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Predatory Protists: The Key Players in the Quest for Sustainable Agricultural Practices

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ABSTRACT

To overcome the global problem of food shortage while supporting sustainable life on Earth, we must appreciate the critical importance of soil microorganisms—the key drivers of essential ecosystem services such as nutrient cycling and plant productivity. Protists are one of the major microbial groups in soil ecosystem including primary producers, decomposers, predators, and symbionts. The diverse morphologies and feeding strategies of predatory protists, including amoebae, ciliates, and flagellates, contribute to their versatility in capturing prey. Particularly, trophic interactions between protists and bacteria play a crucial role in regulating bacterial communities in the soil. Protists selectively prey on bacteria, influencing community composition, and enhancing microbial activity. The impact extends to nutrient cycling, secondary metabolite production, and even antibiotic resistance in soil bacterial communities. Despite recent advances, the field of applied protistology remains underexplored, necessitating further research to bridge the gap between theoretical potential and practical application. We call for increased scientific attention, research efforts, and practical implementations to fully harness the benefits of soil protistology for future agricultural practices. In this article, we introduced the frequently overlooked essential roles of predatory protists in soil ecosystem and their potential usage in sustainable agriculture.

Keywords: Predatory Protist, Soil Protists, Sustainable Agriculture, Trophic Interactions, Plant Growth, Applied Protistology

1. Introduction

Food shortage is one of the most critical global problems. The FAO (Food and Agriculture Organization) predicts that we have to increase overall food production by 70% to feed the world population in 2050, which is challenging with the current agricultural practices (*e.g.*, agrochemicals) (FAO 2024). To overcome the global problem of food shortage while supporting sustainable life on Earth, we must appreciate the critical importance of soil microorganisms—the key drivers of essential ecosystem services such as nutrient cycling and plant productivity (Trivedi et al. 2020). A better understanding of plant-microbe interaction could revolutionize agriculture through manipulating the plant microbiome to sustainably increase crop production (Jansson et al. 2023). Among the soil microbiome, bacteria, fungi, and archaea are well-studied, while protists, the vast majority of eukaryotes, are largely neglected (Gao et al. 2019).

Protists represents the majority of eukaryotes. Indeed, all eukaryotes except fungi, plants, and animals are protists. They are predominantly microscopic, unicellular, and ubiquitous (Adl et al. 2012). Protists includes primary producers, decomposers, predators, and symbionts (Geisen et al. 2017). The predators are taxonomically most diverse functional group of protists, constitutes more than half of the protist diversity (Gao et al. 2019). Their preys include bacteria, archaea, fungi, and even nematodes, which allows them to control soil biodiversity and the population of microorganisms, enhance microbial activity, and promote nutrient cycling and plant productivity (Geisen et al. 2018; Gao et al. 2019; Xiong et al. 2020; Guo et al. 2021). Degradation of organic matter is an important role of decomposer protists, which accelerates nutrient cycling (Geisen et al. 2018). Photoautotrophic protists, mainly algae, play key roles in the global soil carbon balance.

The 6% of the net primary production of the whole terrestrial vegetation come from the carbon fixed by the soil algae (Jassey et al. 2022). Plant pathogenic protists (mainly belonging to Oomycetes) cause enormous negative impacts on agricultural production, while negative effect on the health of animals and microorganisms caused by animal and microbial parasites belonging to protists (Gilbert & Parker 2023). Taken together, soil biodiversity, nutrient cycling, and agricultural productivity are controlled by protists and protist communities are sensitive to environmental factors such as soil physicochemical properties, the rhizosphere effect, organic and inorganic fertilizers, especially nitrogen (Asiloglu et al. 2015; 2016; 2021a; 2021b; Asiloglu 2022; Bodur et al. 2024).

Despite their enormous ecological impact, not everyone today recognizes that protists are the essential component of the microbial world (Caron et al. 2009). This review article explains the basics of predatory protists targeting those who are not specialized in protistology research area. In this article, we aimed to introduce recent studies on predatory protists focusing on their potential in sustainable agriculture. We explained the functional importance of predatory protists, their trophic relationships with their prey communities, and the services they provide to soil and plant health, followed by a highlight on their potential use in sustainable agriculture.

