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REVIEWS

Review

Protists: Puppet Masters of the Rhizosphere Microbiome

Zhilei Gao,[1,4] Ida Karlsson,[1,2,4] Stefan Geisen,[3] George Kowalchuk,[1] and Alexandre Jousset[1,*]

The rhizosphere microbiome is a central determinant of plant performance. Microbiome assembly has traditionally been investigated from a bottom-up perspective, assessing how resources such as root exudates drive microbiome assembly. However, the importance of predation as a driver of microbiome structure has to date largely remained overlooked. Here we review the importance of protists, a paraphyletic group of unicellular eukaryotes, as a key regulator of microbiome assembly. Protists can promote plant-beneficial functions within the microbiome, accelerate nutrient cycling, and remove pathogens. We conclude that protists form an essential component of the rhizosphere microbiome and that accounting for predator–prey interactions would greatly improve our ability to predict and manage microbiome function at the service of plant growth and health.

A Multitrophic Perspective on the Rhizosphere Microbiome

Plant growth, nutrition, and health are to a large extent determined by the activity of associated microorganisms [1]. In particular, plant roots are associated with an active multispecies community, the **rhizosphere microbiome** (see Glossary), providing several important services to the plant. Root-associated microbes, for instance, mineralize nutrients, manipulate plant hormonal balance, and suppress potential pathogens [2]. The species composition of the rhizosphere microbiome is now recognized to have direct effects on host plant traits [3]. However, our understanding of the determinants of microbiome community assembly and composition is still lacunar, restricting our ability to predict and harness microbiome dynamics and functionality. To date, most studies seeking to address the mechanisms underlying microbiome composition, species turnover, and function have focused on bottom-up drivers of microbial community composition, such as plant developmental stage, soil type, and host genotype [4–6]. While **bottom-up control** is certainly crucial, it represents only half of the story (Figure 1). Microorganisms in the rhizosphere are subjected to **top-down control** by a range of bacterial and eukaryotic consumers. Among them, free-living **protists** (Box 1), a highly diverse group of mostly unicellular eukaryotes [7], in our opinion deserve special attention. Protists are highly abundant and active consumers of bacteria and arguably fungi, impact community structure, and play a key role for nutrient cycling in the rhizosphere [8–13]. This review primarily addresses the importance of free-living, heterotrophic protists that feed on other organisms. For the sake of simplicity, we hereafter refer to them as 'protists', deliberately omitting mutualistic or parasitic taxa, including animal parasites or plant pathogens.

Despite their ubiquity and ecological importance for soil functioning, protists are still a relatively misunderstood component of the soil and rhizosphere microbiome [14,15]. This knowledge gap is especially striking given that protists are comparably well investigated in aquatic ecosystems, where they are recognized as an integral part of the microbial food web. However, when it comes to soil, research has long focused on taxonomic species descriptions, with only

[1]Institute of Environmental Biology, Ecology & Biodiversity, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
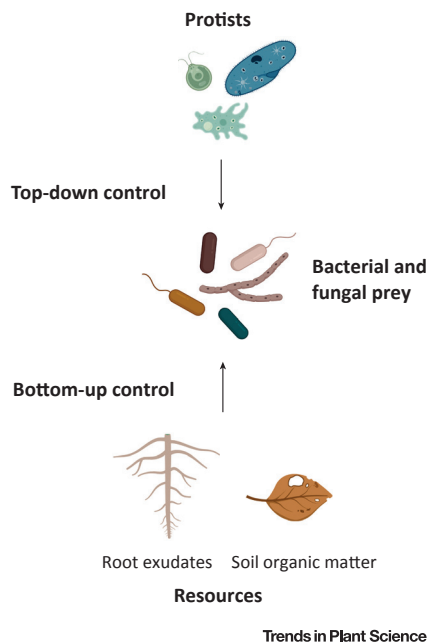[2]Dept. of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, 75007 Uppsala, Sweden
[3]Department of Terrestrial Ecology, Netherlands Institute of Ecology, 6708 PB Wageningen, The Netherlands
[4]These authors contributed equally

*Correspondence:
a.l.c.jousset@uu.nl (A. Jousset).

