



Protists: the hidden ecosystem players in a wetland rice field soil

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Abstract

Irrigated and rain-fed rice fields are unique agroecosystems and anthropogenic wetlands whose main feature is seasonal flooding. Flooded soils are characterized by spatiotemporal shifts and oscillation of the oxygen status and redox potential, sustaining varieties of microbial metabolisms, where bacteria and methanogenic archaea play principal roles and thus have been the major research targets. In this review, we focus on the diversity and ecology of protists—often overlooked biological entities—in wetland rice field soils. Protists with different ecological functions, i.e., phagotrophs, phototrophs, saprotrophs, and parasites, inhabit a rice field soil with a community- and individual-level adaptation to the wide range of oxygen tensions and redox potential. Other agricultural managements like fertilization and char application also influence the protist community. They link to the material cycling in rice soil and affect the activities and community composition of the microorganisms involved in the biogeochemical cycles. Rice roots are the hot spot for protists, which control the rhizospheric bacterial community and could increase the plant productivity through enhancing nutrient release and altering bacterial activities. This review highlights the essential roles of protists in a wetland rice field soil and needs for further research to fill the gaps in knowledge regarding the diversity and functions of the protists in this unique agroecosystem.

Keywords Algae · Methane · Paddy soil · Protozoa · Rice rhizosphere

Introduction

Protists constitute the invisible majority of eukaryotes, including all eukaryotes outside land plants, animals, and, arguably, fungi. They are predominantly unicellular and span the entire eukaryotic tree of life (Adl et al. 2012). Protists are ubiquitous on the planet and play different functional roles in the ecosystems as primary producers, decomposers, predators, and symbionts (Geisen et al. 2017). The recent upsurge in the study of environmental microorganisms has mainly focused on bacteria and fungi and also recently on archaea and viruses. However, not everyone today consciously recognizes that protists form an essential component of the microbial world (Caron et al. 2009).

The oversight regarding the existence, ecological roles, and biogeochemical importance of soil protists had also been

true or even worse than aquatic protists (Geisen et al. 2017). However, the importance of soil phagotrophic protists (protozoa), i.e., regulating the bacterial biomass and community and promoting nutrient cycling and plant growth, was recognized nearly 40 years ago (Clarholm 1985), which is contemporary with the concept of the “microbial loop” in aquatic environments (Azam et al. 1983). After some lag to aquatic protists and soil “non-protist” microorganisms in the era of modern microbiology, soil protists are receiving renotice as the essential players in the soil ecosystem (Geisen et al. 2018; Xiong et al. 2018) and plant growth (Gao et al. 2019; Guo et al. 2021). Methodological advances also give new evidence and insights into the trophic diversity of soil protists; non-bacterivores (fungivores, omnivores, predators of other protists and nematodes), phototrophs, and parasites might be equally important protists in soil ecosystems (Geisen and Bonkowski 2018).

Rice is one of the essential staple foods in the world, together with maize and wheat. Rice agriculture is concentrated in Monsoon Asia, occupying nearly 90% of harvested area and production (FAO 2022). The large majority of rice is cultivated in paddy fields under submerged conditions for a substantial period of rice agriculture. The soil in the

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submerged conditions gives specific physical, chemical, and biological features distinct from the other agricultural lands, such as upland fields and pasture lands (Conrad and Frenzel 2002; Kimura 2000; Kirk 2004; Kyuma 2004). One of the striking features is the spatiotemporal difference in the soil oxygen tension and redox potential caused by water management (Liesack et al. 2000; Zhang and Furman 2021). Waterlogged rice field soil is also regarded as a model ecosystem for wetland soil microbiology, where bacteria and archaea (mainly methanogenic archaea) have been studied in detail (Conrad and Frenzel 2002).

The moisture conditions and redox status in rice field soils range from drained and aerobic conditions to submerged and strongly reduced conditions, which characterize the diversity and multifunctionality of the microorganisms living in paddy soils (Conrad 2020; Kögel-Knabner et al. 2010). The reduction processes sequentially proceed according to the energy yields governed by the thermodynamics (Conrad and Frenzel 2002): denitrification; reduction of manganese, iron, and sulfate; and finally, methanogenesis (Fig. 1). The dynamic biogeochemical cycles in paddy soils, including methanogenesis and methane oxidation for carbon and nitrification, denitrification, and nitrogen fixation for nitrogen, are conducted by prokaryotes (bacteria and archaea), which have been thus the primary target of paddy soil microbiology (Kim and Lee 2020 and references therein). Fungi are another important microbial member but are considered to play less important roles in soil under submerged conditions because the limited availability of oxygen and air space suppresses the activities of fungi, including plant pathogens. Therefore, unlike bacteria and methanogenic archaea, fungi have not been a significant target in paddy soil microbiology with exceptions for fungi in aerobic decomposition

