Protist plant pathogens as drivers of eco-evolutionary dynamics and biodiversity-ecosystem functioning (BEF) relationships in plant communities



Doctoral thesis

for

the award of the doctoral degree

of the Faculty of Mathematics and Natural Sciences

of the University of Cologne

submitted by

Marcel Dominik Solbach

Köln, 2026

(Accepted version of the dissertation.)

This work was conducted in the scope of the DFG-funded Research Unit “The Jena Experiment” (FOR 5000), Subproject 2 – Protist Plant Pathogens (DFG project number 432642583).

Summary

Biodiversity loss and increasing environmental stressors such as climate extremes threaten the functioning and stability of terrestrial ecosystems. Experimental grassland studies consistently show positive relationships between plant species richness and ecosystem functioning that strengthen over time, yet the mechanisms underlying this temporal reinforcement remain poorly understood. In particular, the contribution of soil microbial communities, especially protistan plant pathogens and consumers, to long-term plant–soil feedbacks and biodiversity–ecosystem functioning (BEF) relationships has received comparatively little attention.

This dissertation investigates how plant diversity, environmental stress, and plant–soil history shape soil-borne microbial communities, with a focus on Oomycota, Phytomyxea, and cercozoan consumers, and how these groups contribute to persistent soil microbial legacies in grassland ecosystems. Using taxon-specific DNA metabarcoding, microbial communities were analyzed along the plant species richness gradient (1–60 species) of the long-term Jena Experiment in controlled experimental approaches.

Repeated summer droughts induced long-lasting shifts in soil microbial community composition, including the enrichment of plant species-specific parasites, indicating that climatic stress can weaken plant defense and promote persistent pathogen legacies. Plant communities further generated pronounced and enduring soil microbial legacies that strengthened with increasing duration of shared plant and soil history. These effects were detectable across fungi, Oomycota, Cercozoa, and bacteria, and were characterized not only by microbial enrichment but frequently by the reduction of specific taxa. Increasing plant diversity reduced microbial dominance in some groups, potentially hinting towards dilution effects, but these patterns were not consistent across all microbial groups. Beyond plant pathogens, this dissertation demonstrates that protistan consumers are likely underappreciated participants in legacy formation.

Together, these findings show that plant diversity, time, and environmental stress can create persistent changes in microbial communities. The observed patterns clearly indicate that such microbial legacies can contribute to plant–soil feedbacks and BEF relationships, and the strengthening thereof.

Table of Contents

General Introduction 6

Ecosystem Functions and Biodiversity–Ecosystem Functioning Relationships 6

The Jena Experiment as a Model System 7

Potential Drivers and Strengthening of BEF Relationships 10

Evolution, Diversity, and Ecology of Protists 13

Protist Plant Pathogens in Oomycota and Cercozoa 16

Outline and Aims 19

Results 22

Chapter Overview and Summary 22

Chapter 1: Legacies of Consecutive Summer Droughts on Soil-Borne Plant Parasitic Protists (Oomycota: Stramenopila and Phytomyxea: Rhizaria) and Protistan Consumers (Cercozoa: Rhizaria) Along an Experimental Plant Diversity Gradient 25

Chapter 2: Plant-driven Soil Microbial Legacies Strengthen over Time and Include Widespread Suppression 54

Chapter 3: JenaTron - An Experimental Approach to Study the Effects of Plant History and Soil History on Grassland Ecosystem Functioning 116

Chapter 4: Fungivorous Protists in the Rhizosphere of *Arabidopsis thaliana* – Diversity, Functions, and Publicly Available Cultures for Experimental Exploration 143

Addendum: Further publications 153

General Discussion 156

Synthesis of Main Findings 156

Directionality and Causality in BEF–Microbiome Research 156

Experimental Trade-offs: From the Field to the JenaTron 158

Plant Species Richness and the Dilution Effect 159

Protist Plant Pathogens as Drivers of Eco-Evolutionary Adaptations 161

Protistan Consumers as Underappreciated Modulators of Soil Legacies? 162

Outlook 163

General References 165

General Introduction

Ecosystem Functions and Biodiversity–Ecosystem Functioning Relationships

Earth's ecosystems perform a wide range of functions and provide services essential for human well-being and all life on earth, including primary production, production of breathable oxygen (O_2), nutrient cycling, carbon sequestration, etc. (Field *et al.*, 1998; Tilman, 2001; Chapin *et al.*, 2002; Cardinale *et al.*, 2012; Bengtsson *et al.*, 2019). These services can be summarized as the flow and conversion of energy and matter through the environment, mediated by organisms (Chapin *et al.*, 2002), and are sometimes categorized into provisioning services, such as the production of food, wood, or drinking water, and regulating services, such as climate regulation and pest/disease control (Cardinale *et al.*, 2012).

Approximately half of today's global gross primary productivity can be attributed to terrestrial ecosystems (Crockford *et al.*, 2023), in which plants act as the primary producers and thereby play a key role in ecosystem functioning (Gough, 2012; Pace *et al.*, 2021). Global climate change, as well as other anthropogenic influences, such as pollution, habitat change, and overexploitation, increasingly challenge the functionality of our ecosystems (Mazor *et al.*, 2018). One of the key consequences is the ongoing loss of biodiversity (Kannan & James, 2009; Mazor *et al.*, 2018; Cowie *et al.*, 2022), including plant diversity (Corlett, 2016; Jandt *et al.*, 2022; Partel *et al.*, 2025). Beyond biodiversity loss in natural ecosystems, modern agriculture further exemplifies the consequences of reduced plant diversity, as the majority of global cropland is dominated by monocultures or low-diversity cropping systems, which may be increasingly vulnerable to pests and environmental stress (Tilman *et al.*, 2002; Crews *et al.*, 2018; Kaur *et al.*, 2024). Consequently, understanding how plant biodiversity and the loss thereof influence ecosystem functioning has become a major focus of ecological research, and various studies manipulating plant diversity have been conducted over the past decades (Schmid *et al.*, 2002; Tilman *et al.*, 2014), including forest (Bruelheide *et al.*, 2011; Verheyen *et al.*, 2013) and grassland biodiversity experiments (Tilman, 1996; Hector *et al.*, 1999; Roscher *et al.*, 2004).

These studies have consistently shown that increased plant species richness is most often associated with enhanced ecosystem functioning (Buzhdygan *et al.*, 2020), e.g.,

plant productivity (Balvanera *et al.*, 2006; Weigelt *et al.*, 2009; Tilman *et al.*, 2012; Liang *et al.*, 2016; Wagg *et al.*, 2022), soil fertility (Dybzinski *et al.*, 2008), pollination (Ebeling *et al.*, 2008), water quality (Brisson *et al.*, 2020), or temperature stabilization (Huang *et al.*, 2023). These relationships are known as biodiversity–ecosystem functioning (BEF) relationships.

The Jena Experiment as a Model System

One of the world's largest and longest-running grassland biodiversity experiments is the Jena Experiment (Roscher *et al.*, 2004). Established in 2002, the Jena Experiment tried to overcome shortcomings of earlier grassland biodiversity experiments, such as those erected in the Cedar Creek Natural History Area, Minnesota (Tilman, 1996; Tilman *et al.*, 2001), or the subsequent BIODEPTH experiment erected across multiple sites in Europe (Hector *et al.*, 1999; Spehn *et al.*, 2005). These studies demonstrated that increasing plant species richness enhanced aboveground productivity and stability, providing some of the first causal evidence that biodiversity can directly influence ecosystem functioning.

Despite their pioneering contributions, these early grassland biodiversity experiments have been criticized for several reasons: Species selection was usually random and did not reflect natural communities, and did not account for potential effects of functional groups (Roscher *et al.*, 2004). Therefore, biodiversity effects could not unambiguously be differentiated from selection effects or sampling effects, where the selection of plant species in the experimental setup increases the probability of including highly productive or dominant species that then drive results at higher diversity levels, rather than true complementarity among species (Huston, 1997; Wardle, 1999; Loreau & Hector, 2001). Similarly, if diverse mixtures are more likely to contain certain nitrogen-fixing legumes, then diversity effects and effects of those legumes become confounded (Roscher *et al.*, 2004). Furthermore, most early experiments focused on short-term responses, limiting insights into how biodiversity effects develop over time and how longer-term processes contribute to BEF relationships (Reich *et al.*, 2012).



Figure 1: Aerial view of the Jena Experiment field site in 2016. Shown are Blocks 1 and 2, with the Saale River in the background. Photo credit: The Jena Experiment.

The Jena Experiment (Figure 1) was established in 2002 to explicitly address these limitations (Roscher *et al.*, 2004; Weisser *et al.*, 2017). The plant species pool used in the Jena Experiment was chosen to represent a mesophilic grassland community native to central Europe. A total of sixty plant species was chosen that can be categorized into four plant functional groups (grasses (16), legumes (12), small herbs (12), and tall herbs (20)) based on morphological (above- and belowground) and phenological traits, as well as the capacity for N_2 fixation (Roscher *et al.*, 2004). The main experimental design includes monocultures and mixtures of up to all sixty plant species (1, 2, 4, 8, 16, or 60 plant species per plot; Tables 1 and 2), following an approximately logarithmic (base 2, doubling) design. Plant species richness and functional group richness (1, 2, 3, or 4 plant functional groups per plot) were deliberately crossed (Table 2), with the restriction that functional group richness cannot exceed plant species richness (Roscher *et al.*, 2004; Weisser *et al.*, 2017).

The Jena Experiment is located in the floodplain of the Saale River at the northern edge of the city of Jena (Thuringia, Germany). The site was a grassland before the early 1960s and was then converted to a highly fertilized arable field mainly used for growing vegetables and wheat (Hundt, 1961) before its conversion to an unfertilized experimental grassland (Roscher *et al.*, 2004; Weisser *et al.*, 2017).

Typical of river floodplains, the soil texture at the field site varies with distance from the Saale River, with sandy loam soils occurring closer to the river and transitioning to silt loam further away (Roscher *et al.*, 2004). To account for this environmental gradient, the experiment follows a block design aligned with the river, comprising four blocks with 20 main plots each; Block 1 is closest to the river, and Block 4 is furthest away, and plots with the same plant species richness and functional group richness are distributed approximately evenly across blocks. The plots are mown twice per year (early June and early September) to mimic extensively managed hay meadows, and are weeded during three annual weeding campaigns (spring, summer, and autumn), with summer and autumn weeding conducted after mowing. Weeding is usually performed block-wise, with approximately three blocks weeded per campaign, resulting in each plot being weeded two to three times per year. Plots are generally not being fertilized or re-sown. Plot size was originally 20 m x 20 m, but was progressively reduced in subsequent years for easier maintenance by reducing weeding effort (Weisser *et al.*, 2017).

In addition to the main experimental plots, the field site includes several other plot types, such as succession plots (not mown or weeded since establishment), bare ground plots (kept vegetation-free), and smaller monoculture plots for each of the sixty plant species (Roscher *et al.*, 2004; Weisser *et al.*, 2017). The site regularly hosts stand-alone sub-experiments (Weisser *et al.*, 2017), such as the Trait-Based Experiment (TBE; 2010-2018), which established a reduced plant diversity gradient using communities composed of species with similar or dissimilar functional traits to disentangle selection and complementarity effects (Ebeling *et al.*, 2014). In addition, several split-plot experiments were implemented on subareas of the main biodiversity plots, such as the Drought Experiment (2008–2016), which imposed repeated summer droughts (Vogel *et al.*, 2013a; Vogel *et al.*, 2013b), or the ΔBEF Experiment (“DELTa-BEF Experiment”; DEterminants of Long-Term Biodiversity Effects on Ecosystem Functioning Experiment; 2016–2023), which re-established identical plant communities with differing soil and plant history to investigate how BEF relationships develop over time (Vogel *et al.*, 2019).

Table 1: *Design matrix of the main plots of the Jena Experiment as described in Roscher* et al. *(2004) and Weisser* et al. *(2017). The original setup consisted of 82 plots, but two monocultures (*Cynosurus cristatus *(grass) and* Bellis perennis *(small herb)) were given up due to very low cover (see numbers in parentheses; Weisser* et al. *(2017)), effectively resulting in 80 main plots that have been maintained since 2002.*

Plant Species Richness	1	1	1	1	2	2	2	2	2	2	2	2	4	4	4	4	4	4	4	4	4	4	4	4
Functional Group Richness	1	1	1	1	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	3	3	3	3	4
Grasses	1				2				1		1		4				2		2		2	1	1		1
Small herbs		1				2			1			1		4			2			2	1		1	2	1
Tall herbs			1				2			1	1				4			2	2		1	2		1	1
Legumes				1				2		1		1				4		2		2		1	2	1	1
Number of Replicates	4(3)	4(3)	4	4	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	4
Plant Species Richness	8	8	8	8	8	8	8	8	8	8	8	8	8	16	16	16	16	16	16	16	16	16	16	16	60
Functional Group Richness	1	1	1	1	2	2	2	2	3	3	3	3	4	1	1	2	2	2	2	3	3	3	3	4	4
Grasses	8				4		4		2	3	3		2	16		8		8		5	5	6		4	16
Small herbs		8			4			4	3		3	2	2			8			8	5		5	6	4	12
Tall herbs			8			4	4		3	2		3	2		16		8	8		6	5		5	4	20
Legumes				8		4		4		3	2	3	2				8		8		6	5	5	4	12
Number of Replicates	1	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	4	4

Table 2: *Near-orthogonal crossing of plant species richness and functional group richness in the Jena Experiment as described in Roscher* et al. *(2004) and Weisser* et al. *(2017). The empty cells are the result of impossible combinations, i.e., functional group richness cannot exceed plant species richness, and the 60-species mixtures will always contain the whole species pool and thus all 4 functional groups. Two monocultures (*Cynosurus cristatus *(grass) and* Bellis perennis *(small herb)) were given up due to very low cover (see numbers in parentheses; Weisser* et al. *(2017)).*

		Plant species richness (sown diversity)					
		1	2	4	8	16	60
Functional group richness	1	16 (14)	8	4	4	2	
	2		8	4	4	4	
	3			4	4	4	
	4			4	4	4	4
	Total	16 (14)	16	16	16	14	4

Potential Drivers and Strengthening of BEF Relationships

One of the key findings of long-term plant biodiversity experiments is that BEF relationships, especially the biodiversity-productivity relationship, strengthen over time as ecosystems age (Tilman *et al.*, 2006; Eisenhauer *et al.*, 2012; Reich *et al.*, 2012; Meyer *et al.*, 2016; Guerrero-Ramirez *et al.*, 2017; Wagg *et al.*, 2022), suggesting that short-term experiments likely underestimate the effects of plant diversity on ecosystem functioning (Reich *et al.*, 2012; Vogel *et al.*, 2019). It remains unclear whether this strengthening is attributable to increasing performance of high diversity communities,

reduced performance of low diversity communities, or a combination of both (Eisenhauer *et al.*, 2012; Meyer *et al.*, 2016; Guerrero-Ramirez *et al.*, 2017; Eisenhauer *et al.*, 2019).

Several mechanisms have been proposed as contributors to the strengthening of BEF relationships, including elevated interspecific complementarity (Cardinale *et al.*, 2007; Reich *et al.*, 2012), potentially driven by selection towards increased niche differentiation (Zuppinger-Dingley *et al.*, 2014). Attention has increasingly shifted away from direct plant-plant interactions toward mechanisms that involve biotic interactions with animals (Eisenhauer *et al.*, 2009; Ebeling *et al.*, 2018; Dietrich *et al.*, 2021) and microorganisms in the soil (Eisenhauer *et al.*, 2012; Lange *et al.*, 2015; Eisenhauer *et al.*, 2019; Schmid *et al.*, 2021) that then feed back to the plant communities (Bever *et al.*, 1997; Mitchell *et al.*, 2002; Eisenhauer *et al.*, 2012).