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Protists

Top-down control

Bacterial and fungal prey

Bottom-up control

Root exudates   Soil organic matter

Resources

Trends in Plant Science

Figure 1. Bottom-up versus Top-down Drivers of the Rhizosphere Microbiome Assembly. The microbiome is affected by a range of factors. Plants invest carbon into root exudates, which together with soil organic matter fuel microbial activity in the rhizosphere (bottom-up control). However, top-down control by protist consumers also influences microbiome dynamics and functionality.

a handful of scientists assessing interactions with other microorganisms and plants. This can partly be attributed to methodological constraints in studying protists, as they can be difficult to extract and cultivate, and reliable molecular methods have only recently been developed [16]. Better coverage of protist databases [17] and the emergence of high-throughput sequencing approaches allowing in-depth interrogation of soil protist communities [18] provide new opportunities to explore the diversity and ecological importance of soil-borne protists. The

Glossary

Biocontrol agent: organism that can be applied to reduce pests or diseases.
Biostimulants: (microbial) organisms that promote plant performance, for example, by serving as biofertilizers.
Bottom-up control: the population size is determined by the availability of nutrients for growth or the productivity of primary producers.
Microbial loop: nutrient release through consumption of bacteria or fungi by higher trophic levels such as protists.
Microbiome: the entity of interacting microbial taxa, including bacteria, archaea, fungi, viruses, protists, and other microbial eukaryotes.
Protists: paraphyletic group comprising all eukaryotes, with the exception of plants, fungi, and animals. In the soil this encompasses photoautotrophs (algae), heterotrophs (Protozoa), and mixotrophs.
Rhizosphere: the zone in soils directly influenced by the presence of roots.
Top-down control: mortality due to consumption by organisms at higher trophic levels determines the size of the population.

Box 1. What Are Protists?

Protists represent a paraphyletic, extremely diverse group of unicellular eukaryotes, encompassing by far the majority of eukaryotic phylogenetic diversity [104,105]. Soil protists can be found in all eukaryotic supergroups: Amoebozoa, Obazoa, Archaeplastida, SAR, and Excavata. Protists come in a wide range of morphological shapes and locomotion modes. Soil protistology has formerly focused entirely on heterotrophic taxa (previously 'Protozoa') and have been grouped based on coarse morphological features into naked amoebae, testate amoebae, flagellates, and ciliates. Yet, in-between forms are the rule and phylogenetic work has shown that all these groups, with the exception of ciliates, are paraphyletic, rendering ecological interpretations based on this morphological classification less meaningful. Many protists have a complex life cycle, most consisting of an active and a resting stage, mostly in the form of a cyst, but some intermediate forms are also common [7]. The inactive and persistent cyst stage is formed in response to unfavorable environmental conditions. These protist cysts form an important fraction of the soil microbial seed bank that can readily turn active in response to more favorable conditions, such as increased moisture along with presence of suitable prey [106].

Protists are abundant members of the soil microbiome, typically present at densities of 10^4–10^8 per gram of soil [107]. Protists, like their bacterial and fungal prey, are especially enriched in the rhizosphere, the region directly surrounding, and influenced by, plant roots. The protist community composition is shaped by a number of factors in addition to the presence and composition of prey, including a range of biotic and abiotic factors (such as plant species) and soil properties (such as pH and humidity) [18,47,95]. The functional role of protists in soils is diversely linked to nutrient cycling and includes phagotrophy [consumption of other (micro)organisms such as bacteria], phototrophy, symbiosis, saprotrophy, or a mix of these strategies [7].

time is ripe to shift the perception of plant–microbiome interactions beyond bacteria and fungi, and integrate protistology more solidly into microbiome research.