2. Predatory Protists

Predatory protists, a diverse group of microorganisms, play a crucial role in ecosystems by consuming other microorganisms including bacteria, fungi, other protists, and even nematodes. Their morphology and feeding types vary, allowing diverse trophic interactions with their prey. The outcome of the trophic interactions has profound effects on soil health, nutrient turnover, and plant productivity (Geisen et al. 2018; Gao et al. 2019).

2.1. Morphology and feeding types

Predatory protists are heterotrophs that rely on external sources for nutrients which is obtained through phagotrophy. Predatory protists extract the essential nutrients from their prey by capturing and internalizing their prey. Predatory protists exhibit a range of morphological features with different motility and feeding styles, which results in a variety of ways to consume their prey. Although exceptions are not rare, in general protists' morphology is an important indicator for how they capture their pray. The three major morphological groups are amoebae (naked amoebae and testate amoeba), ciliates, and flagellates (Figure 1).

Amoebae have the ability to alter its shape by extending and retracting pseudopods (Figure 1A). The pseudopodia allow an extraordinary advantage enabling them to navigate their environment with unparalleled precision; therefore, pseudopodia play a crucial role in amoeba's feeding strategy. Thanks to pseudopods, amoeba can reach into small pores that are inaccessible to larger predators such as ciliates. This unique adaptation demonstrates amoeba's ability to exploit the tiniest of resources to survive. Although they are grouped together morphologically, amoeba are not monophyletic as they belong to several taxonomic groups such as Amoebozoa, Rhizaria, and Excavata (Jan 2008; Nikolaev et al. 2024). The major difference between amoeba and the other two morphologic groups, ciliates and flagellates, is that amoeba can crawl on the surface of soil particles, ciliates and flagellates can swim in the water-filled soil pores.

Ciliates are the only monophyletic group of predatory protists belonging to Ciliophara (Gao et al. 2016). They are distinguished with their short hair-like organelles called cilia, which extend from the cell's surface enabling ciliates to perform a wide range of functions (Figure 1B). Ciliates rhythmically move their cilia to swim in aquatic environments or water-filled soil pores. In addition to swimming, cilia are also useful for navigating surfaces and exploring their environment. Ciliates exhibit an exceptional ability, filter-feeding, using their cilia to create water currents that draw in their prey. This filtering process allows ciliates to capture and consume vast amounts of prey microorganisms, effectively serving as efficient predators in their ecosystems. On the other hand, filter-feeding makes ciliates less selective on their prey (Asiloglu et al. 2020). It should be noted that not all ciliates are filter-feeders. Besides cilia's role in prey capturing, cilia also play a crucial role in sensation, while enabling ciliates to detect environmental changes and actively interact with their surroundings.

Another morphological group of predatory protists is flagellates (Figure 1C). They are fast swimmer thanks to their whip-like flagella. The flagella's propulsive action allows flagellates to swiftly explore their surroundings, actively seeking out essential resources; nutrients or prey. Some flagellates exhibit raptorial feeding abilities, using their flagella to capture prey with impressive ability. This predatory strategy allows them to actively pursue and trap smaller microorganisms, making them both fast hunters and elusive. Besides, the flagella allow them to easily escape potential danger (Nielsen & Kiørboe 2021), allowing them to thrive in a variety of ecological environments. Flagellates are polyphyletic, meaning they belong to several taxonomic supergroups of protists. In addition to the three main morphological groups, there is a group of protists that can transform between amoeboid forms and flagellate forms called amoeboflagellates (Mitchell 2007).