Soil microbes are of particular interest since microbial growth is strongly restricted by the availability of carbon in the soil (Blagodatskaya & Kuzyakov, 2013). Plants actively contribute to the growth of soil microbiota by releasing a species-specific mixture of primary and secondary C-metabolites at their growing root tips (Uren, 2000; Oburger & Jones, 2018). These microbes subsequently coevolve along the root axis to species- and even cultivar-specific rhizosphere microbiomes during the lifetime of the plant host (Bakker *et al.*, 2012; Berendsen *et al.*, 2012; Peiffer *et al.*, 2013; Berg *et al.*, 2014; van der Heijden & Schlaeppi, 2015; Pieterse *et al.*, 2016; Rossmann *et al.*, 2020; Rüger *et al.*, 2021). The composition of rhizodeposits leads to plant-specific competitive outcomes in rhizosphere microbiomes (Dennis *et al.*, 2010; Garcia Arredondo *et al.*, 2024), leading to the enrichment of specific microbiota in the rhizosphere (Hu *et al.*, 2018; Sasse *et al.*, 2018; Jacoby *et al.*, 2020; Pang *et al.*, 2021; Schultes *et al.*, 2025). The propagules of these rhizosphere microbial communities remain in place even after plant individuals die and are inherited by following plant generations, thereby generating plant–soil feedbacks (PSFs) (Bever, 1994; Bever *et al.*, 1997; Kulmatiski *et al.*, 2008; Bergmann *et al.*, 2016; Bennett & Klironomos, 2019). PSFs are reciprocal interactions whereby plants modify the biotic (and abiotic) properties of the soil, which in turn influence the performance of subsequent plants growing in that soil (Bever, 1994; Bever *et al.*, 1997). Because plants modify soil communities and properties cumulatively, PSFs can add up over time and give rise to long-lasting soil legacy effects (Frouz, 2024).

PSFs and soil legacy effects are not exclusively mediated by microbes but can also arise from plant-induced abiotic changes to the soil, including alterations in soil moisture, aggregation, pH, and carbon and nitrogen pools (Ehrenfeld *et al.*, 2005). Many of these abiotic modifications are tightly coupled to soil biota, as microbial activity both mediates plant-driven changes (e.g., organic matter turnover and aggregation) and responds to them, creating intertwined feedbacks (van der Heijden *et al.*, 2008; Bardgett & van der Putten, 2014).

Positive PSFs arise when plants promote soil conditions that enhance subsequent plant growth, most commonly through the accumulation of beneficial soil biota such as mycorrhizal fungi, or plant growth–promoting and biocontrol bacteria, while negative PSFs are frequently attributed to the build-up of host-specific antagonists, particularly soil-borne pathogens, which reduce plant performance when host plants repeatedly grow in the same soil (Bever *et al.*, 1997; Ehrenfeld *et al.*, 2005; Kulmatiski *et al.*, 2008; Bever *et al.*, 2010; Latz *et al.*, 2012; van der Putten *et al.*, 2013; Latz *et al.*, 2016; Bennett *et al.*, 2017; Hannula *et al.*, 2021). PSFs are typically quantified using soil-conditioning experiments in which soils are first conditioned by a focal plant species or community and then used to grow the same (conspecific) and different (heterospecific) plants, with feedback strength calculated from the difference in plant performance (Bever *et al.*, 1997; Bever, 2003; Kulmatiski *et al.*, 2008).

In natural ecosystems, PSFs play an important role in shaping plant species coexistence and local biodiversity. Positive PSFs, where plants condition soils in ways that promote the growth of their own offspring, can lead to monospecific dominance, whereas negative PSFs can suppress conspecifics and allow other species to establish, thereby promoting diversity (Bever, 1994; Klironomos, 2002; Petermann *et al.*, 2008). These effects are particularly well documented in the context of invasive plant species (Klironomos, 2002; Zhang *et al.*, 2010; Yelenik & D'Antonio, 2013), and illustrate how belowground biotic interactions can regulate community composition in naturally assembled plant communities.

Importantly, PSFs and soil legacies have been proposed as key drivers underlying the strengthening of BEF relationships over time, as negative feedbacks tend to dominate in monocultures and low-diversity communities through the accumulation of species-specific pathogens, whereas positive feedbacks increasingly prevail in species-rich communities via enhanced mutualisms (Schnitzer & Klironomos, 2011; Eisenhauer *et*

al., 2012; Kulmatiski *et al.*, 2012; Mommer *et al.*, 2018). In monocultures and species-poor communities, proliferation of host-specific pathogens is expected to be facilitated by high frequencies of target hosts, while in diverse plant communities, the reduced frequency of susceptible hosts (and increased frequency of incompetent hosts) should limit the dominance of host-specific pathogens. This process is referred to as the dilution effect or pathogen dilution, which was originally described in animal disease systems (Ostfeld & Keesing, 2000; Schmidt & Ostfeld, 2001) but has since been extended to interactions between plants and their pathogens (Mitchell *et al.*, 2002; Liu *et al.*, 2016; Keesing & Ostfeld, 2021). Together, these processes provide a potential microbial pathway through which plant diversity can influence ecosystem functioning (Kulmatiski *et al.*, 2012).

Studies on soil and rhizosphere microbiomes have traditionally focused on bacteria and fungi, whereas protists were long neglected but are now increasingly recognized as key players in the soil and rhizosphere (Geisen *et al.*, 2018; Xiong *et al.*, 2018; Bonkowski *et al.*, 2019; Xiong *et al.*, 2020).

Evolution, Diversity, and Ecology of Protists

The term “protist” historically encompasses all eukaryotes that do not belong to the kingdoms of animals, plants, or fungi, and thus refers to a paraphyletic grouping out of practical convenience rather than a true phylogenetic clade, representing (mostly) unicellular eukaryotes that do not form tissues (Whittaker, 1969; Bonkowski *et al.*, 2019). Protists include a multitude of lineages with vastly different morphologies and lifestyles, and they are present in almost all ecosystems on Earth, including marine, freshwater, and terrestrial habitats, where they exert an extraordinary range of ecological functions (Geisen *et al.*, 2018; Burki *et al.*, 2021; Singer *et al.*, 2021; Bachy *et al.*, 2022). Yet they remain poorly understood in terms of their true diversity, and thus the eukaryotic tree of life is regularly extended and reshaped (Cavalier-Smith, 2002; Cavalier-Smith, 2003; Ruggiero *et al.*, 2015; Cavalier-Smith *et al.*, 2018; Adl *et al.*, 2019; Burki *et al.*, 2020).

Protist evolution can be traced back at least a billion years to the earliest eukaryotic ancestors that likely emerged as a consequence of the primary endosymbiosis event that led to mitochondria (Martin *et al.*, 2017; Roger *et al.*, 2017; Lopez-Garcia & Moreira, 2020). The (mostly) multicellular kingdoms of animals and fungi have evolved

from protistan ancestors and are thus phylogenetically nested within the protists (Bonkowski *et al.*, 2019). Another key evolutionary event was the primary endosymbiosis of a cyanobacterium giving rise to plastids and to a major photosynthetic lineage (Archaeplastida), which ultimately also resulted in the emergence of the third large kingdom of multicellular eukaryotes, the plants (Keeling, 2010). Beyond the lineage containing primary plastids, the ability for photosynthesis has spread widely within the eukaryotic tree of life via secondary (and tertiary) endosymbioses, in which previously non-photosynthetic eukaryotes engulfed algae that already possessed primary (or secondary) plastids. As a result, plastid-bearing protists can be found in several distantly related groups, including Stramenopila (e.g., diatoms and brown algae), Alveolata (e.g., dinoflagellates), Rhizaria (chlorarachniophytes), etc. (Keeling, 2013).

Functionally, protists can act as primary producers, consumers, decomposers, or symbionts (Geisen *et al.*, 2018; Singer *et al.*, 2021). For example, in marine systems, phytoplankton forms the basis of aquatic food webs and drives carbon sequestration through the biological carbon pump (Ducklow *et al.*, 2001; Nowicki *et al.*, 2022). Marine systems are responsible for roughly half of the Earth's primary biomass and oxygen production (Field *et al.*, 1998; Falkowski, 2012; Sekerci & Petrovskii, 2015), and photosynthetic protists such as diatoms, dinoflagellates, and green algae play a key role in these processes. Equally important are heterotrophic protists such as flagellates, ciliates, and amoebae, which play pivotal roles as predators and grazers of bacteria and other microeukaryotes, including the phytoplankton (Sherr & Sherr, 2002; Bachy *et al.*, 2022). Microbial consumers are key drivers of the "microbial loop" (Azam *et al.*, 1983). Dissolved organic matter (DOM) released by the phytoplankton (Fogg, 1983) is rapidly taken up by bacteria, and these bacteria are then consumed by heterotrophic protists, which closes the loop by partially returning essential nutrients back to the phytoplankton (Azam *et al.*, 1983).

In contrast to transparent water, soils are opaque, and therefore primary production by photosynthetic microorganisms on land is limited to the uppermost layer (Geisen *et al.*, 2018), where they are often involved in the formation of biocrusts on arid soil surfaces (Maier *et al.*, 2018; Samolov *et al.*, 2020). However, as mentioned above, the majority of primary production on land is carried out by vascular plants (Gough, 2012; Pace *et al.*, 2021), which release a variety of root exudates into the surrounding soil. These

organic compounds shape the microbial community in the rhizosphere by attracting certain bacteria and fungi (Sasse *et al.*, 2018). In turn, phagotrophic protists, such as many Amoebozoa and Cercozoa, feed on those bacteria and fungi, thereby regulating microbial populations and facilitating nutrient turnover (Geisen *et al.*, 2018). Similar to aquatic systems, heterotrophic protists are integral to the microbial loop in soils, where they indirectly promote plant growth by releasing nutrients from their consumed prey back into the soil (Coleman, 1994; Bonkowski, 2004), especially nitrogen in the form of ammonium (Clarholm, 1985). The heterotrophic protists are then consumed by predators of higher trophic levels (Adl & Gupta, 2006).

Mutualistic relationships involving protists have also been reported, e.g., dinoflagellate symbionts of the family Symbiodiniaceae sustain coral reef ecosystems (Stat *et al.*, 2008), and green algae such as *Chlorella* spp. form symbioses with ciliates, cnidarians, and flatworms (Karakashian & Karakashian, 1965). In turn, many free-living protists serve as hosts for endosymbiotic bacteria (Gortz, 2001; Park *et al.*, 2004; Hess *et al.*, 2016; Boscaro *et al.*, 2019).

Pathogenic protists can cause a range of diseases in humans, e.g., malaria caused by *Plasmodium* spp. (Carter & Mendis, 2002), African sleeping sickness and Chagas disease caused by *Trypanosoma* spp., and leishmaniasis caused by *Leishmania* spp. (Stuart *et al.*, 2008), while usually free-living amoebae such as *Acanthamoeba* spp. and *Naegleria fowleri* can cause opportunistic infections (Visvesvara *et al.*, 2007). Similarly, protists can also cause significant diseases in animals, e.g., *Perkinsus* spp., which infect mollusks such as clams and oysters (Casas *et al.*, 2008), *Ichthyophthirius multifiliis*, the ciliate responsible for “white spot disease” in freshwater fish (von Gersdorff Jørgensen, 2017), and *Eimeria* spp., apicomplexans that cause coccidiosis in poultry (Shirley *et al.*, 2005).

While protists engage in a wide range of associations with other organisms across ecosystems, those that manifest as plant diseases are of particular importance for terrestrial ecosystems, both ecologically and economically. Some of the most severe plant pathogens are found in the protistan supergroups Stramenopila (phylum Oomycota) and Rhizaria (phylum Cercozoa, class Phytomyxea) (Neuhauser *et al.*, 2014; Kamoun *et al.*, 2015; Derevnina *et al.*, 2016; Bulman & Neuhauser, 2017; Schwelm *et al.*, 2018).

Protist Plant Pathogens in Oomycota and Cercozoa

Oomycota have been traditionally described as being fungus-like, but despite similar morphology and ecological roles, Oomycota differ in several traits from true fungi (Beakes & Thines, 2016): Their vegetative thallus is diploid rather than haploid (Win-Tin & Dick, 1975), their cell walls do not contain chitin but cellulose (Helbert *et al.*, 1997), their hyphae are usually non-septate (Latijnhouwers *et al.*, 2003), and they produce mycolaminarans (β-glucans) rather than glycogen as their primary carbohydrate storage molecule (Wang & Bartnicki-Garcia, 1974). Oomycota can produce biflagellate zoospores with two distinct flagella: a smooth, posterior whiplash flagellum and an anterior tinsel flagellum bearing mastigonemes that enable active swimming (Walker & van West, 2007). In contrast, many true fungi lack motile spores, or they produce zoospores that are most often uniflagellate (Gleason & Lilje, 2009). Molecular phylogenies have confirmed that Oomycota are more closely related to photosynthetic lineages in the Stramenopila, such as brown algae and diatoms, than to true fungi (Adl *et al.*, 2012; Keeling, 2013). This positioning suggests that the ancestors of Oomycota potentially also possessed plastids that were lost during their evolution (Tyler *et al.*, 2006; Beakes *et al.*, 2012), but this is still a point of controversy (Bodył, 2005; Keeling, 2013).

The phylum Oomycota can be separated into several basal lineages and the two major classes Saprolegniomycetes and Peronosporomycetes (Beakes & Thines, 2016). The basal lineages include mostly marine algal parasitoids (Buaya & Thines, 2020; Buaya *et al.*, 2021; Buaya *et al.*, 2023). Saprolegniomycetes are best known as aquatic animal pathogens, especially in aquaculture, where species of *Saprolegnia* and *Achlya* cause infections in fish (Singhal *et al.*, 1987) and *Aphanomyces astaci* causes crayfish plague (Svoboda *et al.*, 2017). This class also contains several terrestrial plant pathogens, e.g., *Aphanomyces euteiches*, the causal agent of root rot in legumes (Gaulin *et al.*, 2007). Approximately 60% of described Oomycota species parasitize plants (Thines & Kamoun, 2010), many of which cause serious plant diseases (Kamoun *et al.*, 2015). The class Peronosporomycetes is particularly rich in plant pathogenic taxa, which includes the *Pythium sensu lato* complex (Uzuhashi *et al.*, 2010) such as *Globisporangium ultimum* (formerly *Pythium ultimum*) causing root rot or damping-off disease in seedlings of various crops (van der Plaats-Niterink, 1981; Eggertson *et al.*, 2023), the downy mildews like *Peronospora* (Thines & Choi, 2016), the white blister

rusts such as *Albugo* (Thines & Voglmayr, 2009), and the genus *Phytophthora* including forest pathogens such as *P. cinnamomi* and *P. ramorum* (Hansen, 2015). The most infamous species of the latter genus is *P. infestans*, the causal agent of potato late blight, which triggered the Great Irish Famine starting in 1845 and remains one of the most economically damaging plant pathogens worldwide (Fry, 2008; Kamoun *et al.*, 2015). Plant host-specificity in Peronosporomycetes appears to have evolved gradually and ranges from generalist opportunists (e.g., *Pythium sensu lato*) to highly specialized obligate biotrophs (e.g., *Peronospora* and *Albugo*) (Thines & Kamoun, 2010).