We aim here to we place the rhizosphere microbiome into a multitrophic perspective. We highlight the importance of free-living protists as an often overlooked but central group of rhizosphere organisms that drive both microbiome structure and interaction with the host plant. Our main goal is to combine the recent advances in protistology, microbiology, and general ecology, fostering exchanges between different disciplines that often address the same topic but have long been disjointed. We synthesize the current knowledge on the roles of protists within the rhizosphere microbiome and propose new roads for future research. We demonstrate that protists pull the strings of several processes in the rhizosphere and steer community structure, function, and evolution. Thanks to their versatility and central role as a major selective pressure on rhizosphere microbes, we argue that protists function as 'puppet masters', steering beneficial plant–microbe interactions that might be exploited to manipulate the rhizosphere microbiome functionality.
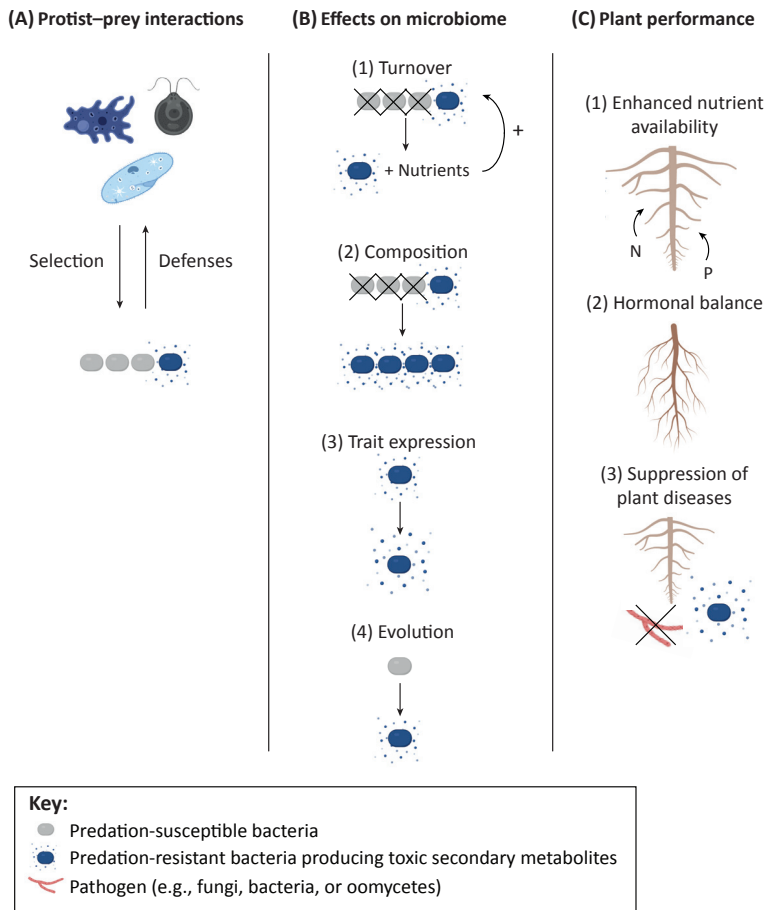
Predator–Prey Interactions in the Rhizosphere Microbiome

Protists interact with their preys in a variety of ways, including trophic interactions and chemical communication (Figure 2A). These different interactions can, in turn, result in important changes in microbiome structure and activity. In this section, we highlight different types of interactions between protists, bacteria, and fungi. We will address the consequences for microbiome functioning and plant growth in the next section.

First, consumption of microorganisms by protists increases nutrient turnover. A reason for this is that protists have a higher C:N ratio than the bacteria or fungi they are consuming. They will therefore excrete the excess N, making it available for other microorganisms or the host plant [8,19]. Further, by consuming dormant cells, protists release limiting micronutrients (that would otherwise remain locked in the microbial seed bank) that do not contribute to microbiome function [20]. This increased nutrient turnover can happen regardless of the traits of protists and their prey. In addition, microbial consumption may have a range of trait-dependent effects on community structure and function when predation correlates with specific prey traits.

Most protists show strong prey-selection patterns based on species-specific sets of traits. For instance, the size ratio between predator and prey restricts which prey can be ingested. Protist feeding mode and motility is also important. Amoeba can, for example, reach for tiny pores in the soil matrix thanks to their extremely plastic body shape and even digest biofilms thanks to the production of extracellular enzymes. Filter-feeding ciliates can eat single bacteria or microcolonies. They show a comparatively low selectivity but can have a high per-capita consumption rate [21]. Different feeding types ae associated with a given level of specialization. For instance, mycophagous Grossglockneriidae, a group of ciliates, have a specialized needle-like feeding structure, only permitting them to feed on fungi [22]. Bacterivorous protists show refined patterns of prey selection and can discriminate bacteria on the base of their size [23], surface properties [24], or the presence of diffusible secondary metabolites [25]. They are further attracted or repelled by volatile compounds, such as terpenes, secreted by microorganisms [26]. Protists respond in a species-specific manner to these volatiles [26].