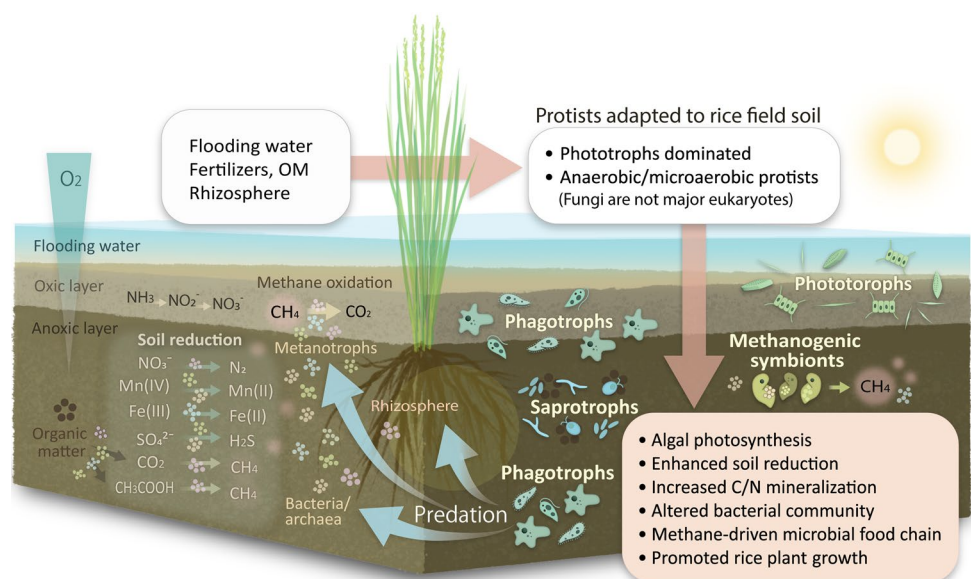
(Yarwood 2018), AM fungi (Herdler et al. 2008), and denitrifying fungi (Ishii et al. 2011).

The features of submerged soils would not be a constraint for protists as they live as aquatic organisms even in soil environments (Fenchel 1987). Water availability in the soil is a critical factor that controls the abundance, community composition, and functions of protists in soil (Anderson 2000; Bates et al. 2013; Geisen et al. 2014). Anoxia and low redox potentials would not suppress all protists but could drive the adaptation and evolution of anaerobic protists (Fenchel and Finlay 1995; Gawryluk and Stairs 2021). This review aims to provide the current body of knowledge regarding the diversity, ecology, and functional roles of protists in wetland rice field soil.

Diversity of protists in rice field soil

Metabarcoding of protists in environments has rapidly developed (Bates et al. 2013; Geisen 2016b; Mahé et al. 2017; Oliverio et al. 2020). As protists include the highly polyphyletic groups of different taxa, universal primers targeting eukaryotic ssu (18S) rRNA genes and their transcripts are often used, as well as the group-specific primers (Vaulot et al. 2022). Automated assignment of high-throughput sequencing (HTS) data with databases should be interpreted carefully (Dupont et al. 2016; Lara et al. 2022), but HTS gives a holistic view of the vast diversity of soil protists with different ecological functions. Shotgun metagenomes (Jacquiod et al. 2016; Oliverio et al. 2020) and metatranscriptomes (Geisen et al. 2015) are further options for studying the diversity and functions of the soil protistan community. RNA-based methods and stable-isotope probing can determine the active community (Murase and Frenzel 2007).

Fig. 1 Ecological and functional features of protists (right) and their interactions with the agricultural managements and microbial processes (left) in a wetland rice field soil



A comparative study of the taxonomic and functional diversity of the protistan community in different environments (soil, freshwater, and marine) demonstrated that the soils from the natural and agricultural fields have distinct protistan communities with the highest species richness of protists, characterized by predators as the dominant functional group (Grossmann et al. 2016; Sieber et al. 2020; Singer et al. 2021; Xiong et al. 2021). The HTS studies also demonstrated the unexpected presence of typically marine protists such as choanoflagellate and foraminiferan groups (Geisen et al. 2015). Climatic conditions (moisture) and pH are the primary environmental factors affecting soil protistan communities' biogeography (Aslani et al. 2022; Bates et al. 2013; Oliverio et al. 2020). Pedogenesis (soil age) may indirectly influence protistan predators' richness and community composition by affecting the bacterial and fungal biomasses (Xiong et al. 2022).

Chen et al. (2021) studied the biogeography of protist communities in rice and maize field soils across eastern China. HTS datasets demonstrated that the protist community of rice field soils was distinct from that of the adjacent maize fields, characterized by the higher relative abundance of Chlorophyceae (green algae), Bacillariophyta (diatoms), and Endomyxa (Rhizaria). The higher abundance of green algae and diatoms may be partly due to the influence of the planktonic species in the flooding water. However, soil algae are also active in the rice field soil as the dominant members in the microeukaryotic community over fungi (Murase et al. 2015), which indicates the importance of phototrophs among the protists in rice field soils. Protists in rice soils show a lower alpha diversity, less association with bacteria, and a significant correlation with the alpha diversity of the fungal community (Chen et al. 2021). The assembly of the fungal and protistan communities is more structured by a selection-based deterministic process than by dispersal-based stochastic processes (Luan et al. 2020), suggesting that the irrigated rice agriculture should foster the distinct protistan community in the rice field soils. The dominance of some protistan groups changes along with latitude; the relative abundance of Bacillariophyta increased with the increasing latitude, while Chlorophyceae showed the opposite trend. The mean annual temperature, soil texture, and nutrients (N and K) are the most important environmental variables to predict the relative abundance of different groups of protists in rice field soils (Chen et al. 2021). The other soil physiochemical properties, such as pH, EC, and exchangeable cations, also shape the protistan community in rice field soils (Asiloglu et al. 2021c).

Besides the molecular-based analysis, culture-based and microscopic studies of the protistan communities in a rice field soil have been conducted, targeting the selective groups. To our knowledge, Sandon (1927) first investigated the "protozoan fauna" (flagellates, ciliates, amoeba,

and testaceous rhizopods) in the rice field soils from Japan, India, and Myanmar (Burma in those days). Since then, however, only a few studies have reported the abundance and community composition of soil protists in rice fields. Takahashi and Suhama (1991) described the species composition of ciliates living in the rice straw and soil in the rice field and reported the dominance of hypotrich species. Madoni (1996) observed the higher numbers of ciliates in the soil-water interface of the Italian rice field compared to the water column dominated by *Coleps*. Prokina et al. (2017) studied the species composition of heterotrophic flagellates and heliozoans in the freshwater habitats of various types in Ethiopia and reported the highest species richness in puddles at the rice field. A novel heterolobosean amoeba was isolated from an Italian rice field soil (Murase et al. 2010).