The Oomycota lifecycle can alternate between asexual and sexual reproduction. Asexual reproduction involves the production of sporangia, which can either germinate directly or release biflagellate zoospores, adapted for dispersal in water films. These zoospores encyst upon contact with a host surface and germinate to form an appressorium, usually penetrating the plant cuticle and pushing into the epidermal cells (Bircher & Hohl, 1997; van West *et al.*, 2003; Fawke *et al.*, 2015; Kamoun *et al.*, 2015). Entry involves mechanical force by pressure (Money *et al.*, 2004), likely combined with cell wall-degrading enzymes (Judelson & Blanco, 2005; Hardham, 2007). Once inside, the pathogen develops coenocytic (multinucleate) hyphae that spread intercellularly, extracting nutrients via specialized feeding structures called haustoria (Latijnhouwers *et al.*, 2003). Under unfavorable conditions, many Oomycota can also form thick-walled asexual chlamydospores, either in the middle or at the tip of a hypha (Meng *et al.*, 2014; Kamoun *et al.*, 2015). Sexual reproduction involves the formation of male antheridia and female oogonia, followed by fertilization of the oogonium by the antheridium, resulting in the development of thick-walled oospores that can persist in soil for years and serve as the primary inoculum for subsequent infection cycles (Turkensteen *et al.*, 2008).

The other group of protistan plant pathogens investigated in this thesis belongs to the class Phytomyxea in the phylum Cercozoa. Cercozoa is a diverse and ubiquitous phylum in the supergroup Rhizaria known to comprise a major part of soil protistan communities (Adl & Gupta, 2006; Lentendu *et al.*, 2014; Fiore-Donno *et al.*, 2024). Rhizarian/cercozoan taxonomy has recently undergone several revisions and changes regarding the placement of the Endomyxa, including the Vampyrellida and Phytomyxea (Cavalier-Smith & Chao, 2003; Cavalier-Smith, 2018; Cavalier-Smith *et al.*, 2018).

Throughout this thesis and all included chapters, the Endomyxa will be treated as a taxonomic sub-clade of Cercozoa as originally defined by Cavalier-Smith and Chao (2003) and in accordance with phylogenetic analyses by Irwin *et al.* (2019) and the current taxonomy in the PR² database (version 5.1.0, https://pr2-database.org/) (Guillou *et al.*, 2013). However, I acknowledge that Cercozoa might be a polyphyletic group according to this definition.

Phytomyxea are obligate biotrophic parasites and comprise two groups: The Phagomyxida, which are primarily marine and parasitize Stramenopila (e.g., brown algae, diatoms), and the Plasmodiophorida, which are mostly terrestrial and infect plants (Neuhauser *et al.*, 2014; Schwelm *et al.*, 2018; Hittorf *et al.*, 2020). Like for Oomycota, most research effort on this group has been spent on a few plant pathogens of economically important plants (Schwelm *et al.*, 2018), e.g., *Plasmodiophora brassicae* causing clubroot disease in cabbage and other Brassicaceae (Aist & Williams, 1971; Dixon, 2009; Rastas *et al.*, 2012), or *Spongospora subterranea* causing powdery scab in potatoes (Falloon *et al.*, 2015; Strydom *et al.*, 2024). Importantly, not all members of the Plasmodiophorida are pathogenic themselves; however, several species play a crucial role as vectors of plant viruses. Well-known examples include *Polymyxa graminis,* which transmits viruses such as the barley yellow mosaic virus (BaYMV) (Adams *et al.*, 1988), and the soil-borne wheat mosaic virus (SBWMV) (Kanyuka *et al.*, 2003), and *Polymyxa betae,* which transmits the beet necrotic yellow vein virus (BNYVV), the causal agent of rhizomania in sugar beets (Tamada & Baba, 1973; Weiland *et al.*, 2019). Recent studies suggested that the Plasmodiophorida also represent a considerable reservoir for plant pathogens in natural ecosystems (Neuhauser *et al.*, 2014) and have already been reported from environmental sequencing studies in grasslands (Fiore-Donno *et al.*, 2019).

The typical plasmodiophorid life cycle is characterized by two main stages: a primary infection of host root hairs and a subsequent secondary infection of cortical tissues (Bulman & Neuhauser, 2017; Wang *et al.*, 2022). Infection begins when long-lived resting spores in the soil germinate to release primary zoospores, which swim using two unequal flagella and penetrate root hairs. They deploy a specialized extrusome apparatus termed the “Rohr” and “Stachel” (tube and sting) (Keskin & Fuchs, 1969; Aist & Williams, 1971; Schwelm *et al.*, 2018). The “Rohr” is a tubular structure that extends from the zoospore, while the “Stachel” is a needle-like protrusion. This

apparatus punctures the host cell wall, enabling direct injection of the cytoplasm into the host cell (Keskin & Fuchs, 1969; Aist & Williams, 1971). Once inside, the zoospore develops into a multinucleate primary plasmodium that eventually forms zoosporangia containing motile secondary zoospores (Bulman & Neuhauser, 2017). During secondary infections, the parasite colonizes cortical cells and forms a secondary plasmodium, which eventually forms resting spores again that are released back into the soil upon decay of the host tissue (Kageyama & Asano, 2009; Bulman & Neuhauser, 2017).

The alternating lifestyles in Oomycota and Phytomyxea combine efficient dispersal via zoospores and long-term persistence via resting stages (oospores and resting spores). Survival rates of several months, years, or even decades have been reported for oospores (Populer, 1981; Turkensteen *et al.*, 2008) as well as Phytomyxea resting spores (Wallenhammar, 1996; Kanyuka *et al.*, 2003; Bulman & Neuhauser, 2017; Schwelm *et al.*, 2018). Thereby, these groups represent excellent candidates for creating PSFs and lasting legacy effects in the soil.

Advances in molecular sequencing techniques such as metabarcoding now allow for the detection and deeper investigation of Oomycota and Cercozoa (Fiore-Donno *et al.*, 2018; Fiore-Donno *et al.*, 2019; Fiore-Donno & Bonkowski, 2021). Importantly, DNA-based metabarcoding captures genetic material from both metabolically active and inactive organisms, including the dormant resting stages persisting in the soil, thereby providing access to the microbial “seed bank”. This approach was deliberately chosen for this thesis to assess the long-term soil-borne reservoir of these protistan lineages rather than only their currently active fraction.

Outline and Aims

Within the Jena Experiment, this thesis examines how soil-borne protists, particularly Oomycota and Cercozoa (including plant-parasitic Phytomyxea), and to a lesser extent bacteria and fungi, respond to the plant diversity gradient, repeated summer droughts, and experimental manipulations of soil and plant history using taxon-specific metabarcoding approaches. Chapter 1 (Solbach *et al.*, 2025a) investigates data from a Drought Experiment (Vogel *et al.*, 2013a; Vogel *et al.*, 2013b) to assess drought-induced legacy effects of soil protist communities, while Chapter 2 (unpublished manuscript) focuses on the ΔBEF Experiment (Vogel *et al.*, 2019) to investigate how

plant-driven soil microbial legacies strengthen over time. Building on these field-based studies, Chapter 3 introduces the experimental setup of the JenaTron Experiment (Madaj *et al.*, 2025), a controlled indoor experiment using soils originating from the Jena Experiment field site to disentangle soil and plant legacy effects in a fully crossed design of soil and plant history manipulations. Finally, Chapter 4 (Estermann *et al.*, 2023) adopts a complementary approach by experimentally investigating fungivorous protists isolated from the rhizosphere of *Arabidopsis thaliana*, providing direct evidence for consumer–fungus interactions that cannot be resolved from field-based metabarcoding alone, and thereby providing a mechanistic perspective on protistan consumers as drivers of microbial legacies.

In particular, this thesis addresses the following hypotheses:

H1 – Host filtering and pathogen dilution: Plant species act as environmental filters for the assembly of soil-borne protistan plant pathogen communities, leading to host-associated community structure. With increasing plant species richness, pathogen communities experience a dilution effect due to reduced availability of compatible hosts, resulting in lower dominance/higher evenness of pathogenic taxa at high plant species richness.

H2 – Stress-mediated weakening of plant defense: Environmental stressors such as repeated summer droughts reduce plant defense against soil-borne pathogens, increasing susceptibility to infection, and thereby amplify the accumulation of specific pathogen propagules, particularly in low-diversity plant communities where disease pressure is highest.

H3 – Legacy accumulation and persistence: Plant-driven soil microbial legacies strengthen over time and are most pronounced in long-established plant communities, and they remain in place after plants are exchanged.

H4 – Eco-evolutionary adaptations to soil-borne pathogens: Plant communities with a shared history are exposed to sustained disease pressure from accumulated soil-borne protistan pathogens, promoting eco-evolutionary adaptation toward increased resistance and resulting in lower pathogen abundance and altered pathogen community composition compared to naïve plant communities.

H5 – Protistan consumers and microbiome complexity: The diversity and composition of cercozoan consumers also respond to plant species richness, and likewise form soil legacies, but these effects are expected to be weaker than for protistan plant pathogens due to a comparatively smaller capacity to form long-term resting stages. Patterns in consumers are linked to those of fungi and bacteria, as they constitute potential prey.

Results

Chapter Overview and Summary

Chapter 1:

Solbach MD, Albracht C, Dumack K, Eisenhauer N, Fiore-Donno AM, Heck N, Vogel A, Wagg C, Bonkowski M. 2025. Legacies of consecutive summer droughts on soil-borne plant parasitic protists (Oomycota: Stramenopila and Phytomyxea: Rhizaria) and protistan consumers (Cercozoa: Rhizaria) along an experimental plant diversity gradient. *New Phytologist* **Early view**. https://doi.org/10.1111/nph.70756

This chapter investigates the long-term legacy effects of consecutive summer droughts on soil-borne protistan communities along the plant diversity gradient of the Jena Experiment, focusing on parasitic Oomycota and Phytomyxea as well as cercozoan consumers. Using environmental sequencing with taxon-specific primers, this study demonstrates that repeated droughts as well as plant diversity can induce lasting shifts in protist community composition. We found an increased number of plant species-specific parasite taxa after repeated droughts, indicating decreased plant defense. By explicitly linking drought as an environmental stressor, plant diversity, and soil-borne protistan parasites, this chapter provides mechanistic insight into how abiotic stress can generate lasting soil legacies that potentially undermine ecosystem stability and functioning under future climate scenarios.

Chapter 2:

Solbach MD, Hennecke J, Chen Y, Wagner K, Albracht C, Bassi L, Eisenhauer N, Fox A, Heintz-Buschart A, Lange M, Mommer L, Pinheiro Alves de Souza Y, Rai A, Weigelt A, Bonkowski M. (n.d.) Plant-driven soil microbial legacies strengthen over time and include widespread suppression. *Unsubmitted manuscript.*

This chapter examines how plant and soil history shape soil microbial communities, including fungi, Oomycota, Cercozoa, and bacteria, along the plant diversity gradient

of the Jena Experiment. By comparing bulk soil collected from plots differing in plant and soil history, this study investigates the strength and temporal development of soil legacy effects by quantifying the effect strength of plant species and plant diversity on microbial communities. We found that microbial legacies were substantially stronger after 19 years of shared plant and soil history than after only 5 years, and were largely independent of plant history. Increasing plant diversity reduced dominance/increased evenness within several microbial groups, potentially indicating dilution effects. Importantly, the reduction of specific taxa by plant species and plant diversity was frequently stronger than enrichment, suggesting suppression as an underappreciated mechanism of long-term soil legacy formation.

Chapter 3:

Madaj A-M, Huang Y, Ebeling A, Ertel L, Gebler A, Gleixner G, Hines J, Roscher C, Weigelt A, Albracht C, Amyntas A, Bassi L, Bonato Asato AE, Bonkowski M, Bröcher M, Buscot F, De Giorgi F, Pinheiro Alves de Souza Y, Doan VC, Durka W, Heintz-Buschart A, Hennecke J, Lange M, Medina-van Berkum P, Meyer ST, Krawczyk S, Rai A, Reitz T, Ristok C, Scheu S, Schloter M, Schulz S, Solbach MD, Unsicker SB, Eisenhauer N. 2025. JenaTron - An Experimental Approach to Study the Effects of Plant History and Soil History on Grassland Ecosystem Functioning. *Journal of Visualized Experiments* **217**: e67496. https://doi.org/10.3791/67496

This chapter presents the design and methodology of the JenaTron Experiment, which investigates the combined effects of plant and soil history on grassland ecosystem functioning using soil from the Jena Experiment field site, either conditioned by plant communities of a previous experiment (Trait-Based Experiment), or originating from bare ground plots, and seeds either originating from plants grown in the Trait-Based Experiment, or from a commercial supplier. By including all four treatment combinations of plant and soil history in a fully crossed design with a controlled plant diversity gradient (1, 2, 3, and 6 plant species), the study allows for precise testing of soil legacy formation and the influence of plant diversity on microbial community composition. This methodological framework complements the field-based ΔBEF Experiment (Chapter 2) and will help to provide further experimental evidence directly

relevant to understanding the eco-evolutionary dynamics of protist plant pathogens and consumers in shaping biodiversity–ecosystem functioning relationships.

Chapter 4:

Estermann AH, Teixeira Pereira Bassiaridis J, Loos A, Solbach MD, Bonkowski M, Hess S, Dumack K. 2023. Fungivorous protists in the rhizosphere of *Arabidopsis thaliana* – Diversity, functions, and publicly available cultures for experimental exploration. *Soil Biology and Biochemistry* **187**: 109206. https://doi.org/10.1016/j.soilbio.2023.109206

This work broadens the scope of the dissertation by examining fungivorous protists within Cercozoa (order Vampyrellida) and Amoebozoa (order Arcellinida) in the rhizosphere of *Arabidopsis thaliana*. While this thesis focuses primarily on parasites in Cercozoa and Oomycota, these two groups represent only a fraction of the true protist diversity present in soil and involved in plant–soil interactions. By characterizing the feeding behavior and functional roles of additional protistan consumers in the rhizosphere, this work provides insight into trophic processes, nutrient cycling, and potential biocontrol mechanisms that could contribute to plant–microbe community dynamics and the shaping of long-term legacies.

Chapter 1: Legacies of Consecutive Summer Droughts on Soil-Borne Plant Parasitic Protists (Oomycota: Stramenopila and Phytomyxea: Rhizaria) and Protistan Consumers (Cercozoa: Rhizaria) Along an Experimental Plant Diversity Gradient

Publication:

Solbach MD, Albracht C, Dumack K, Eisenhauer N, Fiore-Donno AM, Heck N, Vogel A, Wagg C, Bonkowski M. 2025. Legacies of consecutive summer droughts on soil-borne plant parasitic protists (Oomycota: Stramenopila and Phytomyxea: Rhizaria) and protistan consumers (Cercozoa: Rhizaria) along an experimental plant diversity gradient. *New Phytologist* **Early view**. https://doi.org/10.1111/nph.70756

For high-resolution images and additional supplementary material (Datasets S1-S3), readers are referred to the online version of the publication.

Declaration of personal contributions:

I conducted the laboratory work for the sequencing together with N Heck. I processed the Cercozoa sequencing data together with N Heck and K Dumack, and the Oomycota sequencing data together with AM Fiore-Donno. I performed all statistical analyses and visualized the results. I wrote the initial manuscript, with revisions by M Bonkowski and contributions by all other co-authors. I handled the submission and the review process of the article.