Bacteria have evolved a range of defense mechanisms to prevent detection, ingestion, or digestion by protists. These mechanisms can be either constitutive or be triggered by the presence of protists, and this variation in palatability is a fundamental driver of selective feeding by protists. Bacteria sense chemical cues from protists and specifically respond to predation

(A) Protist–prey interactions

Selection | Defenses

(B) Effects on microbiome

(1) Turnover

+ Nutrients

+

(2) Composition

(3) Trait expression

(4) Evolution

(C) Plant performance

(1) Enhanced nutrient availability

N   P

(2) Hormonal balance

(3) Suppression of plant diseases

Key:
- Predation-susceptible bacteria
- Predation-resistant bacteria producing toxic secondary metabolites
- Pathogen (e.g., fungi, bacteria, or oomycetes)

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Figure 2. Protist–Prey Interactions Drive Rhizosphere Microbiome Function and Plant Performance. Protist–prey interactions, specifically the interplay between protist selective feeding and prey defense traits (A), affect several characteristics of the microbiome (B). Here we use production of secondary metabolites as an example of a bacterial defense trait. Protist consumption increases species turnover and subsequent nutrient release, which fuels further microbial activity (1). Moreover, protist feeding is selective and shifts the taxonomic composition of the microbiome as well as the frequency of functional traits (2). Bacteria respond to protist consumption by increased expression of defense traits (3). Finally, protist consumption acts as an evolutionary force on microbial populations (4). These effects on and responses of the microbiome result in a range of changes in plant physiology, with various implications for plant performance (C). Importantly, protist consumption unlocks nutrients bound in rhizosphere microbes, which can be taken up by plant roots and stimulate growth (1). Moreover, protists can increase lateral root branching by promoting auxin-producing microbes (2). A last example is the production of certain antibiotics, which both confer resistance against protists and inhibit plant pathogens, and may thus protect plants against diseases (3).

pressure by adaptations such as changes in cell size and shape [27], increased motility [28], surface properties, and secretion of defensive secondary metabolites [25]. Secondary metabolites known to confer predation resistance against protists include: pigments like violacein [29], polyketide antibiotics, hydrogen cyanide, the exoprotease AprA [25], and cyclic lipopeptides [30,31]. Several of the bacterial responses to predation are expressed on the population level, such as formation of biofilms or filaments that are less accessible for predators than single cells [32,33]. Likewise, several of the secondary metabolites conferring predator resistance are regulated by quorum sensing, such as the pigment violacein produced by *Chromobacterium*

violaceum [29]. In *Pseudomonas* bacteria, coordinated production of several antibiotics with antipredator activity is induced in a density-dependent manner [25,34].

Impact of Protists on the Microbiome

From Biomass to Function

Protist–prey interactions lead to a range of effects on several characteristics of the microbiome (Figure 2B). Predation typically decreases total bacterial biomass [35,36]. By increasing nutrient turnover, protist predation stimulates microbial activity; this is evidenced by increased microbial respiration and nutrient mineralization [37,38]. Simultaneously, selective feeding shifts rhizo-sphere microbiome composition and gives a selective advantage to microbial groups that can avoid predation [36,39]. For instance, predation may promote Gram-positive bacteria, which, thanks to their thick cell wall, are harder to digest [13,40]. Moreover, protist predation can help maintain diversity within bacterial communities by feeding on the dominant taxa and thereby increase the relative abundance of formerly rare bacteria, leading to increased bacterial evenness and complementarity [41,42].

Protist selective predation can further affect functional trait composition of the microbiome. Predation results in an increased abundance of organisms harboring traits conferring resis-tance to protists [43]. Furthermore, predation can stimulate expression of several traits linked to defense [44]. These antipredator traits can be highly relevant for the delivery of microbiome function relevant for plant health. For instance, several secondary metabolites conferring resistance against consumption by protists are also involved in the suppression of plant pathogens and immunity [25,45]. We later discuss in detail how these different effects on microbiome taxonomic and functional composition impact plant performance.