Impact of soil management on protist community

Water regime

Many rice fields have a natural or artificial cyclic process of submergence and drainage (Kyuma 2004), giving dynamic conditions in the soil moisture content, which is the primary factor affecting soil protists' community composition (Geisen et al. 2014). Abdallah et al. (2019) demonstrated in a microcosm incubation experiment that drainage after the flooding period reduces the ssu rRNA abundance of Cercozoa and *Acanthamoeba* while inducing an increase in fungi (Ascomycota and Basidiomycota) absolute and relative ssu rRNA abundance. The impact of mid-summer drainage on the microeukaryotic community was also reported for the organic compartments in rice field soil (Hatamoto et al. 2008; Sugano et al. 2007). Aridity may also affect soil protistan network complexity and stability (Chen et al. 2022). On the other hand, the protist and other microeukaryotic communities of the meadow soil were not significantly affected by flooding (Graupner et al. 2017). The difference may suggest the community-level adaptation of the rice soil protists to the water regime caused by long-term rice agriculture. Change in water regime induced by crop rotation may also influence the protistan community in rice field soil. Asiloglu et al. (2015) reported a distinct difference in the microeukaryotic community at the harvesting stage of rice and wheat in the soil under the double cropping system.

Fertilizers

Soil microorganisms are also strongly affected by other crop management than water management, including fertilization. Bacterial and fungal communities in rice field soils are affected by fertilization treatments associated with the change in soil properties, including soil organic matter, total P, and pH (Geisseler et al. 2017; Kuppasamy et al. 2018).

Organic fertilizer treatments increase total microbial biomass in the rice field soil (Kuppusamy et al. 2018). Protists show a higher sensitivity to nitrogen fertilization than bacteria and fungi in diverse agricultural upland soils (Zhao et al. 2019)

Long-term fertilization shapes the microeukaryotic community composition of rice field soils (Murase et al. 2015). The phosphorus fertilizer primarily affects the community composition of protists in the rice field soil over nitrogen and potassium fertilizers. A high relative abundance of diatoms characterized soils without phosphorus fertilizer, whereas, in phosphorus-fertilized soils, green algae predominated. Different rates of organic fertilizer (rice straw compost) application have distinctive effects on the algal composition. Phagotrophic protists, including Ciliophora, Amoebozoa, Heterolobosea amoeba, and various groups of flagellates, are the largest group among the potentially active (rRNA-based) microeukaryotic community in the soils with organic fertilizer application. Besides the protists, bacterivorous nematodes are dominant in the soil without fertilizers. Studies of the upland soil often demonstrate that chemical fertilizers, including nitrogen, reduce the diversity and relative abundance of phagotrophic protists (Guo et al. 2018; Zhao et al. 2019, 2020). A meta-analysis demonstrates that applying mineral fertilizers increases soil microbial biomass carbon in paddy rice systems, more pronounced than in upland cropping systems (Geisseler et al. 2017), which may enrich phagotrophic protists in a wetland rice soil. Such differences may partly contribute to the distinct effects of fertilization on the protists in the rice field soil. Thus, fertilizer management would be an essential determinant of the structure and function of the microbial food web in the rice field soil (Murase et al. 2015), as demonstrated in the upland soil (Xiong et al. 2018).

Hu et al. (2017) studied the responses of the microbial community at the different trophic levels in the rice rhizosphere to elevated CO₂ and fertilization. Elevated atmospheric CO₂ concentrations reduced the number of flagellates in the rice rhizosphere at the heading stage with no fertilizer application, while amoeba increased with elevated CO₂ at the heading stage. Elevated CO₂, fertilization, and rice cultivars interactively affect the phagotrophic protists and nematode community in the rice field soil and, thus, the microbial-food webs.

Biochar

Biochar has the potential to mitigate the carbon footprint of farming systems. The use of biochar in rice agriculture has been advocated as a potential strategy not only to increase carbon stock but also to reduce methane emission from rice fields and improve soil function and crop productivity, notwithstanding the effects depend on various factors such as

the properties of biochars, soil properties, and land management (Asadi et al. 2021; Mohammadi et al. 2020). Although biochar differently affects microbial communities depending on the raw material, pyrolyzation temperature, and soil type (Cai et al. 2021), our knowledge of the biochar-protist interaction in paddy field soils is limited (Asiloglu 2022). Asiloglu et al. (2020a) studied the short-term effects of two biochars (rice husk and poultry litter biochars) on the protistan community of rice field soil. Applying the biochars altered the taxonomic and functional compositions of the protists, with the increased relative abundance of phagotrophs with the poultry litter biochar and decreased plant parasites with both biochars. The change in the protistan community caused by the biochar application was correlated with the change in the soil properties, including increased pH, nitrogen, phosphate, potassium, magnesium, total volume, and C/N ratio.

Protists in hypoxia/anoxia

Paddy rice fields have a natural or artificial cyclic process of submergence and drainage. Waterlogging limits the oxygen diffusion from the atmosphere to the soil, creating various environments in terms of oxygen tension and redox potential with spatiotemporal heterogeneity. Such heterogeneity sustains various aerobic and anaerobic metabolisms of prokaryotic microorganisms (Kimura 2000; Kirk 2004).

Oxygen status also affects the microeukaryotic community, including protists. Either hypoxia or anoxia not only suppresses the activities of aerobic microeukaryotes like fungi but also supports microaerophilic or anaerobic protists, which has been demonstrated in aquatic environments (e.g., Bernhard et al. 2003; Edgcomb et al. 2011; Fenchel and Finlay 2008; Fenchel and Finlay 1995; Fuchsman et al. 2022; Orsi et al. 2011; Stoeck and Epstein 2003).