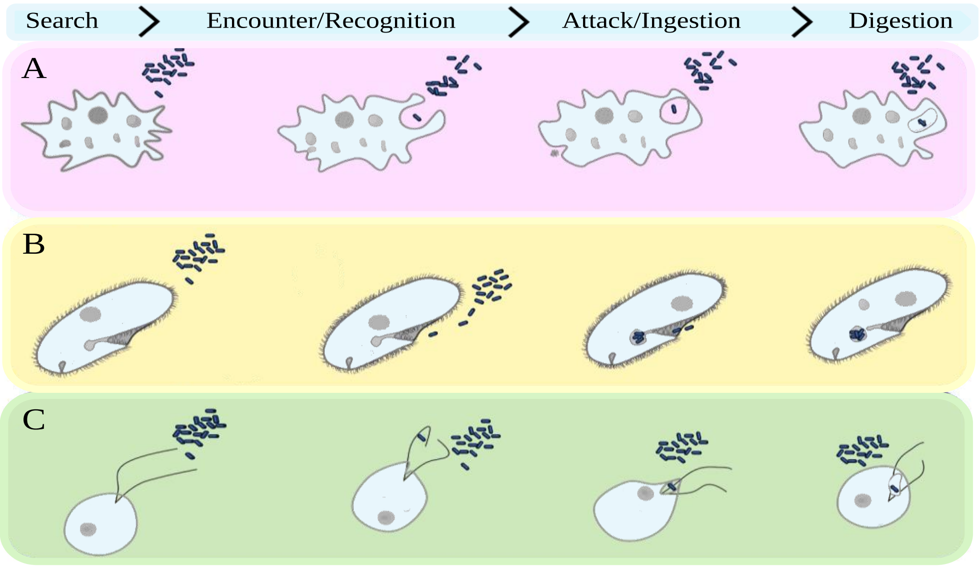

Figure 1- Schematic view of predatory protists' feeding types. A, Amoeba utilizes pseudopodia for precise navigation and resource capture through phagotrophy; **B**, Ciliate engages in filter-feeding using cilia, creating water currents to capture and consume prey microorganisms; **C**, Flagella demonstrates fast-swimming and raptorial feeding abilities using whip-like flagella for prey capture.

2.2. Microbial trophic interaction

Predatory protists engage in complex trophic interactions with various microorganisms, influencing ecosystem dynamics. They have diverse impact on their prey communities depending on the prey type.

2.2.1. Trophic interaction with bacteria

Understanding how protists and bacteria interact in soil is crucial for revealing the complex dynamics of the soil ecosystems. Numerous studies have been conducted on the regulation of bacterial communities concerning various biotic and abiotic factors. When considering trophic levels in ecology, most of these factors primarily constitute the provision of nutrients to bacteria, which are primarily bottom-up factors. The famous ecology paper by Hairston and colleagues (1960) argued that the abundance of prey is controlled by predators, introducing the concept of top-down control. Pernthaler (2005), emphasized structure of microbial communities is shaped by both protistan predation (top-down control) and competition for organic carbon and nutrients (bottom-up control) in aquatic ecosystem. Recent studies have highlighted the significance of top-down regulation by predatory protists on soil bacterial communities, surpassing the impact of bottom-up factors such as nutrient availability. Asiloglu et al. (2021c) studied the top-down and bottom-up factors controlling soil bacterial communities, which revealed that the soil bacterial communities are strongly controlled by predatory protists rather than the bottom-up nutrients in rice field soil. Fujino et al. (2023) revealed a substantial role of protists in shaping the composition of the active bacterial community, emphasizing the pronounced top-down influence of predatory protists on bacterial communities, especially Proteobacteria and Bacteroidota, and likely activities within the soil ecosystems.