Protist Species-Specific Effects

Recent studies have revealed unexpected diversity of soil protists [46,47]. This diversity is also reflected in diverse interactions with their prey. Protists with different feeding modes have distinct effects on biofilm morphology [48,49]. For instance, *Acanthamoeba polyphaga*, which requires attachment to a firm surface to be able to feed, was most efficient in reducing the biomass of biofilms. In contrast, the flagellate *Bodo saltans* stimulated microcolony formation in biofilms, which conferred resistance against this protist [48]. Moreover, protists representing different feeding modes and motility types have species-specific effects on bacterial community structure and diversity [37,42].

In addition to morphology, the phylogenetic affiliation of protists is an important predictor of their effect on microbial communities. Such phylogenetic patterns can emerge at different scales. For instance, when comparing the effects of nine Cercozoa species on model prey communities, protist phylogenetic distance could explain variation in bacterial community structure [50]. In another example, including protist species spanning several eukaryotic supergroups, broad-scale taxonomic affiliation could be correlated with sensitivity to bacterial defense compounds, a crucial characteristic linked to interactions with their prey [51]. Still, most studies investigating the effects of protists on microbial community structure have been conducted with only one, or very few, model species. We advocate that further studies should include more protist species to unravel the links between protist taxonomy and traits and their impact on microbiome structure and function.

Microbiome Evolution

Beyond ecological interactions, protists can also drive the evolutionary dynamics of the rhizosphere microbiome. Predation by protists creates a selection pressure that impacts

the evolution of microbial traits that are relevant for interactions between microorganisms and the host plant [52]. Predation can trigger diversification [53], thereby increasing the phenotypic pool available to the plant. Further, they may also guide the evolution of specific traits. A range of bacterial traits have likely evolved at least partially as an adaptation to protists, including size, surface properties, or the secretion of defensive secondary metabolites [24,25,27]. Alterations in these traits may impact bacterial growth, interactions with competitors, and ultimately the host plant. For instance, surface molecules such as lipopolysaccharides play a central role in adhesion to roots and recognition by the plant immune system. Their alteration to avoid recognition by protists may thus change the way they interact with the plant [54,55].

Protists also affect intraspecific interactions and can, for instance, enforce cooperation by consuming defectors that use plant-derived resources but do not provide plant-beneficial functions in return [43]. Therefore, they ensure the evolutionary stability of social behaviors required for plant growth and health, such as secondary metabolite production [43,56]. Evolution of protist resistance may also have an impact in a multitrophic context, with protists selecting, for instance, for bacteria susceptible to bacteriophages [57].

Evolution of microorganisms within protists may also affect microbiome function. Protists carry several intracellular bacteria, ranging from pathogens to symbionts [58]. Some of these bacteria are also opportunistic pathogens of humans and plants, and it has been proposed that virulence traits such as secretion systems and elicitors have evolved originally as an adaptation to survive within vacuoles [59]. Protists may therefore function as a hotspot of pathogen evolution for both human - and plant-pathogenic bacteria [60]. Vacuoles are also a hotspot for horizontal gene transfer between microorganisms [61], further exacerbated as predation by protists promotes conjunctive plasmids, another central mechanism of microbial evolution [62]. The different eco-evolutionary feedbacks of bacteria and protists still need to be investigated in more detail in the rhizosphere and integrated into the community level, in order to understand protist effects on rhizosphere microbiome evolutionary dynamics.

Impact of Protists on Microbiome Functionality and Plant Performance

The rhizosphere microbiome is increasingly recognized as an essential component, shaping plant physiology, nutrition, and health [2,63]. In the previous sections, we highlighted how protists can affect the functional and taxonomic composition of the rhizosphere microbiome (Figure 2B). Here, we will show that many of these changes can have a concrete impact on plant performance (Figure 2C).