New Phytologist

Research

Legacies of consecutive summer droughts on soil-borne plant parasitic protists (Oomycota: Stramenopila and Phytomyxea: Rhizaria) and protistan consumers (Cercozoa: Rhizaria) along an experimental plant diversity gradient

Marcel Dominik Solbach[1], Cynthia Albracht[2,3], Kenneth Dumack[1,4], Nico Eisenhauer[5,6], Anna Maria Fiore-Donno[1], Nils Heck[1], Anja Vogel[5,6], Cameron Wagg[7,8] and Michael Bonkowski[1]

[1]Terrestrial Ecology, Institute of Zoology, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, Cologne, 50674, Germany; [2]Department of Soil Ecology, Helmholtz-Centre for Environmental Research – UFZ Halle, 06120, Halle, Germany; [3]Institute for Biosafety in Plant Biotechnology, Julius Kühn-Institute, 06484, Quedlinburg, Germany; [4]Aquatic Ecosystem Analyses, Institute for Integrated Natural Sciences, University Koblenz, Koblenz, 56070, Germany; [5]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, 04103, Leipzig, Germany; [6]Institute of Biology, Leipzig University, 04103, Leipzig, Germany; [7]Department of Geography, Remote Sensing Laboratories, University of Zürich, CH-8057, Zürich, Switzerland; [8]Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, Fredericton, E3B 4Z7, NB, Canada

Authors for correspondence:
Marcel Dominik Solbach
Email: marcel.solbach@uni-koeln.de

Michael Bonkowski
Email: m.bonkowski@uni-koeln.de

Received: *27 September 2025*
Accepted: *29 October 2025*

New Phytologist (2025)
doi: 10.1111/nph.70756

Key words: biodiversity loss, biodiversity–ecosystem functioning relationships, Cercozoa, climate change, Jena Experiment, Oomycota, plant parasites, soil biodiversity.

Summary

- Increasing frequencies of severe summer droughts and plant diversity loss disrupt ecosystem functioning and stability of European grasslands. Understanding how these factors interact with pathogens is crucial.
- We investigated the effects of plant diversity and repeated summer drought on soil-borne parasites within a grassland biodiversity experiment. The experiment included plant communities ranging from 1 to 60 species, with a sub-experiment simulating annual droughts for 6 wk in summer over 9 yr. One year after the final drought period, we analyzed the diversity and community composition of two parasitic protistan taxa with many plant-pathogenic members, Oomycota (Stramenopila) and Phytomyxea (Rhizaria), as well as protistan consumers in the Cercozoa (Rhizaria) using amplicon sequencing.
- Both Oomycota and Cercozoa, including Phytomyxea, responded to plant species richness and drought, but not uniformly. Plant species-specific Oomycota were enriched under drought, while Phytomyxea and cercozoan consumers exhibited shifts in both enriched and reduced operational taxonomic units. No mitigating effect of plant diversity against the effects of drought was observed.
- Our findings suggest that repeated summer droughts weaken plant defense against protistan plant parasites, causing long-lasting soil legacy effects across plant communities varying in diversity and community composition, potentially threatening ecosystem stability and functioning under future climate conditions.

Introduction

One of the key consequences of global climate change is the increasing frequency and intensity of extreme weather events, such as prolonged summer droughts (Spinoni *et al.*, 2017; Cook *et al.*, 2018). In addition, the probability of droughts occurring in consecutive years has increased up to sevenfold in Central Europe (Hari *et al.*, 2020). Drought stress is likely to impair plant defenses against biotic stressors, such as pathogens, with increasingly negative feedback effects on plant productivity (Ramegowda & Senthil-Kumar, 2015). Plants usually allocate their limited resources to different traits, resulting in trade-offs, for example, between growth and defense (Cappelli *et al.*, 2020; Monson *et al.*, 2022). During drought, plants may shift resource allocation even further away from defense toward enhancing protection from drought stress, potentially increasing susceptibility to diseases (Haugen *et al.*, 2007; Siemens *et al.*, 2012; Choudhary & Senthil-Kumar, 2024).

Plant diversity loss may facilitate the spread of diseases and thus further exacerbate biotic stress. Most plant pathogens show high host specificity (Gilbert, 2002; Cortois *et al.*, 2016; Francioli *et al.*, 2020). In species-poor communities, the transmission of species-specific plant pathogens is favored by the higher density of susceptible hosts, leading to increased disease pressure (Burdon & Chilvers, 1975), whereas susceptibility declines at higher plant diversity where potential hosts are increasingly

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

diluted (Maron *et al.*, 2011; Ampt *et al.*, 2022; Wang *et al.*, 2023). Soil-borne plant antagonists are therefore increasingly regarded as important drivers of the plant diversity–productivity relationship (Eisenhauer *et al.*, 2012; Kulmatiski *et al.*, 2012; Meyer *et al.*, 2016; Guerrero-Ramírez *et al.*, 2017; Wan *et al.*, 2023; Wang *et al.*, 2023), and may even further reduce plant productivity in concert with drought (Liu *et al.*, 2016; Francioli *et al.*, 2020; Xi *et al.*, 2022).

Some of the most impactful plant pathogens belong to the protistan phyla Oomycota and Cercozoa (Thines, 2014; Derevnina *et al.*, 2016; Schwelm *et al.*, 2018). Both groups exhibit a high diversity in German grasslands (Fiore-Donno *et al.*, 2019, 2020; Oliverio *et al.*, 2020; Fiore-Donno & Bonkowski, 2021). Oomycota are stramenopile protists that have independently evolved fungus-like hyphal structures and live as saprotrophs or parasites on various eukaryotic hosts, including animals, fungi, and diatoms. The majority of described species (*c.* 60%), however, are plant parasites (Thines & Kamoun, 2010). The group includes several marine basal lineages and two major classes: Saprolegniomycetes (primarily aquatic saprotrophs and animal parasites) and Peronosporomycetes (dominating terrestrial ecosystems) (Beakes & Thines, 2016; Thines & Kamoun, 2010). Within Peronosporomycetes, plant associations span diverse lifestyles. *Pythium sensu lato* species (Uzuhashi *et al.*, 2010) are typically necrotrophs and opportunistic pathogens with broad host ranges, though species-specific preferences occur (van der Plaats-Niterink, 1981; Mitchell & Deacon, 1986; Augspurger & Wilkinson, 2007). *Phytophthora* exhibits hemibiotrophy, beginning with biotrophic infection followed by necrotrophy on killed tissue. Downy mildews (*Peronospora*, *Plasmopara*) and white blister rusts (*Albugo*) are obligate biotrophs with narrow host ranges, fully dependent on living plant tissue (Thines & Choi, 2016; Ah-Fong *et al.*, 2019; Pandaranayaka *et al.*, 2019). Plant host specificity in Peronosporomycetes thus ranges from generalist opportunists to highly specialized biotrophs, appearing to have evolved gradually (Thines & Kamoun, 2010).

Plant pathogens within Cercozoa belong to the class Phytomyxea, which are obligate biotrophic parasites comprising two groups: Phagomyxida primarily parasitizing marine stramenopiles like brown algae and diatoms, and Plasmodiophorida mostly infecting land plants (Neuhauser *et al.*, 2014; Hittorf *et al.*, 2020). Economically important examples of pathogenic Plasmodiophorida are *Plasmodiophora brassicae*, the causal agent of clubroot disease in cabbage (Brassicaceae); *Spongospora* spp. causing powdery scab in potato (Solanaceae); and *Polymyxa* spp. as transmitters of viruses in beets, cereals, and grasses (Neuhauser *et al.*, 2010; Dixon, 2014; Garvetto *et al.*, 2023). The remaining Cercozoa (i.e. the core Cercozoa/"Filosa" and Vampyrellida) are dominant consumers in soil food webs (Fiore-Donno *et al.*, 2019) and likewise establish plant species-specific protistan rhizosphere microbiomes (Rossmann *et al.*, 2020; Bonkowski *et al.*, 2021; Rüger *et al.*, 2021; Dumack *et al.*, 2022). These consumers were shown to promote defense mechanisms and/or to suppress some pathogens in rhizosphere communities (Jousset, 2012; Xiong *et al.*, 2020), thereby enhancing the biocontrol potential of bacterial rhizosphere microbiomes against pathogen infections (Li *et al.*, 2021).

Oomycota and Phytomyxea form robust resting spores and cysts (Turkensteen *et al.*, 2008; Bulman & Neuhauser, 2017) that generate negative soil feedbacks on plants through the accrual of their persistent propagules (Petermann *et al.*, 2008; Hannula *et al.*, 2021). On the other hand, drought may impair the spread of Oomycota and Phytomyxea, as pathogen dispersal of these protists in soil generally requires a swimming flagellate stage (zoospore) (van West *et al.*, 2003; Neuhauser *et al.*, 2014; Amponsah *et al.*, 2021; Kasteel *et al.*, 2023). While few studies directly examine drought impacts on Phytomyxea, available research suggests their resting stages can not only survive drought conditions but may actually show enhanced viability in dry vs moist soils (MacFarlane, 1952; Takahashi, 1994). Conversely, reduced soil moisture can inhibit *Plasmodiophora brassicae* infection even when resting spore densities remain high (Wallenhammar *et al.*, 2021). Similarly, the impact of drought on oomycete activity appears context-dependent, showing variable outcomes depending on the specific pathogen and plant host involved (Markell *et al.*, 2008; Del Castillo Múnera *et al.*, 2019; González *et al.*, 2020; Neupane *et al.*, 2022). However, all these studies focused on the interaction between a single plant species and a single pathogen in controlled experimental settings, with no comprehensive community-wide studies available so far.

To address this research gap, we investigated the long-term effects of repeated summer droughts in combination with plant diversity loss and their potential interaction effects on Oomycota and Cercozoa (including Phytomyxea) communities. To this end, a sub-experiment was established on the plots of the Jena Experiment in 2008 that simulated regular summer drought periods over nine consecutive years (Vogel *et al.*, 2012, 2013). One year after the Drought Experiment had ended, soil samples were collected and analyzed by Illumina amplicon sequencing to test drought legacy effects along the plant diversity gradient.

We hypothesize that: (1) the dominance of host-specific parasites is highest in monocultures and gradually declines with increasing plant species richness (dilution effect), leading to a log-linear increase in evenness-sensitive alpha diversity indices such as inverse Simpson index with increasing plant species richness, and an associated shift in beta diversity with plant species richness; (2) drought increases the susceptibility of plants to parasites, resulting in increased dominance of specific operational taxonomic units (OTUs), especially at low plant species richness due to a greater proliferation of host-specific parasites in species-poor plant communities; (3) the higher proliferation of specific parasites on drought-stressed plants leads to an accrual of host-specific propagules, measurable as significant log-fold changes when comparing drought vs control treatments.

Materials and Methods

Study site and experimental setup

The study was conducted in the 'Jena Experiment', a grassland biodiversity experiment composed of a species pool of 60

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

New Phytologist

Research 3

plant species representative of European mesophilic grasslands (Roscher *et al.*, 2004; Weisser *et al.*, 2017; for a full list of species, see Supporting Information Table S1). The main experiment consists of 80 communities with 1–60 plant species (1, 2, 4, 8, 16, or 60 species; Table S2) that have been maintained by weeding and mowing since their establishment in 2002. Each plant species belongs to one of four plant functional groups (grass, legume, small herb, or tall herb). The plots are arranged in four blocks (Blocks 1–4) with increasing distance from the river Saale to account for potential spatial differences in soil properties (Roscher *et al.*, 2004). The full size of the main Jena Experiment plots was originally 20 m × 20 m, but the plot size was reduced to 6 m × 5.5 m in 2010 for easier maintenance. The Drought Experiment was originally nested within the core area of the main plots but was located outside of the core area after plot size reduction (Weisser *et al.*, 2017).

For the Drought Experiment, part of each plot was split into two subplots (drought vs control), and transparent roofs were installed for 6 wk in summer (mid-July until the end of August) in subsequent years from 2008 to 2016. The roof constructions consisted of a wooden frame (3 m × 2.5 m) with corrugated polyvinyl chloride sheets on each plot. All measurements and samplings were conducted in a 1 m × 1 m square, at least 0.5 m away from the edges, effectively resulting in a size of 1 m^2 per subplot. During the roofing period, the control subplot was watered after each rain event with the amount of rainwater withheld by the roof constructions, while the drought subplot was not watered (Fig. S1, see also Vogel *et al.*, 2012, Vogel *et al.*, 2013). Roofed controls were used to account for potential artifacts (e.g. microclimatic effects) of the roof constructions (Vogel *et al.*, 2013). Summer precipitation in the drought treatment was reduced by *c.* 42% during the experiment (Wagg *et al.*, 2017).

Sampling, DNA extraction, and sequencing

The experimental plots were sampled for soil microbial analyses in August 2017, 1 yr after the Drought Experiment had been terminated. Three to five soil cores (0–15 cm soil depth) of bulk soil were sampled from random positions within the central 1 m × 1 m square on each of the 160 subplots (80 plots × 2 treatments) to account for spatial variation, and were pooled to obtain *c.* 50 g of soil per subplot. Plant material was removed, the soil homogenized by sieving (2 mm), and subsamples were stored at −80°C. Subsequently, DNA was extracted from 0.4 g of each sample using the DNeasy PowerSoil Kit (Qiagen, Venlo, Netherlands) and stored at −20°C until use.

Barcoding regions of Oomycota and Cercozoa were amplified using taxon-specific semi-nested PCR approaches to increase specificity and reduce amplification of nontarget taxa, following the protocols from Fiore-Donno & Bonkowski (2021) and Fiore-Donno *et al.* (2020). Oomycete community composition was assessed by sequencing the ITS1 region using the primers S1777F and 58SOomR, and subsequently barcoded primers S1786StraF and 58SOomR (Fiore-Donno & Bonkowski, 2021), resulting in a fragment of *c.* 250 bp in length. Cercozoan community composition was analyzed by sequencing the hypervariable V4 region of the 18S ribosomal RNA gene (SSU rDNA) with the primers S615F_Cerco and S615F_Phyt (1 : 1 mixture) and S963R_Phyt, and subsequently barcoded primers S615F_Cer and S947R_Cer (Fiore-Donno *et al.*, 2020), resulting in a fragment of *c.* 350 bp in length. The first PCR step was conducted with 1 µl of extracted DNA as the template and 10 µl of PCR master mix containing 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), 1 µl Thermo Scientific DreamTaq Green Buffer, 0.2 µl dNTPs (10 mM), 0.1 µl Thermo Scientific DreamTaq Polymerase, and 6.7 µl nuclease-free water. The second, semi-nested PCR was conducted with 1 µl of the first PCR as the template, and 17 µl of master mix containing 1.7 µl of barcoded forward primer, 1.7 µl of barcoded reverse primer (dual-indexing; for the sample list and their respective barcode combinations, see Dataset S1), 1.7 µl Thermo Scientific DreamTaq Green Buffer, 0.34 µl dNTPs (10 mM), 0.17 µl Thermo Scientific DreamTaq Polymerase, and 11.4 µl nuclease-free water. The following PCR conditions were used: initial denaturation at 95°C for 2 min, 24 cycles (denaturation at 95°C for 30 s, annealing at 58°C (Oomycota)/52°C (Cercozoa) for 30 s, elongation at 72°C for 30 s), terminal extension at 72°C for 5 min, and cooling at 15°C. Two negative controls (containing nuclease-free water instead of DNA extract) were added per PCR batch. They received random combinations of barcoded primers during the second PCR round. PCR success was verified by gel electrophoresis. The PCR batches were discarded if there was contamination in the negative controls. PCRs were repeated until two successfully amplified PCR products were obtained per sample.