Trophic interactions between protists and bacteria significantly influence the overall community composition in soil (Figure 2A). Additionally, most of protists exhibit selective feeding behaviours, preying on specific microbial taxa within the bacterial community (Gonzalez et al. 1990; Verity 1991; Šimek et al. 1994). A study by Matz et al. (2002) showed that bacterial phenotypic traits affect the feeding preferences of the heterotrophic nanoflagellate, *Spumella* sp. Pernthaler (2005) emphasized that protists exhibit selective feeding, favour certain bacterial species over the others. Murase & Frenzel (2008) compared the edibility of different methanotrophs for soil protist in rice field soil. The study found that protist showed a grazing preference for different methanotrophs, with some strains being more edible than others. This suggests that selective grazing by protists may impact not only bacteria but also the methanotrophic community of archaea. Taking together protist have a species-specific effect on bacterial communities, thus the presence of protist species in soil ecosystem is important to understand how soil microbiome is shaped. In addition, Asiloglu et al. (2020) showed that mix culture of protists has a bigger impact on bacterial communities than single protists species, suggesting that the effect of protists may be much more than expected in the soil ecosystem where hundreds of protist species inhabits.

Prey traits play crucial role in the survival, thus endurance and diversification of bacteria. Important bacterial traits include but not limited to prey size, prey motility, prey biochemical composition, cell surface characteristics, and prey's ability to produce toxins, form microcolonies and biofilm (Jürgens & Massana 2008). Matz & Kjelleberg (2005) studied the dietary niche breadth on eight protist isolates and 20 bacterial species. The researchers found that each protist showed a distinct feeding pattern depending on the bacterial traits. In addition to these direct interactions based on bacterial traits, protists can also respond to volatile organic compounds (VOCs) produced by bacteria. A recent study demonstrated that predatory protists can sense bacterial volatile organic compounds (VOCs) and likely to use them to locate and select prey in soil ecosystems (Schulz-Bohm et al. 2017). This selectivity may be a critical factor in detecting distinct bacterial species and thus shaping the composition of bacterial communities.

Protists exert influence not only by reducing specific bacterial populations through selective predation, but also several bacterial species benefit the protist predation through reduced bacterial competition and release of nutrients (Figure 2B) (Clarholm, M., 1985; Bonkowski et al. 2000; Creevy et al. 2016). Previously, Asiloglu et al. (2020) showed that co-inoculation of protists and a plant growth promoting rhizobacteria (PGPR), *Azospirillum* sp. B510, enhanced the survival of PGPR, most likely through regulating the bacterial communities. Understanding how protists affect its environment is essential for comprehending the broader implications for nutrient cycling, ecosystem stability, and plant-microbe interactions.

Beyond shaping bacterial community structure, trophic interactions with protists impact the bacterial activity (Figure 2C). This influence extends to the production of secondary metabolites and plant growth hormones (Krome et al. 2010). Some protists can directly prey upon plant pathogens and contribute to disease suppression. (Guo et al. 2022). For instance, they can enhance the production of antibiotics in PGPRs. Recent studies show that soil protists, as the key predators of soil bacteria, play an important role in driving the abundance and diversity of antibiotic resistance in soil bacterial communities (Nguyen et al. 2023). The researchers inoculated soil microcosms with low, medium, and high concentrations of indigenous soil protist suspensions. They found that an increase in protistan predation pressure was strongly associated with a higher abundance and diversity of soil antibiotic resistance genes.

Taken together, protists regulate bacterial communities and activities in species-specific manner depending on the bacterial traits. The trophic relationships between soil predatory protists and bacteria are a cornerstone in the intricate web of soil ecology. The interdependence between these microorganisms not only shapes the composition of bacterial communities but also has far-reaching effects on ecosystem functionality. Further research in this field will contribute to our understanding of soil ecosystem dynamics, providing valuable insights for sustainable agriculture, environmental conservation, and microbial ecology.