Plant Nutrition

Soil microorganisms play essential roles in plant nutrition by fixing nitrogen, mineralizing soil organic matter, or solubilizing organically bound nutrients that would otherwise remain inaccessible to the plant [64]. Protists impact these activities in several ways. One central hypothesis, the **microbial loop**, postulates that most biomass turnover occurs at a microscopic level. Protist consumption releases nutrients from bacterial biomass and makes them available to plants [8], resulting in increased plant nutrition and growth [65]. While much attention has been directed to the role of protists in nitrogen cycling, recent work has emphasized their importance for phosphorous mineralization in soil [66]. The effects of protists are not restricted to plant biomass, but can also influence nutrition and biomass allocation, increasing, for instance, resource allocation to reproductive organs [65,67]. The effect of protists on plant nutrition is more pronounced in the presence of arbuscular mycorrhizal fungi, which on their own have a limited ability to produce the enzymes required for soil organic matter breakdown. Protists

increase nutrient mineralization by hyphae-associated microorganisms, which can then be taken up by the mycorrhiza and transferred to the host plant [68].

Protists may also affect nutrition by selecting for or against specific bacterial groups that are important for nutrient cycling and plant nutrition, such as nitrifiers [36] or phosphate solubilizers [69]. Protist predation can moreover induce bacterial traits important for nutrient cycling, such as siderophores, which chelate iron and thus modulate iron availability in soil [70]. Finally, some protists such as testate amoebae are involved in the cycling of silica, an element required for plant stress tolerance [71,72] (Table 1).

Plant Hormonal Balance

Plants use various hormones to regulate their life history, including flowering time, root morphology, and stress resistance [73]. Each of these traits is linked with specific costs and benefits and a tight regulation is necessary to match the plant's phenotype to the specific environmental conditions it is facing. Root-associated microorganisms can influence plant hormonal balance in several ways. Several rhizosphere microorganisms can produce or degrade hormones such as ethylene, auxin, cytokinin, or gibberellin, with broad repercussions on plant phenotype and fitness [74,75]. Protists can impact the effect of microorganisms on the plant hormonal balance by altering both the abundance and activity of the involved microorganisms: protists promote, for instance, auxin-producing bacteria [76], thereby stimulating lateral root branching [77]. Protists also increase cytokinin concentrations in plants, possibly as a result of the increased nitrate concentration that occurs when excess nitrogen is secreted [77]. Finally, protists could alter the plant's hormonal balance indirectly by affecting microbiome functions, for instance by increasing the production of bacterial metabolites such as 2,4-diacetylphloroglucinol (DAPG) [44], an antimicrobial compound that also interferes with auxin signaling [78]. The strong effect of protists on the plant metabolome can most likely be linked to these multiple hormonal changes [79].

Plant Health

Plants are confronted with a broad range of pathogens. Plant-associated microbes are unanimously recognized to be a central determinant of plant health by inhibiting pathogens and stimulating plant immunity, with a power equaling the defense traits encoded in the plant genome [2]. However, not all microbiomes suppress disease equally, with impacts ranging from

Table 1. Effect of Protists on Rhizosphere Microbiome Functions

Function	Impact of protists	Refs
Nutrient turnover	Increased plant carbon uptake	[65,67,98]
	Increased nitrogen release	[8,98]
	Increased nitrogen mineralization	[35,68]
	Increased plant nitrogen uptake	[35,65,98–101]
	Increased plant phosphorus uptake	[102]
	Increased plant magnesium and calcium	[103]
	Increased silica mineralization	[71,72]
Plant hormones	Increased plant free auxin	[77]
	Increased plant cytokinin levels	[77]
Disease suppression	Higher abundance of pathogen-suppressing bacteria	[44]

full disease suppression to disease promotion [80]. Further, even if great strides have recently been made in correlating patterns of community structure to the presence of specific taxa or particular functional genes to disease suppression [81,82], the mechanisms underlying the presence or absence of these microbiome characteristics remain elusive. As a result, disease suppression by microbial communities is still unpredictable and hard to manage, as we only partially understand why a plant-protective microbiome configuration can emerge and be preserved. Predation by protists may be a missing link to understand soil suppressiveness.