Anaerobic protists also inhabit terrestrial environments, being found mainly in swamps and floodplains, where the soil is under continuously anoxic conditions (Foissner 1998). However, the frequent cyclic process of submergence and drainage is not a constraint for the anaerobic protists in rice field soil. Schwarz and Frenzel (2003) reported the high diversity of ciliates in anoxic rice field soil, particularly in the initial period of anoxic conditions. The ciliate community in the anoxic rice soil consisted of different species of obligate (*Metopus* spp.) and facultative (e.g., Oxytrichidae and Spathidiidae) anaerobes, shifting with time. An incubation experiment with RNA-based molecular analysis demonstrated that the anoxically incubated rice field soil included the active microeukaryotic community distinct from the oxic rice soil. Heterolobosea amoeba (*Harpagon*), ciliates (*Metopus*), and oomycetes (*Pythiaceae*, *Lagenidium*) were enriched as active members in the anoxic soil with organic amendment (Murase et al. 2014). The anaerobic protists

may respond differently to the redox potentials (Asiloglu and Murase 2016).

Phagotrophic protists (amoeba and flagellates) in a rice field soil adapt to a wide range of oxygen tensions, to which the phagotrophic protists show a community shift (Take-nouchi et al. 2016). The amoebas isolated from the rice field soil keep their activities under the oxygen tension, ranging from the atmospheric level (21%) to below the Pasteur point (0.21%). The versatility of these amoebas suggests their ability to search for food in soil environments such as the oxic-anoxic interface of flooded soil or inside soil aggregates that are inaccessible to ciliates with larger sizes. The amoeba *Acanthamoeba castellanii* (Amoebozoa) and amoeboflagellates *Naegleria gruberi* and *Naegleria fowleri* (Discoba) are facultative anaerobes that are often found in soil environments. These amoebas have mitochondrion-related organelles (MROs) where the functional genes associate with anaerobiosis, like [FeFe]-hydrogenase (Gawryluk and Stairs 2021), besides the typical mitochondrion machinery of oxidative phosphorylation. Protists with such MROs would adapt to the range of oxygen tensions.

Functional roles of protists in rice field soil

Soil protists are categorized into four groups according to their ecological functions: phagotrophs, saprotrophs, phototrophs, and symbionts (Geisen et al. 2018). All the functional groups of protists also inhabit a rice field soil.

Phagotrophs

Paddy soils include higher amounts of microbial biomass than upland soils (Wei et al. 2022), which would benefit phagotrophic protists. Production of ciliates in the rice field soil is estimated to be comparable to that in the eutrophic pond (Madoni 1996).

The impact of phagotrophic protists on bacterial populations is often studied by re-inoculating the bacterial assemblage to the sterilized soil with or without protists to compare different microbial parameters between the two treatments: with vs. without protists. Meta-analysis of the relevant literature demonstrated that the global effect of grazing of bacterivores (protists and nematodes) on the soil microbial biomass and bacterial abundance was – 16 and – 17% of control (without grazers), respectively (Trap et al. 2016). The study through the same approach demonstrated that microbial biomass assessed by the amount of extracted DNA reduced by 47% in the top layer, including the oxic zone (0–3 mm in depth) of water-saturated rice field soil, when the natural assemblage of protists was present (Murase et al. 2006). This finding suggests a higher impact of phagotrophic protists on microbial biomass in rice field soil than in upland soil.

Phagotrophic protists functioning as a shaping force on the bacterial community have been well acknowledged in the soil ecosystems (Rønn et al. 2002; Singh 1941). Protists also shape the bacterial community in a wetland rice field soil, particularly in the surface layer where oxygen is available (Murase et al. 2006). Protists increased relative abundance of Clostridia, while Betaproteobacteria became highly dominant without protists, which can be explained by the recent study that grazing preferences are modulated by bacterial cell-wall structure and growth rate (Thompson et al. 2021). Preferential grazing on soil bacteria was also demonstrated for the protists isolated from rice field soils (Asiloglu et al. 2020b; Murase and Frenzel 2008). The top-down effects of phagotrophic protists are more significant than the bottom-up effects of fertilizers on the formation of bacterial communities in rice field soil (Asiloglu et al. 2021a). Protists also prey on fungi (Geisen 2016a; Geisen and Bonkowski 2018). Network analysis of the eukaryotic community of rice field soils under different climatic zones in China suggests that protist grazing may also control the fungal community in the rice field soils (Huang et al. 2021).

Different soil amoebas show bactericidal effects on rice bacterial pathogens (*Xanthomonas oryzae*) through direct grazing and suggested production of extracellular bactericidal compounds (Long et al. 2018).

Saprotrophs

Bacteria and fungi are the major drivers of soil carbon cycling through their decomposing activity of plant-derived carbon, but protists are also involved in the decomposition process. Cellulose-derived carbon is assimilated by micro-eukaryotes, including phagotrophic and mixotrophic protists, in oxic slurries of agricultural soil (Chatzinotas et al. 2013). Involvement of protists in the decomposition of organic matter has been well documented in the terrestrial ecosystems. Resource partitioning between bacteria, fungi, and protists is reported in the detritosphere of upland soil, where saprotrophic and fungivorous protists are involved (Kramer et al. 2016). Soil protists could also function as “catalyzers” of microbial litter breakdown and carbon cycling (Geisen et al. 2021; Kuikman et al. 1990). Protist grazing can change the functional profiles of the bacterial community (Flues et al. 2017). In addition, protists (microflagellates) may contribute to a substantial part of potential CO₂ efflux from soil under moss-rich environments (Roger Anderson 2008).