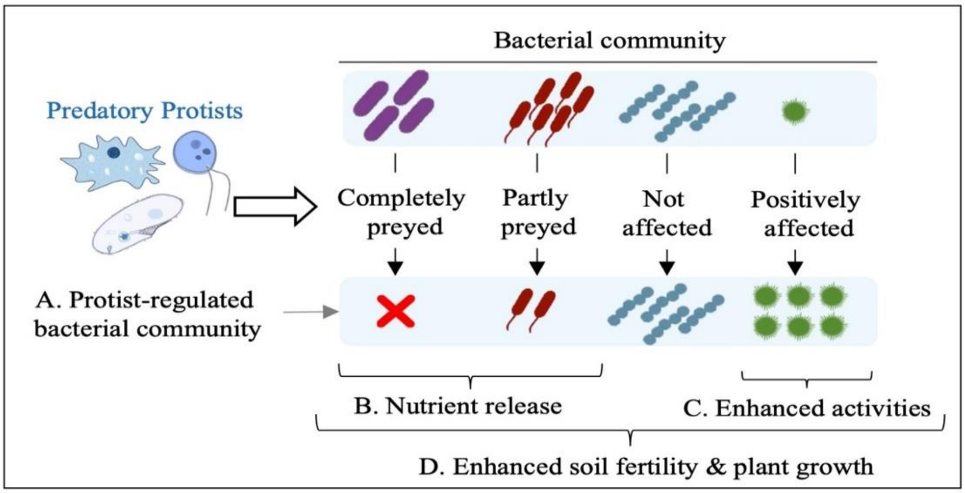

Figure 2- Effects of predators of bacteria (predatory protists) in the soil ecosystem. A, Predatory protists selectively feed on bacteria and alter their community composition; **B**, Nutrients are released from the preyed bacterial biomass into the soil; **C**, Predatory protists enhance activities of several bacterial species; **D**, Consequently, the soil fertility and plant growth are enhanced by the predatory protists

2.2.2. Trophic interaction with fungi

Although protists are considered mainly bacterivorous, it has been long recognized that protists feed on fungi as well (Heal 1963; Old & Darbyshire1978; Bunting et al. 1979). Nevertheless, most of the studies focused on protist-bacteria interaction and fungivorous protists are mainly ignored until recently. Geisen et al. (2016) re-visited the protist-fungi interaction and showed that many bacterivorous protists also feed on fungi, which makes it possible for protists to affect fungal communities and activities. In addition to bacterivorous protists that feed on fungi, obligate fungivorous protists are abundant in soils (Geisen at al. 2016). For instance, *Lecythium terrestris* is identified by Dumack et al. (2016) as a fungivorous and algivorous protist, feeding on fungi and algae in soil environments. Huang et al. (2021) studied effects of different biotic and abiotic factors on fungal communities in paddy soils across climatic zones. The study showed that among the several biotic and abiotic factors, protists are the most important factor shaping fungal communities. In addition, studies showed that the trophic relationship between predatory protists and fungi is driven by biotic and abiotic factors such as land use intensification (Xue et al. 2023), organic fertilizer addition (Hu et al. 2024), the rhizosphere effect (Wang et al. 2024). Even though not directly proven, studies suggested that these protists might control fungal communities. Protist-fungi interaction is a relatively new research area open to discoveries. Understanding these interactions is essential for unravelling the dynamics of soil microbial communities and their influence on ecosystem functioning.

2.2.3. Trophic interaction with nematode and other microorganisms

Intriguingly, predatory protists can interact with other microorganisms like nematodes. This trophic relationship can have cascading effects on soil food webs and nutrient dynamics, highlighting the interconnectedness of microorganisms in the soil ecosystem. Geisen et al. (2015) investigated the predatory behaviour of a common soil amoeba on nematodes. In this study they showed feeding procedure of *Cryptodifflugia operculata* on the nematode *Acrobeloides buetschlii*. The findings revealed a specific attack pattern of the protist, wherein the amoeba immobilized nematodes by attaching to their tails and anterior ends. Subsequently, other amoebae cooperatively immobilized the nematode. This cooperative feeding process involved multiple amoebae disintegrating and absorbing the nematode prey within 12 hours. Additionally, the study underlined that protist could serve as potential biocontrol agents against plant pathogenic nematodes.

In addition to all these, there are various findings that protists prey on other protists. In a study (Seppey et al. 2017) proposing that phagotrophic protists play a significant role in contribute to an alternative pathway for nutrient cycling in soil ecosystems by consuming algae.