Both duplicates of the second PCR (barcoded) were pooled (12.5 µl each), and then purified and normalized using the SequalPrep Normalization Plate Kit (Invitrogen, Thermo Fisher Scientific, USA) to obtain a concentration of 1–2 ng μl^{-1} per sample. The purified PCR products of each Oomycota sample and the PCR products of each Cercozoa sample were separately pooled and then sequenced in two separate sequencing runs. Library preparation, including ligation of Illumina adapters, and sequencing with a MiSeq v3 Reagent kit of 600 cycles on a MiSeq Desktop Sequencer (Illumina Inc., San Diego, CA, USA) was performed by the Cologne Center for Genomics (CCG, Cologne, Germany).

Bioinformatic data processing

Illumina adapters were trimmed by the sequencing facility, and sequence reads were provided in FASTQ format. The quality of the raw reads was checked using FASTX-TOOLKIT (v.0.0.13, http://hannonlab.cshl.edu/fastx_toolkit/). Reads were then processed in MOTHUR v.1.45.3 (Schloss *et al.*, 2009) if not stated otherwise. Forward and reverse reads were assembled and demultiplexed according to their barcode sequences (Dataset S1) using the make.contigs command with standard parameters, not allowing for any differences in the barcode or primer sequences, and the barcode and primer sequences were trimmed. Quality filtering parameters were optimized separately for each marker based on assembly reports. For Oomycota (ITS1 region): no

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

mismatches and no ambiguities in the overlap were permitted; merged sequences < 170 bp, or with an overlap < 70 bp, were discarded. For Cercozoa (V4 region): a maximum of one mismatch with no ambiguities in the overlap was allowed; sequences < 300 bp, or with an overlap < 200 bp, were discarded. Contigs were clustered into OTUs by abundance-based greedy clustering (agc) with a similarity threshold of 97% and standard parameters using VSEARCH v.2.7.1 (Rognes *et al.*, 2016). Sequences were clustered together with sequences from other Jena Experiment studies (unpublished) to optimize comparability. OTUs representing < 0.005% of all reads (globally across 1035 samples of all studies; equivalent to 1525 reads for Oomycota, 1703 reads for Cercozoa) were removed as potential amplification or sequencing artifacts (Bokulich *et al.*, 2013; Nelson *et al.*, 2014). For Oomycota, we constructed a custom ITS1 database based on the reference database from Fiore-Donno & Bonkowski (2021) and supplemented with additional sequences from NCBI GenBank, resulting in *c.* 40 000 sequences (Dataset S2). Oomycota sequences were searched against this custom database using the blastn algorithm with standard parameters in BLAST+ (Camacho *et al.*, 2009) with an *E*-value cutoff of $1e^{-10}$. Cercozoan sequences were searched against the PR^2 database (Guillou *et al.*, 2013) with an *E*-value cutoff of $1e^{-50}$. Only the best BLAST hit was retained for each OTU. OTUs whose top match corresponded to a nontarget taxon (nonoomycete or noncercozoan, respectively), or that failed to return a hit under the specific *E*-value cutoffs, were discarded. Cercozoan OTU sequences were aligned against a curated template alignment of 78 representative cercozoan V4 sequences spanning all major cercozoan clades (Fiore-Donno *et al.*, 2018) using the align.seqs command in MOTHUR with a penalty for opening gaps of −5. Query sequences with a similarity of < 50% to the template sequences were discarded. Sequences that started after the position that 90% of the sequences do, ended before the position that 90% of the sequences do, or had gaps of more than five nucleotides, were also removed. Reference-based chimera detection was performed using UCHIME (Edgar *et al.*, 2011) as implemented in MOTHUR, with the same 78 representative cercozoan sequences from the template alignment (Fiore-Donno *et al.*, 2018) serving as the reference database. Because no suitable reference alignment exists for oomycete ITS1 sequences, and *de novo* chimera detection with UCHIME frequently produced false positives and negatives, we instead discarded sequences with query coverage < 70% based on BLAST alignment results (Masigol *et al.*, 2025).

The taxonomic assignment of both OTU databases was manually checked. The Oomycota OTU database required manual curation due to recent taxonomic revisions of *Pythium*, which has been divided into several new genera (Uzuhashi *et al.*, 2010) that are not yet fully implemented into the NCBI database. Functional traits were assigned to OTUs at the genus level based on the trait tables from Fiore-Donno & Bonkowski (2021) and Dumack *et al.* (2020). These resources compile literature-based functional classifications for oomycete and cercozoan genera, including trophic strategies (e.g. parasitic, saprotrophic), host associations, and ecological preferences.

Data analysis

All data analyses were conducted in R v.4.2.1 (R Core Team, 2022) and mostly visualized using GGPLOT2 (Wickham, 2016) if not stated otherwise. Sankey diagrams displaying relative abundances at different taxonomic levels were calculated with the RIVERPLOT package (Weiner, 2021) using a custom function from Freudenthal *et al.* (2024). Rarefaction curves were constructed using iNEXT (Chao *et al.*, 2014; Hsieh *et al.*, 2016) to investigate the sequencing depth of the datasets (Fig. S2).

The Oomycota dataset was split into *Pythium sensu stricto* (in the following referred to as '*Pythium*') and the remaining Oomycota (dominated by the genus *Globisporangium*; in the following referred to as '*Globisporangium*'). The Cercozoa dataset was split into plant-parasitic Plasmodiophorida (class Phytomyxea) and the remaining Cercozoa (microbial consumers). Alpha and beta diversity analyses were conducted individually for the resulting four datasets.

Alpha diversity was quantified from OTU tables using Hill numbers (effective number of species) with the extrapolation methods from iNEXT (Chao *et al.*, 2014; Hsieh *et al.*, 2016): OTU richness (Hill q = 0) was estimated using iNEXT::ChaoRichness, exponential Shannon index (Hill q = 1) with iNEXT::ChaoShannon, and inverse Simpson index (Hill q = 2) with iNEXT::ChaoSimpson. Additionally, Pielou's evenness was calculated as Shannon entropy divided by log(OTU richness). For each of these diversity metrics, we analyzed the interactive effect of plant species richness, plant functional groups, and drought treatment using a set of linear mixed models (NLME::lme) (Pinheiro & Bates, 2000; Pinheiro *et al.*, 2022). Plant species richness (as sown diversity, linear, log-transformed), presence/absence of functional groups (grasses, legumes, small herbs, tall herbs), drought treatment (drought vs control), and their interactions entered the model as explanatory variables, while 'block' (experimental block on the field site) and 'plot' were added as random terms to account for differences in edaphic variation of the field site, and the nested split-plot design. The models were designed to reflect the experimental split-plot design (Jones & Nachtsheim, 2009): Whole-plot factors were fitted first and were tested against the whole-plot error, and the split-plot factor (drought treatment) was fitted after, followed by the interactions, and tested against the residuals (split-plot error).

Due to inherent correlations among factors in our study (Fig. S3) resulting from experimental design constraints (e.g. monocultures only containing one plant functional group), we calculated multiple models with varying fitting sequences as recommended by Hector *et al.* (2010) and general biodiversity experiment design principles (Schmid *et al.*, 2017). These models were analyzed using ANOVA with type I SS (sequential sums of squares), and the results were summarized. We also analyzed the models using ANOVA with type III SS (marginal sums of squares), but this approach can be misleading in unbalanced designs and has been widely criticized (Milliken & Johnson, 1984; Hector *et al.*, 2010; LaMotte, 2019). Therefore, we do not discuss these results; detailed ANOVA tables based on type I and type III SS are provided in Dataset S3.

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

New Phytologist

Research 5

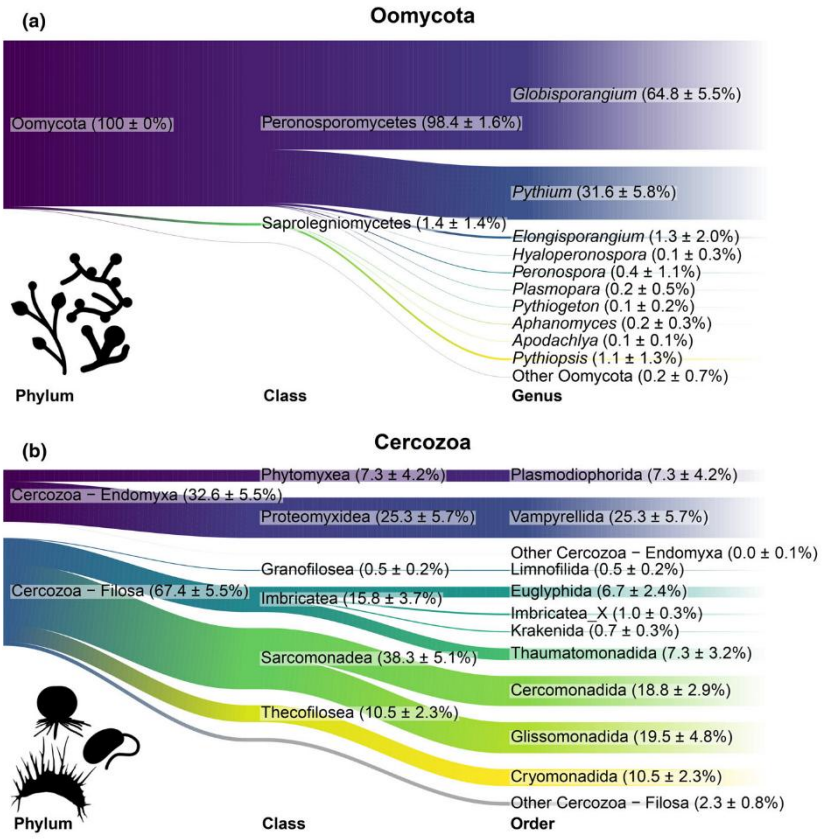


Fig. 1 Sankey diagrams displaying an overview of the relative abundances (based on sequence reads). Only the 10 most abundant genera or orders, respectively, are shown. The remaining taxa are summarized in 'Other'. Numbers in parentheses refer to the average relative abundances ± SD in percent, rounded to one decimal place. (a) Oomycota; (b) Cercozoa.

To analyze differences in community compositions (beta diversity), total abundances of OTUs were converted to relative abundances and log(+1)-transformed. Nonmetric multidimensional scaling (NMDS) was performed based on Bray–Curtis dissimilarities (VEGAN::metaMDS; Oksanen *et al.*, 2022) and tested by permutational multivariate analysis of variance (PERMANOVA, 999 permutations, VEGAN::adonis2). For the explanatory factors that were manipulated on the plot level (plant species richness, presence/absence of plant functional groups), statistical testing was performed using 'Plot' as the error term, and permutations were only allowed between plots. For the drought treatment and its interactions, the residuals were used as the error term, and permutations were only allowed within plots (Bakker, 2024). The PERMANOVA models were designed in the same way as the linear mixed models. Group dispersion was examined with VEGAN::betadisper. Aboveground plant biomass of the target plant species from August 2017 was obtained from Albracht *et al.* (2023) and fitted to the NMDS plots using VEGAN::envfit.

Differential abundance analysis of the main factors was performed in DESEQ2 (Love *et al.*, 2014) using the poscounts parameter for size factor estimation to account for zeros in the datasets. The number of differentially abundant OTUs (0.05 significance level) was counted for each main factor and summarized. Furthermore, differential abundance analysis was performed for each of the 60 plant species, always comparing all plots with the respective plant species vs all plots without the respective plant species. This analysis was performed independently for drought and control treatments; then the number of differentially expressed OTUs was counted, summarized, and statistical differences between drought and control treatments were determined by Wilcoxon signed-rank tests for paired samples.

Results

Overall community composition

The final OTU databases contained 300 Oomycota OTUs and 912 Cercozoa OTUs. Oomycota were by far dominated by the Peronosporomycetes, in particular the necrotrophic and opportunistic genera *Globisporangium* (64.8%) and *Pythium* (31.6%) (Fig. 1a). Conversely, highly host-specific obligate biotrophs like *Plasmopara* and *Peronospora* occurred at extremely low abundances (< 1% of reads), suggesting that propagules of these specialized leaf pathogens were nearly absent from our soil samples. Cercozoan parasites consisted exclusively of Plasmodiophorida (class Phytomyxea), comprising 7.3% of cercozoan reads. The remaining cercozoan community was dominated by microbial consumers: fungivorous and predatory Vampyrellida (naked amoebae, 25.3% of reads), and primarily bacterivorous

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

New Phytologist

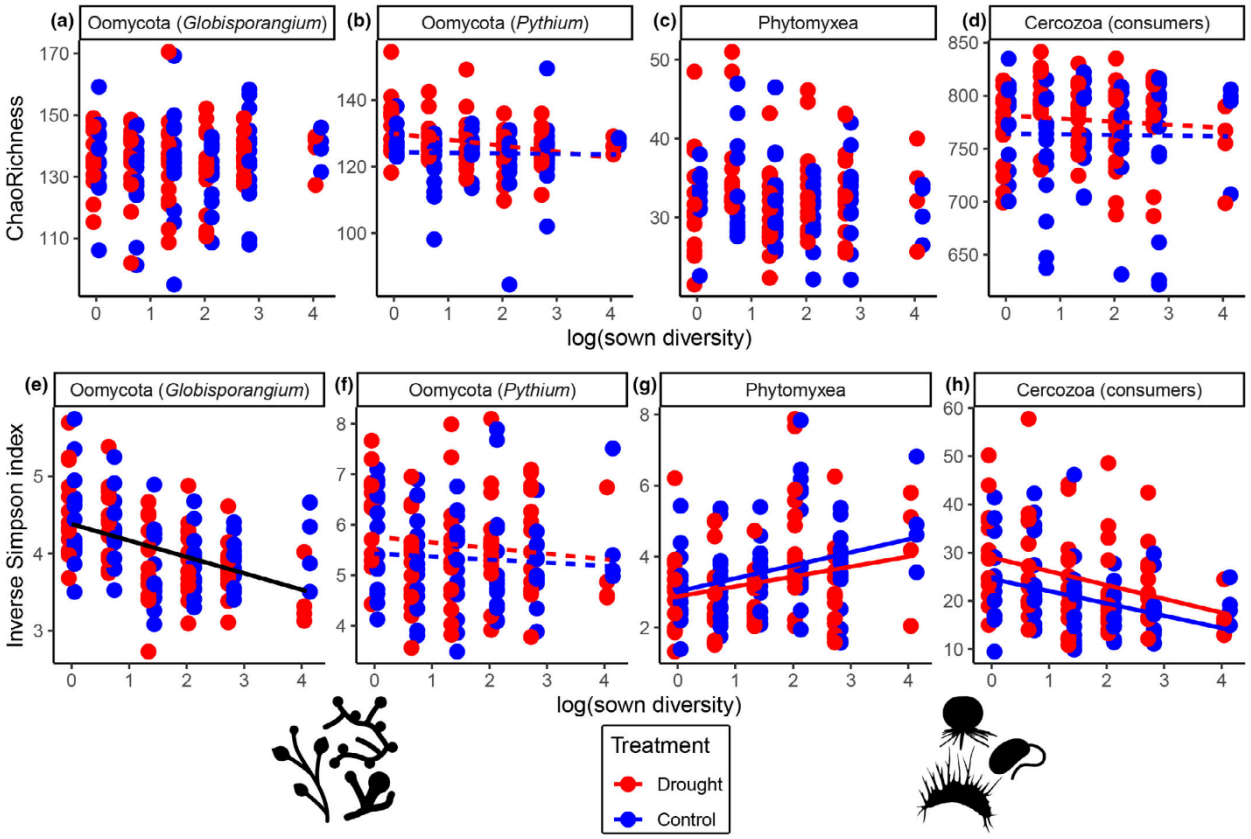


Fig. 2 ChaoRichness (asymptotic OTU richness) and inverse Simpson index as affected by plant species richness (as log(sown diversity)) and drought treatment (drought vs control). Results of the respective statistical analyses are given in Table 1. Solid lines indicate a significant effect and dashed lines indicate a nonsignificant effect of log(sown diversity). Two separate lines indicate a significant effect of the drought treatment. When no lines are displayed, neither the log(sown diversity) nor the drought treatment had a significant effect. (a–d) ChaoRichness; (e–h) inverse Simpson index. (a, e) Oomycota (*Globisporangium*); (b, f) Oomycota (*Pythium*); (c, g) Phytomyxea; (d, h) Cercozoa (consumers).