Sugano et al. (2007) reported that fungi (Ascomycota) are the dominant microeukaryotes inhabiting rice straw incorporated in a Japanese rice field soil under drained conditions, while phagotrophic and saprotrophic protists, including *Cercomonas* and *Phytophthora*, inhabited under the flooded conditions. Hatamoto et al. (2008) reported the succession of the microeukaryotic community in the rice straw compost

placed in a Japanese rice field. Mid-season drainage affected the community, in which different groups of ciliates and testate and naked amoebas characterized the community in the late stage of rice cultivation, suggesting their indirect contribution to the decomposition of the compost through preying on bacteria proliferated in the compost. The stable isotope probing approach showed direct evidence that not only fungi but also oomycetes and Amoebozoa proliferated on ^{13}C -labeled plant residue in a rice field soil (Murase et al. 2012). *Polymyxa*, usually regarded as a plant parasite, was also demonstrated to utilize the plant residue.

Phototrophs

The floodwater and floodwater-soil interface in wetlands are optimal environments for terrestrial phototrophic microorganisms due to the light and hydrological conditions. The net primary production is often more remarkable in wetlands than in drylands located in similar climate zones (Kirk 2004). Indeed, the protist community of a rice field soil is characterized by the dominated phototrophs, including green algae and diatoms (Murase et al. 2015), implying the importance of primary production by phototrophic protists in a rice field soil. The cell density of diatoms and the viable count of other microalgae markedly increased in the soil collected at a depth of 0–1 cm during the flooding period, dominated by *Chlorella* spp., *Nitzschia* spp., and *Navicula* spp., whereas *Scenedesmus* spp. and *Hantzschia* spp. were predominant during the drainage period (Fujita and Nakahara 2006). The shift of dominant algae demonstrates the community-level adaptation to the fluctuating hydrological conditions in rice field soil. Despite the unavailability of light, many microalgae survive in the subsurface soils throughout the annual cultivation cycle (Fujita and Nakahara 2006). The biomass of benthic algae far exceeds that of planktonic algae (Yamagishi et al. 1980).

Recently, the significance of CO_2 fixation by soil algae in the global scale carbon cycle is suggested through machine learning modeling (Jassey et al. 2022). Liao et al. (2023) reported a higher potential of CO_2 fixation in rice field soils than in forest and upland soils. Feng et al. (2011) demonstrated by a stable-isotope probing approach that illumination of the rice field soil in a microcosm under illumination induces a phototrophy-driven microbial food web, in which green algae (*Scenedesmus*), Amoebozoa, and Cercozoa were involved in the utilization of ^{13}C -labeled formate.

Symbionts (parasites)

Rice stripe necrosis virus (RSNV) is a soil-borne benyvirus and causes severe viral disease in rice-growing areas in Africa and America (Wang et al. 2022). RSNV is vectored by *Polymyxa graminis* (Cercozoa, Plasmodiophoridae), a

root obligate biotrophic plant parasite. *P. graminis* is commonly detected in rice roots at the nursery stage and the early stage after transplantation, followed by the abrupt disappearance (Ikenaga et al. 2004). *P. graminis* transmits different viruses that cause diseases in other crops (Kanyuka et al. 2003).

High-throughput DNA metabarcoding revealed a diverse community of protists parasitizing soil metazoan (Geisen et al. 2015), but no such evidence has been provided for rice field soils. Scanning electron microscopic observation detected epibiotic protists, likely flagellates, on benthic crustaceans (Ostracoda) (Taniguchi et al. 1999). A ciliated protist (*Chilodonella uncinata*) infects mosquito larvae in the surface water of paddy fields and causes high mortality in susceptible hosts (Das 2003).

Linking to biogeochemical cycles in a rice field soil

Covering a rice field with flooding water creates a steep gradient of dissolved oxygen from the soil-water boundary to 3–6 mm in the depth of the surface soil (Liesack et al. 2000). Such a spatial shift of oxygen status with temporal oscillations controls the biogeochemical cycles of different elements, including carbon, nitrogen, manganese, iron, and sulfur (Kirk 2004; Kögel-Knabner et al. 2010).

Soil respiration and sequential reduction processes

Murase et al. (2006) demonstrated the effect of protists on the biogeochemistry of wetland rice field soil by comparing an untreated control soil with a γ -radiation-sterilized soil that had been reinoculated with a natural bacterial assemblage. In order to verify that the observed effects were due to protistan grazing and did not result from sterilization, they set the third set of microcosms containing sterilized soil that had been reinoculated with natural assemblage bacteria plus protists. The presence of protists induced a steeper vertical profile of dissolved oxygen in the soil, indicating enhanced microbial respiration, inconsistent with the more active exchange rate of O_2 and CO_2 ; the presence of protists enhanced CO_2 emission from water-saturated rice field soil up to 100% compared to the conditions without protists. The extractable SO_4^{2-} was less with protists than without protists, suggesting that the protists stimulated the sequential reduction processes.

Nitrogen cycles

The soil food web significantly affects the nitrogen cycle in the soil. Predation by microfauna, predominantly protists, could contribute to 30–40% of the annual nitrogen mineralization in grass and cropland soils (Whalen et al. 2013). Predation by protists enhances nitrogen mineralization in a

water-saturated rice field soil by up to 50% (Murase et al. 2006). Such promoted nitrogen mineralization by protists may also be because the water-saturated rice field soil is a preferable environment for soil protists as the soil pores are filled in water where protists freely locomote, seeking their foods with less restriction by hypoxia. Another possible reason may be that the flooded soil suppresses the fungal activity and thus promotes the bacterial-dominated food web, which is linked to a faster rate of N-cycling than fungal-dominated food webs (Whalen et al. 2013).