2.3. Protists and soil health

Soil protists, like all other soil microorganisms, are essential for soil fertility and ecosystem functioning. They exert various impacts on soil health by influencing nutrient turnover, microbial communities, as well as activities related to nutrient decomposition and mineralization. In soil ecosystems, predatory protists serve a fundamental role in nutrient turnover, as outlined by the microbial loop concept (Clarholm M 1985; Bonkowski 2004). By preying on diverse microorganisms, protists release the excess nutrients. As nitrogen is excreted as a waste product from protist-consumed microbial biomass, protists are known to increase nitrogen availability in soil (Sherr et al. 1983; Bonkowski et al. 2000).

By increasing nutrient availability (Figure 2B) protist leads to increase microorganism activity. The predatory activity of protists on bacteria and fungi, they influence the composition and abundance of these microbial populations (Figure 2C). This control over microbial communities can have far-reaching impact for the functioning of the soil ecosystems. Jousset (2017) has been suggested that heterotrophic protists increase the activity and survival of PGPR. Asiloglu et al. (2020) showed that protist enhance the survival of *Azosprillum* sp. which is one of the most widely used commercial biofertilizer. Additionally, some studies are showing that the protist stimulates the production of siderophores, an iron-chelating compound, by *Pseudomonas* (Levrat et al. 1992), the cyclic biosynthesis of an antibiotic lipopeptide (Mazzola et al. 2009), and the anti-phytopathogenic compound 2,4-diacetylphloroglucinol (Jousset & Bonkowski 2010; Jousset et. al. 2011).

The regulation of specific microbial groups by protists can indirectly influence bacterial activities (Figure 2D) such as nitrogen mineralization and carbon mineralization (Kuikman et al. 1991; Hervey & Greaves 1941; Ratsak et al. 1996; Rønn et al. 2001). The first evidence for an effect of protists on the sulfur cycle showed by Murase and colleague (2006). The protists are thought to influence SO_4^{2-} reduction directly by grazing on SO_4^{2-} reducing bacteria or indirectly via creating more reduced soil conditions by stimulating the overall microbial activities. Gluconic acid plays a crucial role in phosphorous mineralization, serving as an efficient strategy for *Enterobacter* to avoid protist grazing, that was demonstrated by Gómez et al. (2010). In addition, protists feed on dormant bacteria leading to increase the relative abundance of the active bacteria. Taking together protists are considered as an important compound for soil fertility.

2.4. Protists and plant productivity

Protists are one of the most abundant microorganisms in rhizosphere (Murase & Asiloglu 2023) that densely inhabit the plant roots (Asiloglu & Murase 2017; Bonkowski et al. 2004). Plant health is influenced by especially the rhizosphere soil microbiome, in which protists are one of the most abundant microorganisms. The interactions between predatory protists and microorganisms have implications for plant productivity (Bonkowski 2004; Rosenberg et al. 2009). A pivotal aspect of this influence is the enhancement of nutrient availability through protist predation, a critical factor in meeting the nutritional needs of plants. Xiong et al. (2020) and Gao et al. (2019) showed the importance of protist, which is one of the main groups of the rhizosphere, as top-down controllers of functioning linked to plant health. The research by Guo et al. (2021) emphasizes the positive impact of cercozoan protists on plant growth. Additionally, gluconic acid which is produced by enterobacter against *Colpoda steini* (Gómez et al. 2010), has been proven to increase fertility by they participate actively in mobilizing phosphorus between sparingly soluble mineral phosphates and the soil solution, thus increasing the fertility of phosphorus deficient soils (Rodríguez & Fraga 1999).

The production of plant hormones by PGPR is known to stimulate plant growth. Bonkowski & Brandt (2002) showed that the presence of *Acanthamoeba castellanii* correlated with a greater and more branched root system in watercress seedlings. This change was accompanied by an increase in the ratio of auxin, indole-3-acetic acid (IAA) producing rhizospheric bacteria in the presence of protists. This has been attributed to changes in the composition and activity of the microbial community. Kreuzer et al. (2006), supported this information by showing that *Acanthamoeba castellanii* can stimulate rice root growth and elongate lateral roots. The effects of a rhizosphere bacterial community and *A. castellanii* on root branching and on IAA metabolism in *Lepidium sativum* and *Arabidopsis thaliana* were investigated by Krome et al. (2010). In the study, concentrations of bioactive-free IAA in *Lepidium sativium* shoots were strongly increased by the predation activity of protists on bacteria. Increased nitrate concentrations by presence of amoeba in the rhizosphere are thought to cause the accumulation of cytokinin in plants and interactions with free auxin and resulted to increasing root growth.

