhao, B., Ma, M., Guan, D., Li, J., Chen, S., Cao, F., Shen, D., Qin, J., 2016. Thirty four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. *Soil Biol. Biochem.* 95, 135–143. <https://doi.org/10.1016/j.soilbio.2015.12.012>.
- Zhou, X., Liu, X., Zhang, P., Du, Z.G., G., 2019. Increased community compositional dissimilarity alleviates species loss following nutrient enrichment at large spatial scales. *J. Plant Ecol.* 12, 376. <https://doi.org/10.1093/jpe/rt035>.
- Zhu, L., Luan, L., Chen, Y., Wang, X., Zhou, S., Zou, W., Han, X., Duan, Y., Zhu, B., Li, Y., Liu, W., Zhou, J., Zhang, J., Jiang, Y., Sun, B., 2024. Community assembly of organisms regulates soil microbial functional potential through dual mechanisms. *Glob. Change Biol.* 30, e17160. <https://doi.org/10.1111/gcb.17160>.