Denitrification would proceed faster in the presence of protists than in their absence (Murase et al. 2006). However, it is unclear whether it is the direct effect on denitrifying microorganisms or the indirect effect of protist grazing that enhances oxygen consumption and fastens the sequential reduction process in the water-saturated rice soil. Dissimilatory nitrate reduction by protists is known in marine environments, where ciliates (*Loxodes* sp.), foraminifers, and diatoms are involved in the process (Kamp et al. 2015). Foraminifera, as well as ciliates and diatoms, are widespread in freshwater and soils (Holzmann et al. 2021), but it remains to be studied if the soil protists have denitrification activities.

The presence of protists increases the abundance of the putative bacterial genes in a rice field soil involved in nitrogen cycles, including mineralization, dissimilatory nitrate reduction to ammonium, and nitrate assimilation (Asiloglu et al. 2021b). Protists could activate the activity of nitrifying bacteria (Verhagen et al. 1993) and nitrogen-fixing bacteria (Martinez-Reyes et al. 2022), but no such evidence is yet given in a rice field soil ecosystem.

Methane production

Anoxic rice field soil supports the growth of different species of anaerobic ciliates *Metopus* (Schwarz and Frenzel 2003). *Metopus* spp. harbor hydrogenotrophic methanogens as endosymbionts in the cytoplasm (Fenchel and Finlay 1995). The symbiotic methanogens significantly contribute to methane production in the initial period after flooding a rice field soil when the bulk soil redox potential is still high, and other anaerobic bacteria, like iron reducers and sulfate-reducing bacteria, outcompete the activity of non-symbiotic methanogens (Schwarz and Frenzel 2005). The symbiotic methanogens play a minor role in total methanogenesis in the rice field soil after the prolonged flooding, consistent with the results in Dutch freshwater sediments (van Hoek et al. 2006). However, *Metopus* also stimulates the methanogenic activity of soil by grazing (Biagini et al. 1998) and becomes an essential member in anoxic soils with a supply of acetate, as suggested in anaerobic sediments (Holmes et al. 2014). Anaerobic protists also produce different fermentation products by predation: acetate,

propionate, butyrate, and hydrogen (Hirakata et al. 2020), which a methanogenic microbial consortium could utilize.

Microbial food chain driven by methane oxidation

Methane oxidation is a crucial process that controls methane emission from a flooded rice field as a substantial part of methane produced in anoxic environments is oxidized in the oxic rice rhizosphere and surface soils (Conrad 2007). Methane-oxidizing bacteria (methanotrophs) are exclusively involved in aerobic methane oxidation and utilization of methane carbon (Conrad 2007), but methane-carbon assimilated and metabolized by methanotrophs can link to a microbial food web in a rice field soil. Sultana et al. (2022) reported that 24–60% of methane-derived carbon accumulated as soil organic carbon in rice field soils after incubation under air enriched with methane (5% [v/v]).

Murase and Frenzel (2007) conducted a stable-isotope probing of the microbial community involved in using methane carbon through a microcosm study that simulated the oxic-anoxic boundary layer to expose a rice field soil to an opposing gradient of oxygen and methane. The sequences of ^{13}C -enriched heavy RNA were affiliated not only with methane-oxidizing bacteria but also with phagotrophic protists, including specific representatives of amoebae, ciliates, and flagellates. Another stable-isotope probing experiment using ^{13}C -labeled methanotroph cells demonstrated that methanotroph biomass added to oxic rice field soil was incorporated into protist phospholipid fatty acids (PLFAs) within 24 h (Murase et al. 2011). Methanol, an intermediate metabolite of methane oxidation, supports the growth of the microeukaryotic community in the rice field soil, including predatory soil flagellates of Cercozoa (Lueders et al. 2004).

The number of phagotrophic protists grazing on methane-oxidizing bacteria estimated by the most probable number (MPN) method using methanotroph isolates was often at the level of 10^4 -per-gram dry weight of soils, which is comparable to the number estimated using *Escherichia coli*, a commonly used food bacterium (Murase and Frenzel 2008). On the other hand, some *Methylocystis* spp. yielded fewer numbers of methanotrophs-grazing protists. Selective predation on methanotrophs by phagotrophic protists was verified by an incubation experiment using amoebas and flagellates isolated from the rice field soil. Strain-specific incorporation of methanotrophic biomass into eukaryotic grazers in a rice field soil was also demonstrated by PLFA-SIP (Murase et al. 2011). Predation by protists could alter the methanotrophic community in a rice field soil toward the increased dominance of type-II methanotrophs due to the preferential grazing of protists on type-I methanotrophs (Murase and Frenzel 2010). A species-level difference in resistance to predation by protists was also demonstrated in soil pseudomonads (Amacker et al. 2020). It has been reported that bacterial

traits related to the suppression of plant pathogens (fungal pathogens and *Pythium ultimum*) are related to protection against protists (see references in Amacker et al. 2020), but such traits remain to be explored for methanotrophs.

Ecology of protists in rice rhizosphere

The majority of the plant-microbe interactions occur in the rhizosphere: a narrow zone of soil surrounding the roots of living plants. The plant roots deposit carbon-containing organic compounds, including sloughed-off root cells and tissues, water-soluble and volatile compounds, and mucilages, collectively known as rhizodeposits (Hütsch et al. 2002). The rhizodeposits promote microbial life in the rhizosphere (Tian et al. 2020), which results in altered and, often, enhanced microbial communities compared to the non-rhizosphere (bulk) soil. Although the rhizosphere effect of the roots depends on plant- and soil-originated factors, all plants can show much or less rhizosphere effect on all microbial groups, including protists. The rhizosphere effect on protist communities has been demonstrated for several plant species, including forest trees (Fiore-Donno et al. 2022), maize (Taerum et al. 2022), wheat (Rossmann et al. 2020), soybean (Zhang et al. 2021), sorghum (Li et al. 2021), switchgrass (Ceja-Navarro et al. 2021), and rice (Asiloglu et al. 2015; Asiloglu and Murase 2016; Asiloglu et al. 2021c).