The enhanced nutrient turnover facilitated by protists has the potential to improve plant health by increasing plant resistance to disease and stress. Moreover, protists actively contribute to the suppression of plant pathogens, creating a protective effect on plants (Guo et al. 2022). Not only bacteria but also fungal pathogens impacted by protist. The study by Bahroun et al. (2021) showed that bacterivorous protist has been shown to induce soil suppressiveness against the fungal pathogen *Fusarium oxysporum*. However, very less is known about protist-fungi interaction.

Overall, by enhancing nutrient availability, altering microbial community composition, influencing root development, and fortifying plants against pathogens, protists emerge as key players in the intricate relationship of factors shaping plant productivity.

3. Application of Predators in Sustainable Agriculture

The application of microorganisms and the knowledge about them has been used for centuries in curing diseases, improving food production, and protecting the environment from pollution. The application of soil protists in agriculture is promising as they can improve agricultural yield while, potentially, reducing the input of chemical fertilizers or pesticides. In applied protistology, predatory protists deserve special attention as they can be applied as biostimulants and biocontrol agents. Various strategies can be employed for the application of predatory protists, similar to the methods used for bacterial and fungal biocontrol agents. While specific application methods were not explicitly tested, there is potential efficacy in approaches like seed coating or introducing protists into irrigation water and drip irrigation systems. Notably, predatory protists serve as catalysts for the decomposition of organic matter. Consequently, there is a prospective application of protists in organic fertilizers, including biochar and compost, to optimize their effects when integrated into agricultural fields. However, applied protistology has received little attention despite its vast potential. The relatively few applied protistology researches in the last decade that were mainly conducted in Europe and the United States obtained exciting results. For instance, recently, protist-based biostimulants and plant protection products can be seen in the European market (Protoplus, ECOstyle, Belgium).

As different protists have distinct impact on the bacterial community composition, soil fertility, and the plant health (Gao et al. 2019), selection of protist species to be applied should be considered carefully. For instance, depending on the predatory protists' feeding types (Figure 1), their usage can be varied. Protists such as ciliates with filter-feeding behaviour can feed on huge number of bacteria in a short time (Figure 1B), resulting in higher nutrient turnover rates and lower selective impact on bacterial community composition. On the other hand, protists such as amoeba and flagellates (Figure 1A and C) feed on relatively less bacteria than ciliates, while having a higher selectivity on the prey bacteria. Therefore, filter-feeders can be beneficial for overall soil health due to higher nutrient turnover rates, while the protists with the other feeding types (Figure 1) can be used to manipulate soil microbial communities and functionalities.

4. Conclusions

This review highlights the roles of predatory protist in soil microbial ecosystems, which has been overlooked for many years. Briefly we introduced the trophic interaction between predatory protists and microbiomes and their impact on soil health and plant productivity, which indicate a robust potential for the use of predatory protists in sustainable agriculture. However, uncertainties exist regarding the adoption of this new perspective in practice and its integration into standard agricultural practices. Methods for implementing protist-based solutions have not been clearly defined yet. Despite exciting recent findings, limited scientific interest in protistology hampers progress in this field. There is a need for increased scientific attention and participation to bridge the gap between the potential benefits of soil protistology and tangible, applicable solutions. At this juncture, more research is needed on how protist-based solutions can be integrated into agricultural practices, the impact of this integration on agricultural productivity and sustainability, and the scientific community's allocation of more resources to this field. Encouraging the scientific community to understand the significance of soil protistology and engage in further research can play a crucial role in shaping future sustainable agricultural practices.

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