There are several potential means by which protists can influence the ability of the microbiome to suppress diseases. Predation by protists can select for bacteria producing compounds linked to disease suppression, such as cyclic lipopeptides, polyketides, alkaloids, or hydrogen cyanide [25,30,83] and stimulate the expression of these traits [44,45]. The overlap between the suite of bacterial traits linked with predation resistance and pathogen suppression can be used as a tool to promote specific functions throughout the microbiome. It may also provide a stepping stone to enhance the performance of introduced biocontrol bacteria, which are implemented as a sustainable alternative to pesticides but often fail to establish under field conditions. The establishment of such **biocontrol agents** may be facilitated by protists. Selective feeding by protists may favor introduced, secondary metabolite-producing biocontrol *Pseudomonas protegens* [39] by preferentially consuming less defended resident species that compete with the biocontrol agents. In addition, protists may even affect plant immunity: some secondary metabolites overproduced by bacteria in their defense response to predators [84] can prime plant immunity via the jasmonic acid pathway [85], thereby enhancing resistance to a range of belowground and aboveground pathogens.

Protists may also contribute to disease suppression by directly consuming pathogens, reducing their survival in soil and potentially protecting plants [86]. Mycophagous protist groups such as vampyrellid amoebae and grossglockneriid ciliates are more widespread and abundant in different soils than previously thought [87]. Moreover, several species previously considered bacterivorous were recently discovered to feed on a range of fungal spores and yeast cells, including plant pathogenic fungi [87]. This widespread mycophagy suggests that mycophagous protists constitute a reservoir of biocontrol agents that could directly consume fungal pathogens. In addition to direct consumption, protists secrete several extracellular compounds, some of which show bactericidal effects that may prevent the growth of bacterial pathogens [88]. In conclusion, evidence from experiments in controlled environments suggest that protists can influence the disease suppressive ability of microbial communities directly or via changes in the microbiome composition. The next step is to investigate whether and how these promising findings can be translated into applications to control plant diseases.

Protists as a Microbiome Optimization Tool for Sustainable Agriculture

Protists hold promise for future strategies to enhance microbiome function and contribute to sustainable, high yield agricultural practices. One challenge of applying beneficial microbes is achieving stable formulations, which is easier for organisms forming resistant structures, such as spores [89]. Similarly, the ability of protists to form cysts can facilitate efficient large-scale production efforts for industrial applications, such as seed coatings or soil amendments. The ability of protists to enhance nutrient cycling and promote plant growth make them interesting as **biostimulants**. Protists may, for instance, be used to speed up the mineralization of organic fertilizer and increase the survival and activity of beneficial microbes [39,90]. The first protist-based biostimulants and plant protection products have already hit the market [Ecostyle (https://www.ecostyle.nl/groensector/protoplusr)] or are under development [amoéba (http://www.amoeba-biocide.com/en/news/w-magna-90-efficacy-mildew)]. We suggest that