Effects of rice roots on protists

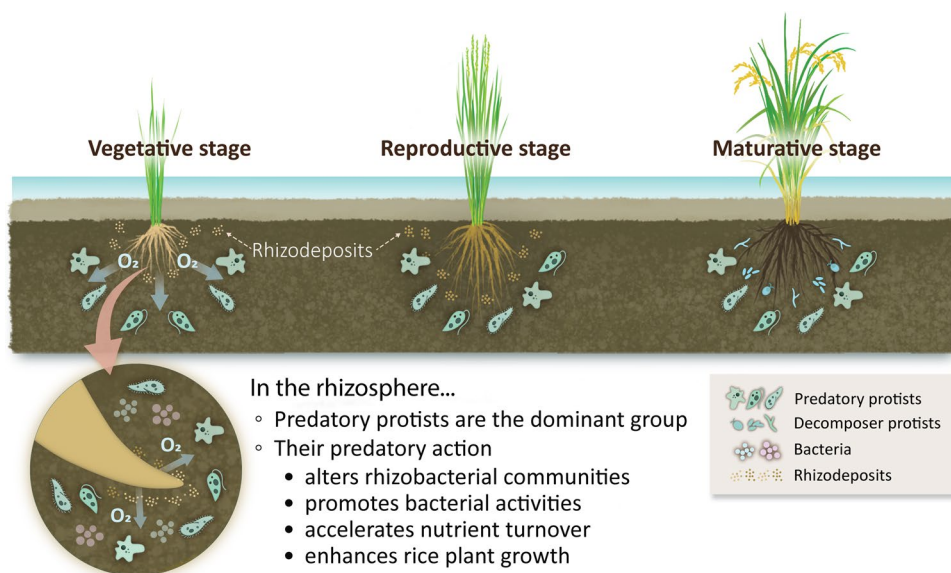
Paddy fields are not only biogeochemically distinct from the upland fields, but also the rhizosphere effects of rice plants are different from that of upland plants (Fig. 2). For instance,

a field study under the rice-wheat rotation system showed distinct protist communities in the rice rhizosphere from the wheat rhizosphere (Asiloglu et al. 2015). In addition to the effects of rhizodeposits, which account for the majority of the rhizosphere effect in upland fields, the rice roots release oxygen to the rhizosphere depending on the root segment and the growth stage (Ando et al. 1983)(Fig. 2). The balance between the oxidizing power of the rice roots and the oxygen consumption by rhizosphere microorganisms controls the redox conditions in the rice rhizosphere (Ando et al. 1983). In general, the oxidizing power of young rice plants is higher than that of the older plants, making the rice rhizosphere more oxic than bulk soil in the early plant growth stage (Asiloglu and Murase 2016, 2017) (Fig. 2).

Although the exclusive effect of oxygen released from rice roots on protist communities is yet to be revealed, previous studies suggested that both the rhizodeposits and the oxygen released from rice roots play essential roles in shaping rhizosphere protist communities (Asiloglu and Murase 2016, 2017). An in vitro study under defined conditions by Murase et al. (2014) showed that oxygen availability is a primary factor shaping the microeukaryotic community, especially protists, in a submerged paddy field soil. Indeed, a mini-rhizobox experiment, in which the redox conditions were monitored with methylene blue, showed that the rice root tips, where the active oxygen release was demonstrated, had a more substantial rhizosphere effect on predatory protists (Asiloglu and Murase 2017). Less oxygen is released as rice plants mature (Fig. 2), and the oxygen is quickly used up by the aerobic and facultative rhizosphere microorganisms, resulting in a rapid decrease in the soil redox potential (Asiloglu and Murase 2016).

The oxygen supply by rice roots is almost out of the equation after the early plant growth stage, but the rhizosphere

Fig. 2 Illustration of the dominant functional groups of protists in the rice rhizosphere at the vegetative, reproductive, and maturative stages of rice growth (upper part). The below part shows the known roles of predatory protists at the vegetative stage



effect on protists is still remarkable as rhizodeposits are continuously released (Fig. 2). Therefore, the growth stage of rice is crucial for understanding the rhizosphere effect on protists (Asiloglu et al. 2015; Asiloglu and Murase 2016). Taken together, the rhizosphere effect of rice plants is mainly explained by the rhizodeposits and oxygen released from rice roots, which depends on the growth stage and the root segment. In addition, the content and amount of rhizodeposits as well as the oxidizing power of rice variable between plant varieties (Armstrong 1969) suggests that the rhizosphere effect of different rice varieties is likely to have distinct effects on the protist communities. However, to date, no information is available on how different rice varieties affect protist communities, which should be further studied.