Cercomonadida and Glissomonadida (flagellates, roughly 19% of reads each) (Fig. 1b).

Alpha diversity

Results of inverse Simpson index (Hill q = 2) were fairly consistent with the results of exponential Shannon index (Hill q = 1, Fig. S4) and Pielou's evenness index (Fig. S5), indicating that the changes in Simpson diversity were mostly caused by changes in the dominance structure/proportions of taxa, that is evenness, rather than OTU richness. The inverse Simpson index quantifies diversity by considering both the number of OTUs (or species) and how evenly sequence reads (or individuals) are distributed among them. Unlike simple richness measures, it gives more weight to the most abundant OTUs, making it particularly sensitive to dominance. High values indicate a more even distribution of reads across OTUs (high evenness, low dominance), whereas low values indicate that a few OTUs dominate the community (low evenness, high dominance). Therefore, we mostly report the results of the inverse Simpson index, but the described patterns are mostly concordant with those of the exponential Shannon index and Pielou's evenness index (Dataset S3).

The inverse Simpson index of *Globisporangium* decreased with increasing plant species richness ($F_{1,71} = 31.7$, $P < 0.001$, Fig. 2e). In all models, plant species richness (as log(sown diversity)) remained significant even if tested after all plant functional groups in the model (Dataset S3), indicating an additional effect of plant species diversity affecting the inverse Simpson index of *Globisporangium* that cannot be explained by the presence of certain plant functional groups alone. Grasses ($F_{1,71} = 4.3$, $P < 0.05$) slightly increased the inverse Simpson index, while the presence of small herbs ($F_{1,71} = 12.6$, $P < 0.001$) and tall herbs ($F_{1,71} = 7.6$, $P < 0.01$) decreased the inverse Simpson index of *Globisporangium*. Drought further decreased the inverse Simpson index of *Globisporangium* in plots with legumes (Legume : Drought interaction, $F_{1,73} = 7.0$, $P < 0.01$).

In contrast to our hypothesis, the inverse Simpson index of *Pythium* was not influenced by plant diversity. Only the presence of small herbs caused a significantly decreased inverse Simpson index in *Pythium* ($F_{1,71} = 22.8$, $P < 0.001$, Table 1), indicating

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

New Phytologist

Table 1 Summary table of linear mixed models for the alpha diversity measures OTU richness and inverse Simpson index.

			Oomycota (*Globisporangium*)			Oomycota (*Pythium*)			Phytomyxea			Cercozoa (consumers)		
	numDF	denDF	*F*-value	*P*-value		*F*-value	*P*-value		*F*-value	*P*-value		*F*-value	*P*-value	
OTU richness (ChaoRichness, Hill q = 0)														
log(sown diversity)	1	71	0.134	0.715		3.3	0.0737		0.211	0.6476		0.355	0.5532	
Grass	1	71	0.439	0.5099		0	0.9517		0.672	0.4151		0.005	0.944	
Legume	1	71	0.818	0.3689		0.57	0.4536		0.717	0.4001		0.034	0.8549	
Small Herb	1	71	0.247	0.6204		0	0.9694		1.512	0.223		3.102	0.0825	
Tall Herb	1	71	0.347	0.5577		0.08	0.7812		1.692	0.1976		0.093	0.7618	
Drought	1	73	0.022	0.8817		6.52	**0.0127***	↑	0.841	0.3622		4.078	**0.0471***	↑
log(sown div.) : Drought	1	73	0.023	0.8793		2.2	0.1423		0.752	0.3886		0.139	0.7107	
Grass : Drought	1	73	2.321	0.132		0.31	0.5823		0.146	0.7031		0.38	0.5396	
Legume : Drought	1	73	0.118	0.732		0.2	0.6572		0.276	0.6011		6.611	**0.0122***	↓
Small Herb : Drought	1	73	0.03	0.8635		2.47	0.1205		0.214	0.6449		0.548	0.4614	
Tall Herb : Drought	1	73	0.497	0.483		4.92	**0.0297***	↓	0.138	0.7116		1.104	0.2968	
Inverse Simpson index (effective number of dominant OTUs, Hill q = 2)														
log(sown diversity)	1	71	31.6718	**<0.0001*****	↓	1.4808	0.2277		9.75334	**0.0026****	↑	18.71352	**<0.0001*****	↓
Grass	1	71	4.2854	**0.0421***	↑	4.9042	**0.03***	↑	3.44862	0.0675		14.53729	**0.0003*****	↓
Legume	1	71	0.0923	0.7622		0.0361	0.8499		1.42093	0.2372		9.35908	**0.0031****	↑
Small Herb	1	71	12.6249	**0.0007*****	↓	22.7929	**<0.0001*****	↓	6.31591	**0.0142***	↑	7.7552	**0.0069****	↓
Tall Herb	1	71	7.5843	**0.0075****	↓	3.5568	0.0634		0.20859	0.6493		7.57251	**0.0075****	↓
Drought	1	73	0.0283	0.8668		5.3913	**0.023***	↑	6.23751	**0.0148***	↑	16.55771	**0.0001*****	↑
log(sown div.) : Drought	1	73	3.6937	0.0585		0.2542	0.6157		0.85565	0.358		0.21276	0.646	
Grass : Drought	1	73	1.2405	0.269		3.3268	0.0723		5.59496	**0.0207***	↓	0.29131	0.591	
Legume : Drought	1	73	7.0479	**0.0097****	↓	0.1467	0.7028		0.41891	0.5195		0.09094	0.7638	
Small Herb : Drought	1	73	0.1008	0.7518		1.7463	0.1905		6.38289	**0.0137***	↓	0.04871	0.8259	
Tall Herb : Drought	1	73	1.1537	0.2863		0.1393	0.7101		0.28788	0.5932		2.16668	0.1453	

log(sown diversity): plant species richness as sown diversity, log-transformed, linear; Grass, Legume, Small Herb, Tall Herb: presence/absence of the respective functional group; Drought: drought vs control treatment. Arrows indicate the direction of the significant effects based on the model coefficients (↑ for positive effects, ↓ for negative effects). For log(sown diversity) (continuous), arrows indicate whether an increase is associated with an increase or decrease in the respective alpha diversity metric. For the plant functional groups (Grass, Legume, Small Herb, Tall Herb), the direction indicates changes relative to plots without the respective plant functional group (baseline: absence of the respective plant functional group), and for the factor Drought relative to the control treatment (baseline: control). Directions of the interaction terms represent how the effect of one variable changes depending on the level of the interacting factor, using the same baselines. Significance code: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$. Significant *P*-values are highlighted in bold. numDF, numerator degrees of freedom; denDF, denominator degrees of freedom.

that specific OTUs gained dominance, whereas the presence of grasses enhanced the inverse Simpson index ($F_{1,71} = 4.9$, $P < 0.05$). Drought further increased the inverse Simpson index in *Pythium* ($F_{1,73} = 5.4$, $P < 0.05$, Fig. 2f). OTU richness of *Pythium* also increased in the drought treatment ($F_{1,73} = 6.52$, $P < 0.05$, Fig. 2b; Table 1), especially in plots lacking tall herbs (Tall Herb : Drought interaction, $F_{1,73} = 4.9$, $P < 0.05$).

Finally, Phytomyxea responded as predicted with the inverse Simpson index increasing with plant species richness ($F_{1,71} = 9.8$, $P < 0.01$, Fig. 2g), indicating the dominance of certain taxa in monocultures and a gradual mixing with increasing richness of potential plant hosts, leading to enhanced evenness. The overall effect of drought on the inverse Simpson index (Fig. 2g) was driven by grasses and small herbs, where the presence of either functional group under drought caused a strong decline in the inverse Simpson index of Phytomyxea (Grass : Drought interaction, $F_{1,73} = 5.6$, $P < 0.05$; Small Herb : Drought interaction, $F_{1,73} = 6.4$, $P < 0.05$).

The responses of Cercozoa (consumers) along the plant species richness gradient resembled those of *Globisporangium*. The inverse Simpson index of cercozoan consumers decreased with higher plant species richness ($F_{1,71} = 18.7$, $P < 0.001$, Fig. 2h), especially in the presence of grasses, small herbs, and tall herbs, but it increased in the presence of legumes (Table 1). Drought led to an increased inverse Simpson index of cercozoan consumers ($F_{1,73} = 16.6$, $P < 0.001$, Fig. 2h) and slightly enhanced their OTU richness ($F_{1,73} = 4.1$, $P < 0.05$, Fig. 2d), especially in plots without legumes (Legume : Drought interaction, $F_{1,73} = 6.7$, $P < 0.05$).

Beta diversity

The community composition of all investigated protistan groups changed along the plant species richness gradient (Fig. 3; Table 2), while group dispersion did not change significantly (betadisper: ns). Especially the communities of *Globisporangium*

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

New Phytologist

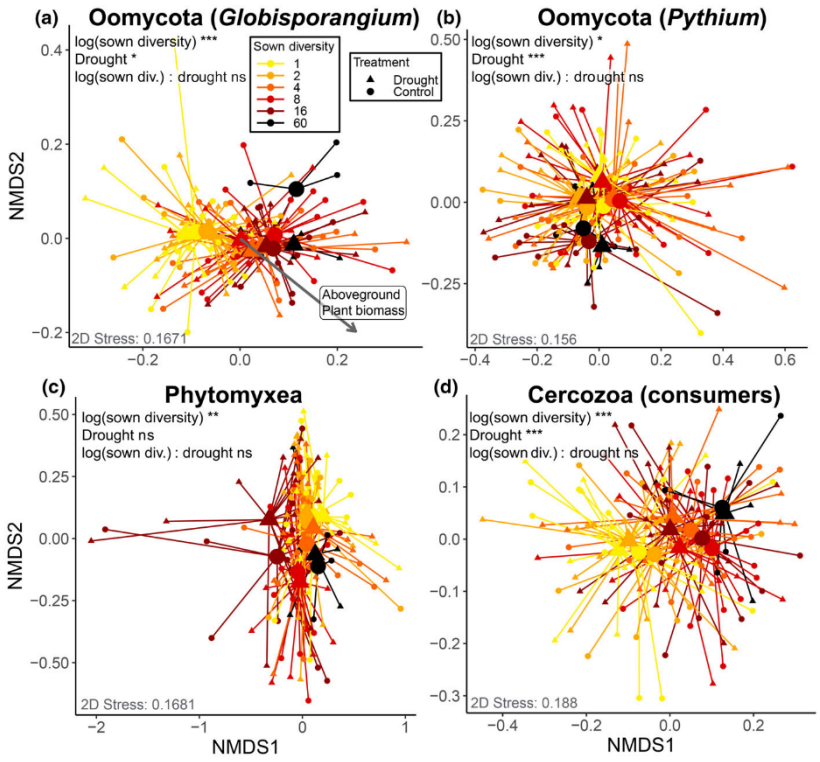


Fig. 3 Nonmetric multidimensional scaling (NMDS) plots based on Bray–Curtis dissimilarities. Displayed is the interactive effect of the drought treatment (symbols) and sown plant diversity (color gradient). Large symbols represent the group centroids of each sown diversity × drought treatment combination, and individual datapoints are connected to their respective group centroids. The vector in (a) represents a significant correlation of aboveground plant biomass with the community composition. Statistical significances were obtained from PERMANOVA. For the full statistical results, see Table 2. Significance code: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, $P > 0.05$ (not significant). (a) Oomycota (*Globisporangium*); (b) Oomycota (*Pythium*); (c) Phytomyxea; (d) Cercozoa (consumers).

Table 2 Treatment effects on differences in community composition (beta diversity) of Oomycota and Cercozoa (PERMANOVA).

			Oomycota (*Globisporangium*)			Oomycota (*Pythium*)			Phytomyxea			Cercozoa (consumers)		
	numDF	denDF	R^2	*F*-value	*P*-value	R^2	*F*-value	*P*-value	R^2	*F*-value	*P*-value	R^2	*F*-value	*P*-value
log(sown diversity)	1	70	0.1112	16.4144	**0.001*****	0.0257	3.6369	**0.007****	0.0314	3.6734	**0.004****	0.0618	9.4579	**0.001*****
Grass	1	70	0.0512	7.5614	**0.001*****	0.0748	10.5884	**0.001*****	0.0093	1.0818	0.395	0.0404	6.1911	**0.001*****
Legume	1	70	0.0144	2.1251	0.07	0.0837	11.8509	**0.001*****	0.0256	2.9896	**0.01****	0.014	2.141	**0.018***
Small Herb	1	70	0.0599	8.8439	**0.001*****	0.0472	6.6835	**0.001*****	0.0218	2.5453	**0.029***	0.0321	4.9189	**0.001*****
Tall Herb	1	70	0.0249	3.6697	**0.01****	0.0065	0.9253	0.427	0.0132	1.5381	0.156	0.0196	2.9964	**0.005****
Drought	1	73	0.0077	2.0104	**0.048***	0.0094	3.4054	**0.001*****	0.004	1.6103	0.159	0.019	5.223	**0.001*****
log(sown div.) : Drought	1	73	0.003	0.7737	0.738	0.0019	0.6907	0.691	0.003	1.1926	0.352	0.0032	0.8706	0.601
Grass : Drought	1	73	0.0065	1.6929	0.093	0.0033	1.1933	0.284	0.0057	2.3002	**0.027***	0.0033	0.9035	0.546
Legume : Drought	1	73	0.0036	0.9323	0.499	0.0019	0.6714	0.733	0.002	0.8153	0.564	0.0039	1.0852	0.309
Small Herb : Drought	1	73	0.0022	0.5806	0.8	0.0025	0.9187	0.497	0.0036	1.4537	0.18	0.0033	0.8963	0.554
Tall Herb : Drought	1	73	0.0038	0.9988	0.456	0.0032	1.1391	0.33	0.0021	0.8395	0.536	0.0044	1.2091	0.221

log(sown diversity): plant species richness as sown diversity, log-transformed, linear; Grass, Legume, Small Herb, Tall Herb: presence/absence of the respective functional group; Drought: drought vs control treatment. Significance code: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$. Significant *P*-values are highlighted in bold. numDF, numerator degrees of freedom; denDF, denominator degrees of freedom.