a targeted approach focusing on determining which protist traits are linked to enhanced plant performance may prove more fruitful in identifying beneficial protist taxa than the traditional screening of a large number of species (Figure 3). We foresee that, thanks to the overlap between predator defense and pathogen suppression, protists may be a promising soil-health improvement technology, alone or in combination with introduced biocontrol microorganisms. Further, thanks to their key function as a regulator of the rhizosphere microbiome, protists may be an excellent target for soil enhancement practices. Protists readily respond to agricultural practices such as soil tillage [91], fertilization [92], or pesticide application [93] as well as sown plant species [94,95]. Protists may thus form an important leverage between management practices and microbiome, helping to manage microbiome function in a more targeted and efficient way. For instance, addition of organic fertilizer was shown to increase the relative abundance of heterotrophic protist taxa at the expense of parasites and pathogens [96]. Protists could also be the target of conservation biocontrol strategies where management
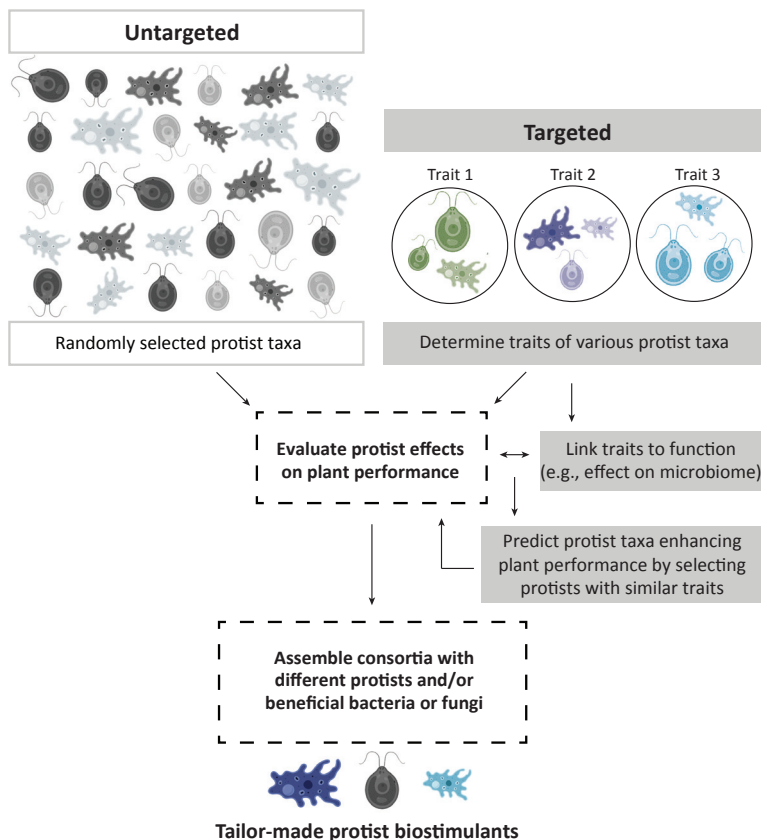
Figure 3. Strategies to Identify Beneficial Protist Taxa. In order to identify protist taxa that are suitable as biostimulants, a targeted approach based on, for example, traits can be applied. In such an approach, protist traits are characterized and linked to functional information. Based on that information, a guided taxon choice can be made for future tests based on traits associated with beneficial effects on plants. In comparison, untargeted approaches randomly test the effect of a wide variety of protists on plant performance. We suggest the targeted approach to be more efficient in finding taxa with the desired effects over time, as it reduces the number of taxa that need to be screened as it guides targeted screening for additional cultures. The aim of both approaches is to identify beneficial protist taxa that can be applied alone or in combination to enhance plant performance. Protists may also be combined with other beneficial microbes in order to reach synergistic effects.

practices, for example different cultivars, are applied to promote indigenous taxa with biocontrol activity. Future research should focus on identifying management practices that increase the abundance and positive effects of specific protists that in turn foster desired traits in the rhizosphere microbiome.

Concluding Remarks and Future Perspectives

In this review, we have summarized recent developments on soil protists, pointing to their role as long-overlooked 'puppet masters' of the rhizosphere, with broad implications for microbiome function and services to plants. Protists may be a missing link that helps us predict and enhance microbiome function. We advocate that future efforts targeting the rhizosphere microbiome should include free-living protists as top-down regulators of microbiome composition, balancing and completing the current prevailing research focus on bottom-up drivers such as root exudates and plant genotype (see Outstanding Questions). Such a multitrophic approach could combine species distribution patterns retrieved from metabarcoding surveys with food-web modelling, providing testable predictions on the impact of given protists on microbiome structure [97]. By better deciphering the rules underlying soil microbiome assembly and function, this approach will allow for designing improved strategies harnessing the beneficial functions of the rhizosphere microbiome. The time has come for protists to get out of their scientific niche and become the next biotechnological tool to engineer microbiomes to promote the functions that are needed to guarantee sustainable and resilient food production.

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Outstanding Questions

What is the relative importance of protist predation and resource competition for rhizosphere microbiome dynamics?

What are the direct effects of protists on plant physiology?

What are general and species-specific effects of protists on the rhizosphere microbiome and plant performance?

What is the power of phylogeny and functional traits as predictors of the impact of protists on microbiome functioning and plant performance?

What are the ecological functions of unknown protist taxa recently discovered via sequencing-based surveys that have not yet been isolated?

How do free-living protists interact with microorganisms in the mycosphere?

How can protists be used as a leverage to enhance microbiome function?

What are the optimal methods for protists as biostimulants?

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