Protist communities inhabiting the rice rhizosphere consist of diverse taxonomies with versatile functionalities. Protist species belonging to Amoebozoa, Cilipohora, and Rhizaria (Cercozoa) dominantly inhabit the rice rhizosphere in the vegetative stage (Asiloglu et al. 2015; Asiloglu and Murase 2016; Asiloglu and Murase 2017; Asiloglu et al. 2021c). The versatile functional groups consist of microbial predators, decomposers, pathogenic plant symbionts, and autotrophs (including mixotrophs) (Asiloglu et al. 2015; Asiloglu and Murase 2016; Asiloglu and Murase 2017; Asiloglu et al. 2021c). Among them, predatory protists deserve special attention since they represent the most abundant functional group of protists (Asiloglu et al. 2021c)(Fig. 2). Predatory protists feed on microorganisms, mainly bacteria. The rhizodeposits, the preferred nutrient source by bacteria, make the rhizosphere a nutrient-rich habitat, increasing bacterial populations in the rice rhizosphere (Hernández et al. 2015). As bacteria are the primary food source of the predatory protists, the bacteria-enriched rhizosphere attracts protists, creating everlasting prey-predator dynamics. Indeed, the differences in the predatory protist communities between the rhizosphere and bulk soil are bacterial prey depended (Zhang and Lueders 2017). In addition, Somasundaram et al. (2008) showed that rice rhizodeposits (mucilage—root border cell complex) mediated the prey-predator interactions between predatory protists and bacteria. Taken together, the rice roots have both direct (altering the chemical properties of the rhizosphere soil such as pH and redox potential) and indirect (altering their prey communities) effects on predatory protists. A direct rhizosphere effect can be observed for decomposer protists (mainly oomycetes), as their growth depends on organic materials, including rhizodeposits (Semchenko et al. 2022). Another important functional group of protists is the plant pathogens, which are often associated with rice roots (Van Buyten and Höfte 2013). DNA-based molecular studies (Asiloglu et al. 2015; Asiloglu et al. 2021c) detected potentially pathogenic species belonging to oomycetes in the rice rhizosphere. Oomycetes include species ranging from opportunistic up to highly virulent

plant pathogens, including *Pythium* species that mainly infect young plants causing unhealthy growth and damping off (Chun and Schneider 1998; Van Buyten and Höfte 2013).

Effects of protists on rice plant performance

As protists are affected by the rice plants simultaneously, the activities of protists affect rice growth and productivity. Predatory protists enhance rice plant growth by accelerating nutrient turnover and altering rhizobacterial communities and activities (Fig. 2). Although protist-enhanced plant growth has long been recognized (Jentschke et al. 1995), to the best of our knowledge, the first study showing a positive effect of predatory protists on rice plant growth was conducted with a common soil amoeba, *Acanthamoeba castellanii* (Kreuzer et al. 2006). The presence of the amoeba stimulated the rice root growth and elongated lateral roots, revealing a potential indirect mechanism: amoeba's predatory effects enhanced rhizobacteria's positive effects on the root architecture (Kreuzer et al. 2006). For instance, although the mechanism is yet to be clarified, Bonkowski and Brandt (2002) showed that amoeba enhances indole-3-acetic acid (IAA)-producing bacteria, which may partly explain the effect of protists on root growth. The amoeba-enhanced lateral root growth was confirmed for several rice cultivars, showing that the prey-predator interaction and its positive outcome can be linked to the functional role of rice root exudates (Somasundaram et al. 2008).

Although most predatory protist research was conducted with a model amoeba (*Acanthamoeba*), predatory protists have species-specific effects on bacterial communities (Rønn et al. 2002). For instance, the impact of predatory protists on rhizobacterial community composition, nitrogen uptake by rice, and plant growth depend on protist species, and a combination of the protist species had a much more substantial impact (Asiloglu et al. 2020b). Although the mechanism is yet to be clarified, protists' co-application with a plant growth-promoting rhizobacteria (PGPR), *N*₂-fixing *Azospirillum* sp. B510, enhanced the survival and positive effects of the PGPR. The protist-enhanced PGPR effect on rice plant growth was later confirmed (Chandarana and Amare-san 2022), who studied the interactive effects of a ciliate (*Kreyellidae* sp.) and two PGPR species (*Pseudomonads* sp. and *Enterobacter* sp.) on the rice plant growth. These findings suggest that protist-enhanced rice plant growth may be partly attributed to the promotion of beneficial bacterial species in the rhizosphere, in addition to the accelerated nutrient turnover. The accelerated nutrient turnover by predatory activities of protists is often used to explain the enhanced nitrogen uptake by the plants; for instance, plants can utilize considerable amounts of NH₄⁺ released from consumed bacterial biomass as a result of protist predation (Bonkowski et al. 2000; Griffiths 1994). The increased putative bacterial

gene abundances involved in nitrogen mineralization in the rice rhizosphere by protists (Asiloglu et al. 2021b) suggest that an increased amount of nitrogen may be partly explained by the enhanced activities of nitrogen-mineralizing bacteria.

Furthermore, predatory protists can be used as biocontrol agents due to their suppressive effects on plant pathogens (Gao et al. 2019). Long et al. (2018) showed that several amoeba species had bactericidal effects of suppressing rice plant pathogenic bacteria, *Xanthomonas oryzae*. Although no direct evidence is available, predatory protists have the potential to suppress plant diseases caused by fungal pathogens as many protist species feed on fungi, including plant pathogenic *Fusarium sp.* (Geisen et al. 2016).

Conclusion and future perspectives

Irrigated rice fields have a unique environment as agricultural land and anthropogenic wetland, where different functional groups of protists are involved in the biogeochemical cycles and plant growth (Fig. 1). Flooding and anoxia would limit the activities of other eukaryotes, including not only fungi but also many soil animals. Thus, protists could play a primary role in the microbial food web in a rice field soil. Our knowledge about the protists in wetland rice soil is still in its infancy, but the review highlights their great potential. New molecular methods used ahead for protist studies in hydrosphere and terrestrial soils (Geisen and Bonkowski 2018) should be applied to rice soil with standardization. Clarification of the taxonomic and functional diversities of paddy soil protists would be another task. Further studies would provide deep and comprehensive insights into the sustainability and high productivity of irrigated rice production. The importance of protists as primary producers, predators, decomposers, and parasites in a rice field soil should be quantitatively assessed compared to other groups of microorganisms. In particular, phototrophic protists would play an essential role in the carbon cycle, nutrient dynamics, and plant growth in rice fields. Multitrophic network analysis would help understand the diversity and multifunctionality of the protists (Jiao et al. 2022). Protists also can be a potential biostimulant in rice production; thus, isolation and agricultural application of beneficial protists would be a significant challenge.

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Declarations

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