and cercozoan consumers showed a directional shift along the plant species richness gradient (Fig. 3a,d). Also, plant functional group identity affected the community compositions of all investigated protistan groups (Table 2), but the effects were taxon-specific. Beta diversity of *Pythium* was affected by the presence of grasses, legumes, and small herbs, while the community

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

New Phytologist

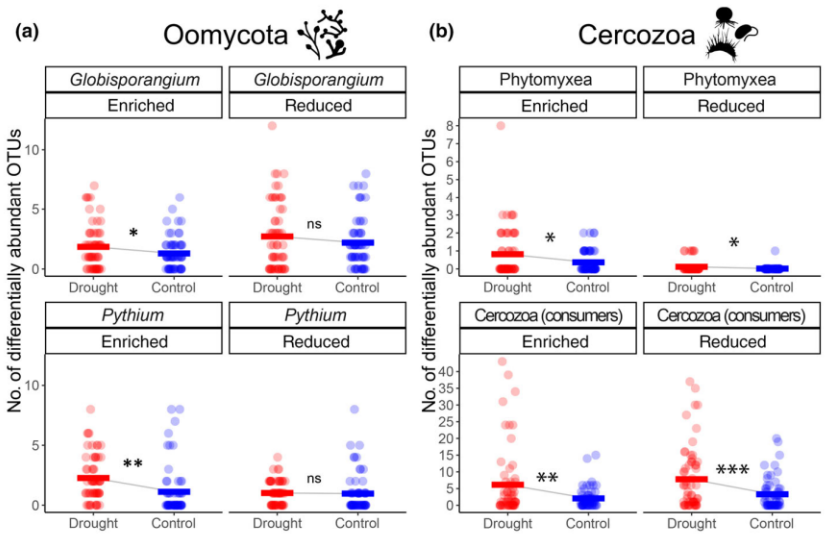


Fig. 4 Summary of differential abundance analysis. The dots represent the number of differentially abundant operational taxonomic units (OTUs) in dependence on the 60 plant species (see Supporting Information Fig. S7), differentiated by drought (red) and control (blue) treatments. Horizontal bars represent group means. 'Enriched' OTUs were more abundant in plots with the respective plant species/less abundant in plots without the respective plant species, and 'reduced' OTUs were less abundant in plots with the respective plant species/more abundant in plots without the respective plant species. Statistical significances were obtained from Wilcoxon signed-rank tests for paired samples. Significance code: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, $P > 0.05$ (not significant). (a) Oomycota; (b) Cercozoa.

composition of *Globisporangium* was influenced by small and tall herbs. Phytomyxea communities differed in the presence of legumes and small herbs, while each of the plant functional groups contributed significantly to the community composition of cercozoan consumers (Table 2). *Globisporangium* was the only investigated group whose beta diversity correlated significantly with aboveground plant biomass (Fig. 3a).

Drought affected the community composition of Oomycota (*Globisporangium* and *Pythium*) and cercozoan consumers. Drought effects on beta diversity of Phytomyxea became only visible in the presence of grasses (Grass : Drought interaction, $F_{1,73} = 2.3$, $P < 0.05$) (Table 2).

Differential abundance analysis

The number of differentially abundant OTUs of the main factors (plant species richness, presence/absence of functional groups, and drought treatment) generally corresponded to the beta diversity analysis results (Fig. S6, compare with Table 2). However, the drought treatment showed a notable exception: only one *Globisporangium* OTU responded to drought, while no *Pythium* or Phytomyxea OTUs showed differential abundance, and cercozoan responses were also minimal. Since all plots contain different plant communities (Table S2), different microbial taxa appeared to respond to the drought treatment depending on the specific plant species present, rather than showing consistent drought responses across all plots. Consequently, we conducted plant species-specific differential abundance analyses (Figs S7, 4).

Analysis of differentially abundant OTUs in dependence on the 60 plant species revealed a significant enrichment of specific OTUs within all parasitic protistan groups under drought legacy compared with the control (Fig. 4a,b). Additionally, Phytomyxea and cercozoan consumers showed a greater number of reduced OTUs in the drought treatment (Fig. 4b).

Discussion

Plant biodiversity as well as 9 yr of consecutive droughts over 6 wk in summer left a significant imprint on soil microbial biodiversity of the Jena Experiment that was clearly discernible even 1 yr after the drought treatment had stopped, among bacteria (de Souza *et al.*, 2024), total and arbuscular-mycorrhizal fungi (Albracht *et al.*, 2023), and also among Oomycota, Phytomyxea, and cercozoan consumers as presented in this study.

However, the different protistan communities did not react in a uniform way to the plant diversity gradient. Shifts in beta diversity of *Globisporangium*, by far the most dominant oomycete genus in the Drought Experiment, were most strongly influenced by plant species richness (Table 2; Fig. 3a). Further, it was the only investigated group whose community composition correlated with aboveground plant biomass (Fig. 3a), indicating that community turnover in this genus was indeed plant host density-dependent. We hypothesized a lower inverse Simpson index (i.e. higher dominance) at low plant diversity, due to a relatively higher richness and abundance of host-specific taxa in the parasite communities, and accordingly a higher inverse Simpson index at higher plant diversity due to dilution of susceptible host plants (Maron *et al.*, 2011; Eisenhauer *et al.*, 2012; Ampt *et al.*, 2022; Wan *et al.*, 2023; Wang *et al.*, 2023). *Globisporangium* exhibited a diametrically opposite pattern with the inverse Simpson index declining from low to high plant diversity, indicating that certain taxa became dominant at higher plant species richness. This suggests a lack of host specificity, consistent with *Globisporangium*'s capacity for rapid adaptation to novel hosts, extensive cryptic speciation, and broad host ranges (Garrido *et al.*, 2022; Eggertson *et al.*, 2023; Zhang *et al.*, 2023). This pattern may also reflect the flexible life cycle of *Globisporangium* and *Pythium*, which alternate between saprophytic soil growth and opportunistic plant tissue colonization (van der Plaats-Niterink, 1981; Lévesque *et al.*, 2010). These genera can switch between parasitic and

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

saprophytic lifestyles, even outcompeting saprophytic fungi in decomposer communities (Kramer *et al.*, 2016). Since soil organic matter content and root exudation increase substantially with increasing plant species richness in the Jena Experiment (Lange *et al.*, 2015, 2023), intensified competition for these resources may have favored the dominance of fewer, competitively superior saprophytic taxa.

Although *Pythium* spp. are also necrotrophs or opportunistic pathogens, their inverse Simpson index did not mirror the pattern seen in *Globisporangium*. This suggests that the genus *Pythium* exhibits a weaker saprophytic behavior than *Globisporangium*. If portions of the *Pythium* community responded to the soil organic matter gradient, while others showed opposite responses to plant species richness, as hypothesized, these divergent strategies could account for the ambiguous, nonsignificant pattern observed. The increased dominance (i.e. significantly decreased inverse Simpson index) among *Pythium* in plots containing small herbs may indicate that these plants serve as nonselective hosts that indiscriminately attract a wide variety of *Pythium*. Grasses, by contrast, enhanced the inverse Simpson index of *Pythium*, potentially reflecting a preferential proliferation of specific OTUs associated with this plant functional group (Mitchell & Deacon, 1986). This pattern aligns with grass characteristics – extensive, high turnover root systems (Roscher *et al.*, 2012) that also attract pathotrophic fungi (Sweeney *et al.*, 2021). The inverse Simpson index of Phytomyxea increased with increasing plant species richness as hypothesized, indicating a decline in certain dominant taxa.

Also, responses to repeated drought were not uniform across parasite groups. We had hypothesized that drought would lead to increased dominance of specific OTUs due to weakened plant defenses, resulting in a decreased inverse Simpson index. However, *Pythium* showed the opposite trend, while the inverse Simpson index of *Globisporangium* remained unaffected. These contrasting patterns may reflect group-specific ecological strategies caused by changes in host or prey availability. The expected pattern occurred only in Phytomyxea, supporting the concept of enhanced proliferation of dominant taxa under plant stress. Since Phytomyxea preferentially colonize the root hairs of their plant hosts (Neuhauser *et al.*, 2014), and root hair water uptake plays a crucial role in stabilizing plant productivity under drought (Marin *et al.*, 2021), parasitism of these structures may significantly impair plant performance during drought stress. This may explain why drought enhanced the dominance of certain Phytomyxea in the presence of small herbs and grasses. Further, root hairs of grasses are frequently colonized by the genus *Polymyxa*, which may cause additional secondary plant damage as a transmitter of plant viruses (Barr, 1979; Littlefield *et al.*, 1998; Kanyuka *et al.*, 2003).

Community compositions of all investigated groups showed weak but detectable reactions to drought. Many parasites exhibit host plant preferences, so different OTUs would be expected to respond to repeated drought depending on the plant species present. Accordingly, we found that drought legacy led to a significant enrichment of plant species-specific OTUs with a significant increase in their log-fold changes based on relative abundances after DESeq2 normalization among all investigated protistan parasite groups (Fig. 4). Apparently, the drought-stressed plants faced a trade-off, with drought stress decreasing their resistance to certain parasites (Seleiman *et al.*, 2021; Lozano *et al.*, 2022), which were then able to multiply more vigorously (Choudhary & Senthil-Kumar, 2024). The thick-walled oospores of Oomycota, as well as resting spores of Phytomyxea, remain viable in soil for many years, with documented survival periods of 2–10 yr depending on the species and environmental conditions (Populer, 1981; Wallenhammar, 1996; Turkensteen *et al.*, 2008; Kanyuka *et al.*, 2003; Bulman & Neuhauser, 2017; Schaap & Schilde, 2018). Their accrual in soil appears to have created long-term soil legacy effects detectable even 1 yr after the last drought manipulation, potentially causing lasting impacts on plant–microbe interactions and plant performance through negative plant–soil feedback effects in subsequent years. It should be noted, however, that not all Oomycota or Phytomyxea are pathogenic in the strict sense of causing visible disease. Nevertheless, even without symptoms, parasites generally impose metabolic costs on plants (Bever, 1994; Newton *et al.*, 2010; Looseley & Newton, 2014). Notably, cercozoan consumers also showed strong plant species-specific reactions to the drought treatment (Fig. 4b). Our results thus indicate a general increase in specificity relationships between plant species and their associated microbiomes in response to repeated droughts.

Plants have been found to modify their root exudation both under drought stress and in the subsequent recovery phase (Williams & de Vries, 2020; Chen *et al.*, 2022), likely contributing to changes in bacterial communities (Canarini *et al.*, 2021; de Souza *et al.*, 2024) and, in turn, their associated consumers, as the diversity of cercozoan consumers tightly correlates with the bacterial (and fungal) microbiomes of plants (Rossmann *et al.*, 2020; Dumack *et al.*, 2022; Degrune *et al.*, 2024; Zhang *et al.*, 2024). For example, in the Drought Experiment, the inverse Simpson index of bacterial taxa strongly decreased with increasing plant species richness in the Drought Experiment, likely because the plant species-specific exudates caused different bacterial taxa to dominate under different plant species, which also led to a clear shift of bacterial beta diversity along the plant species richness gradient (de Souza *et al.*, 2024). Drought generally led to an increased inverse Simpson index of bacterial taxa (de Souza *et al.*, 2024), and all these patterns were exactly mirrored by the cercozoan consumers. It must be noted that the great majority (25%) of the cercozoan consumers belonged to the order Vampyrellida, which are important consumers of other microeukaryotes, fungal spores, and hyphae, as well as predators of nematodes (Weber *et al.*, 1952; Chakraborty *et al.*, 1983; Chakraborty & Warcup, 1984; Hess *et al.*, 2012; Hess & Suthaus, 2022), which indicates a significant biocontrol potential in this group that needs further investigation.

While infection by Oomycota and Phytomyxea likely contributes to the plant biomass decrease observed under drought (Wagg *et al.*, 2017), plant decay induced by pathogens and viruses, followed by decomposition by saprotrophic Oomycota and fungi, also supports nutrient cycling. In parallel, consumption of bacteria and other microeukaryotes by Cercozoa and other protistan consumers facilitates nutrient release via microbial

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

New Phytologist

Research 11

loop mechanisms (Bonkowski, 2004). Together, these processes may participate in the recovery and overcompensation of biomass productivity (de Vries *et al.*, 2012) as observed in the spring following each drought period in the Drought Experiment (Wagg *et al.*, 2017).

Overall, our analyses showed that protistan plant parasites exhibit different strategies in relation to plant diversity and in response to repeated drought, but all investigated taxa showed shifts in community compositions caused by both factors. According to our results, Oomycota and Phytomyxea appear as important drivers of soil legacy effects, potentially contributing to the strengthening of plant diversity–productivity relationships over time (Eisenhauer *et al.*, 2012; Meyer *et al.*, 2016; Dietrich *et al.*, 2021). Consecutive summer droughts led to a significant increase in plant species-specific taxa among all protistan parasites as well as cercozoan consumers. The broad absence of interaction effects of plant species richness with drought indicates that plant species richness itself did not compensate for any drought-induced changes in microbial communities, with drought effects being mostly additive, and not being buffered or reinforced by plant diversity. However, several functional group × drought interactions suggest that plant identity and functional traits may influence drought responses in ways that warrant further investigation.

Acknowledgements

We thank Anne Ebeling, Alexandra Weigelt, and Michael Scherer-Lorenzen for their contribution to the experimental design and setup of the Drought Experiment, Anna Heintz-Buschart and François Buscot for their supervision and funding for the DNA extractions, Bernhard Schmid for his feedback on the statistical analysis, and the staff of the Cologne Center for Genomics (CCG) for their sequencing services. We further thank the technical staff Steffen Eismann, Ute Köber, Gerlinde Kratzsch, Katja Kunze, and Heike Scheffler for their work in maintaining the field site and also many student helpers for weeding the experimental plots. We thank the central coordination team and the data management team, especially Yuanyuan Huang. The Jena Experiment is funded by the Deutsche Forschungsgemeinschaft (DFG, FOR 5000), with additional support by the Friedrich Schiller University Jena, the Max Planck Institute for Biogeochemistry, and the German Centre for Integrative Biodiversity Research (iDiv) Halle–Jena–Leipzig (DFG – FZT 118, 202548816). The current study was funded by the DFG project number 432642583. Open Access funding enabled and organized by Projekt DEAL.

Competing interests

None declared.

Author contributions

MB designed and supervised the study. NE, AV and CW designed the field experiment. AV carried out the soil sampling. CA extracted DNA. MDS and NH performed PCRs and sequencing preparations. MDS, NH and KD processed the Cercozoa sequencing data. MDS and AMF-D processed the Oomycota sequencing data. MDS performed the data analysis and wrote the initial manuscript. All authors contributed to the revision of the manuscript.

ORCID

Cynthia Albracht https://orcid.org/0000-0002-8779-3749
Michael Bonkowski https://orcid.org/0000-0003-2656-1183
Kenneth Dumack https://orcid.org/0000-0001-8798-0483
Nico Eisenhauer https://orcid.org/0000-0002-0371-6720
Anna Maria Fiore-Donno https://orcid.org/0000-0001-6265-1907
Marcel Dominik Solbach https://orcid.org/0000-0001-7073-7428
Cameron Wagg https://orcid.org/0000-0002-9738-6901

Data availability

The raw FASTQ files of the Illumina sequencing runs were submitted to the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home) under the project accession number PRJEB77805. This work is based on data elaborated by Subproject 2 – Protist Plant Pathogens of the Jena Experiment, which is funded by the Deutsche Forschungsgemeinschaft (FOR 5000). The datasets are publicly available in the Jena Experiment database (https://jexis.idiv.de/) under the following dataset IDs: 90 (plot info, doi: https://doi.org/10.25829/XFG6-WD77), 104 (aboveground plant biomass, doi: https://doi.org/10.25829/X1SH-3K57), 233 (plant species info, doi: https://doi.org/10.25829/CJMH-AW38), 523 (Oomycota, OTU table, doi: https://doi.org/10.25829/PMQK-J962), 518 (Oomycota, taxonomic and functional assignment, doi: https://doi.org/10.25829/7YPD-FM92), 519 (Cercozoa, OTU table, doi: https://doi.org/10.25829/0DYA-X475), 517 (Cercozoa, taxonomic and functional assignment, doi: https://doi.org/10.25829/FJPF-3D41), 737 (R code, doi: https://doi.org/10.25829/ET0X-JX88).

References

Ah-Fong AMV, Kagda MS, Abrahamian M, Judelson HS. 2019. Niche-specific metabolic adaptation in biotrophic and necrotrophic oomycetes is manifested in differential use of nutrients, variation in gene content, and enzyme evolution. *PLoS Pathogens* **15**: e1007729.

Albracht C, Eisenhauer N, Vogel A, Wagg C, Buscot F, Heintz-Buschart A. 2023. Effects of recurrent summer droughts on arbuscular mycorrhizal and total fungal communities in experimental grasslands differing in plant diversity and community composition. *Frontiers in Soil Science* **3**: 1129845.

Amponsah J, Tegg RS, Thangavel T, Wilson CR. 2021. Moments of weaknesses – exploiting vulnerabilities between germination and encystment in the Phytomyxea. *Biological Reviews of the Cambridge Philosophical Society* **96**: 1603–1615.

Ampt EA, Francioli D, van Ruijven J, Gomes SIF, Maciá-Vicente JG, Termorshuizen AJ, Bakker LM, Mommer L. 2022. Deciphering the interactions between plant species and their main fungal root pathogens in mixed grassland communities. *Journal of Ecology* **110**: 3039–3052.

Augspurger CK, Wilkinson HT. 2007. Host specificity of pathogenic *Pythium* species: implications for tree species diversity. *Biotropica* **39**: 702–708.

Bakker JD. 2024. *Restricting permutations. Applied multivariate statistics in R.* Seattle, WA: University of Washington. [WWW document] URL https://uw.

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

pressbooks.pub/appliedmultivariatestatistics/chapter/restricting-permutations/ [accessed 16 May 2025].

Barr DJS. 1979. Morphology and host range of *Polymyxa graminis, Polymyxa betae*, and *Ligniera pilorum* from Ontario and some other areas. *Canadian Journal of Plant Pathology* **1**: 85–94.

Beakes GW, Thines M. 2016. Hyphochytriomycota and Oomycota. In: Archibald JM, Simpson AGB, Slamovits CH, eds. *Handbook of the protists*. Cham, Switzerland: Springer, 435–505.

Bever JD. 1994. Feedback between plants and their soil communities in an old field community. *Ecology* **75**: 1965–1977.

Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods* **10**: 57–59.

Bonkowski M. 2004. Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist* **162**: 617–631.

Bonkowski M, Tarkka M, Razavi BS, Schmidt H, Blagodatskaya E, Koller R, Yu P, Knief C, Hochholdinger F, Vetterlein D. 2021. Spatiotemporal dynamics of maize (*Zea mays* L.) root growth and its potential consequences for the assembly of the rhizosphere microbiota. *Frontiers in Microbiology* **12**: 461.

Bulman S, Neuhauser S. 2017. Phytomyxea. In: Archibald JM, Simpson AGB, Slamovits CH, eds. *Handbook of the protists*. Cham, Switzerland: Springer, 783–803.

Burdon JJ, Chilvers GA. 1975. Epidemiology of damping-off disease (*Pythium irregulare*) in relation to density of *Lepidium sativum* seedlings. *Annals of Applied Biology* **81**: 135–143.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* **10**: 421.

Canarini A, Schmidt H, Fuchslueger L, Martin V, Herbold CW, Zezula D, Gundler P, Hasibeder R, Jecmenica M, Bahn M *et al.* 2021. Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. *Nature Communications* **12**: 5308.

Cappelli SL, Pichon NA, Kempel A, Allan E. 2020. Sick plants in grassland communities: a growth-defense trade-off is the main driver of fungal pathogen abundance. *Ecology Letters* **23**: 1349–1359.

Chakraborty S, Old KM, Warcup JH. 1983. Amoebae from a take-all suppressive soil which feed on *Gaeumannomyces graminis tritici* and other soil fungi. *Soil Biology and Biochemistry* **15**: 17–24.

Chakraborty S, Warcup JH. 1984. Populations of mycophagous and other amoebae in take-all suppressive and non-suppressive soils. *Soil Biology and Biochemistry* **16**: 197–199.

Chao A, Gotelli NJ, Hsieh TC, Sander EL, Ma KH, Colwell RK, Ellison AM. 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs* **84**: 45–67.

Chen Y, Yao Z, Sun Y, Wang E, Tian C, Sun Y, Liu J, Sun C, Tian L. 2022. Current studies of the effects of drought stress on root exudates and rhizosphere microbiomes of crop plant species. *International Journal of Molecular Sciences* **23**: 2374.

Choudhary A, Senthil-Kumar M. 2024. Drought: a context-dependent damper and aggravator of plant diseases. *Plant, Cell & Environment* **47**: 2109–2126.

Cook BI, Mankin JS, Anchukaitis KJ. 2018. Climate change and drought: from past to future. *Current Climate Change Reports* **4**: 164–179.

Cortois R, Schröder-Georgi T, Weigelt A, van der Putten WH, De Deyn GB, van der Heijden M. 2016. Plant–soil feedbacks: role of plant functional group and plant traits. *Journal of Ecology* **104**: 1608–1617.

Degrune F, Dumack K, Ryo M, Garland G, Romdhane S, Saghai A, Banerjee S, Edlinger A, Herzog C, Pescador DS *et al.* 2024. The impact of fungi on soil protist communities in European cereal croplands. *Environmental Microbiology* **26**: e16673.

Del Castillo Múnera J, Belayneh B, Lea-Cox J, Swett CL. 2019. Effects of set-point substrate moisture control on oomycete disease risk in containerized annual crops based on the tomato-*Phytophthora capsici* pathosystem. *Phytopathology* **109**: 1441–1452.

Derevnina L, Petre B, Kellner R, Dagdas YF, Sarowar MN, Giannakopoulou A, De la Concepcion JC, Chaparro-Garcia A, Pennington HG, van West P *et al.* 2016. Emerging oomycete threats to plants and animals. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **371**: 20150459.

Dietrich P, Eisenhauer N, Otto P, Roscher C. 2021. Plant history and soil history jointly influence the selection environment for plant species in a long-term grassland biodiversity experiment. *Ecology and Evolution* **11**: 8156–8169.

Dixon GR. 2014. Clubroot (*Plasmodiophora brassicae* Woronin) – an agricultural and biological challenge worldwide. *Canadian Journal of Plant Pathology* **36** (sup1): 5–18.

Dumack K, Feng K, Flues S, Sapp M, Schreiter S, Grosch R, Rose LE, Deng Y, Smalla K, Bonkowski M. 2022. What drives the assembly of plant-associated protist microbiomes? Investigating the effects of crop species, Soil type and bacterial microbiomes. *Protist* **173**: 125913.

Dumack K, Fiore-Donno AM, Bass D, Bonkowski M. 2020. Making sense of environmental sequencing data: Ecologically important functional traits of the protistan groups Cercozoa and Endomyxa (Rhizaria). *Molecular Ecology Resources* **20**: 398–403.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

Eggertson QA, Rintoul TL, Levesque CA. 2023. Resolving the *Globisporangium ultimum* (*Pythium ultimum*) species complex. *Mycologia* **115**: 768–786.

Eisenhauer N, Reich PB, Scheu S. 2012. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic and Applied Ecology* **13**: 571–578.

Fiore-Donno AM, Bonkowski M. 2021. Different community compositions between obligate and facultative oomycete plant parasites in a landscape-scale metabarcoding survey. *Biology and Fertility of Soils* **57**: 245–256.

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

Fiore-Donno AM, Richter-Heitmann T, Degrune F, Dumack K, Regan KM, Marhan S, Boeddinghaus RS, Rillig MC, Friedrich MW, Kandeler E *et al.* 2019. Functional traits and spatio-temporal structure of a major group of soil protists (Rhizaria: Cercozoa) in a temperate grassland. *Frontiers in Microbiology* **10**: 1332.

Fiore-Donno AM, Rixen C, Rippin M, Glaser K, Samolov E, Karsten U, Becker B, Bonkowski M. 2018. New barcoded primers for efficient retrieval of cercozoan sequences in high-throughput environmental diversity surveys, with emphasis on worldwide biological soil crusts. *Molecular Ecology Resources* **18**: 229–239.

Francioli D, van Ruijven J, Bakker L, Mommer L. 2020. Drivers of total and pathogenic soil-borne fungal communities in grassland plant species. *Fungal Ecology* **48**: 100987.

Freudenthal J, Dumack K, Schaffer S, Schlegel M, Bonkowski M. 2024. Algae-fungi symbioses and bacteria-fungi co-exclusion drive tree species-specific differences in canopy bark microbiomes. *The ISME Journal* **18**: wrae206.

Garrido PA, Proaño-Cuenca F, Flor FJF, Benítez EAD, Torres Ivanna FS, Kaiser ARK, Sain L, Peñaloza YAM, Marek SM, Melouk H *et al.* 2022. Identification and characterization of *Pythium, Globisporangium*, and *Phytopythium* species present in floricultural crops from long Island, NY. *Phytopathology* **113**: 1335–1346.

Garvetto A, Murúa P, Kirchmair M, Salvenmoser W, Hittorf M, Ciaghi S, Harikrishnan SL, Gachon CM, Burns JA, Neuhauser S. 2023. Phagocytosis underpins the biotrophic lifestyle of intracellular parasites in the class Phytomyxea (Rhizaria). *New Phytologist* **238**: 2130–2143.

Gilbert GS. 2002. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* **40**: 13–43.

González M, Romero M-Á, García L-V, Gómez-Aparicio L, Serrano M-S. 2020. Unravelling the role of drought as predisposing factor for *Quercus suber* decline caused by *Phytophthora cinnamomi*. *European Journal of Plant Pathology* **156**: 1015–1021.

Guerrero-Ramírez NR, Craven D, Reich PB, Ewel JJ, Isbell F, Koricheva J, Parrotta JA, Auge H, Erickson HE, Forrester DI *et al.* 2017. Diversity-dependent temporal divergence of ecosystem functioning in experimental ecosystems. *Nature Ecology & Evolution* **1**: 1639–1642.

Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, Boutte C, Burgaud G, de Vargas C, Decelle J *et al.* 2013. The protist ribosomal reference database

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

(PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Research* **41**: D597–D604.

Hannula SE, Heinen R, Huberty M, Steinauer K, De Long JR, Jongen R, Bezemer TM. 2021. Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. *Nature Communications* **12**: 5686.

Hari V, Rakovec O, Markonis Y, Hanel M, Kumar R. 2020. Increased future occurrences of the exceptional 2018–2019 Central European drought under global warming. *Scientific Reports* **10**: 12207.

Haugen R, Steffes L, Wolf J, Brown P, Matzner S, Siemens DH. 2007. Evolution of drought tolerance and defense: dependence of tradeoffs on mechanism, environment and defense switching. *Oikos* **117**: 231–244.

Hector A, von Felten S, Schmid B. 2010. Analysis of variance with unbalanced data: an update for ecology & evolution. *Journal of Animal Ecology* **79**: 308–316.

Hess S, Sausen N, Melkonian M. 2012. Shedding light on vampires: the phylogeny of vampyrellid amoebae revisited. *PLoS ONE* **7**: e31165.

Hess S, Suthaus A. 2022. The vampyrellid amoebae (Vampyrellida, Rhizaria). *Protist* **173**: 125854.

Hittorf M, Letsch-Praxmarer S, Windegger A, Bass D, Kirchmair M, Neuhauser S. 2020. Revised taxonomy and expanded biodiversity of the Phytomyxea (Rhizaria, Endomyxa). *Journal of Eukaryotic Microbiology* **67**: 648–659.

Hsieh TC, Ma KH, Chao A, McInerny G. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution* **7**: 1451–1456.

Jones B, Nachtsheim CJ. 2009. Split-plot designs: what, why, and how. *Journal of Quality Technology* **41**: 340–361.

Jousset A. 2012. Ecological and evolutive implications of bacterial defences against predators. *Environmental Microbiology* **14**: 1830–1843.

Kanyuka K, Ward E, Adams MJ. 2003. *Polymyxa graminis* and the cereal viruses it transmits: a research challenge. *Molecular Plant Pathology* **4**: 393–406.

Kasteel M, Ketelaar T, Govers F. 2023. Fatal attraction: how *Phytophthora* zoospores find their host. *Seminars in Cell & Developmental Biology* **148-149**: 13–21.

Kramer S, Dibbern D, Moll J, Huenninghaus M, Koller R, Krueger D, Marhan S, Urich T, Wubet T, Bonkowski M *et al.* 2016. Resource partitioning between bacteria, fungi, and protists in the detritusphere of an agricultural soil. *Frontiers in Microbiology* **7**: 1524.

Kulmatiski A, Beard KH, Heavilin J. 2012. Plant-soil feedbacks provide an additional explanation for diversity-productivity relationships. *Proceedings of the Royal Society B: Biological Sciences* **279**: 3020–3026.

LaMotte LR. 2019. A formula for type III sums of squares. *Communications in Statistics - Theory and Methods* **49**: 3126–3136.

Lange M, Eisenhauer N, Chen H, Gleixner G. 2023. Increased soil carbon storage through plant diversity strengthens with time and extends into the subsoil. *Global Change Biology* **29**: 2627–2639.

Lange M, Eisenhauer N, Sierra CA, Bessler H, Engels C, Griffiths RI, Mellado-Vázquez PG, Malik AA, Roy J, Scheu S *et al.* 2015. Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications* **6**: 6707.

Lévesque CA, Brouwer H, Cano L, Hamilton JP, Holt C, Huitema E, Raffaele S, Robideau GP, Thines M, Win J *et al.* 2010. Genome sequence of the necrotrophic plant pathogen *Pythium ultimum* reveals original pathogenicity mechanisms and effector repertoire. *Genome Biology* **11**: R73.

Li J, Wang C, Liang W, Liu S. 2021. Rhizosphere microbiome: the emerging barrier in plant-pathogen interactions. *Frontiers in Microbiology* **12**: 772420.

Littlefield L, Whallon J, Doss P, Hassan Z. 1998. Postinfection development of *Polymyxa graminis* in roots of *Triticum aestivum*. *Mycologia* **90**: 869–882.

Liu X, Lyu S, Zhou S, Bradshaw CJ. 2016. Warming and fertilization alter the dilution effect of host diversity on disease severity. *Ecology* **97**: 1680–1689.

Looseley M, Newton A. 2014. Assessing the consequences of microbial infection in field trials: seen, unseen, beneficial, parasitic and pathogenic. *Agronomy* **4**: 302–321.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**: 550.

Lozano YM, Aguilar-Trigueros CA, Ospina JM, Rillig MC. 2022. Drought legacy effects on root morphological traits and plant biomass via soil biota feedback. *New Phytologist* **236**: 222–234.

MacFarlane I. 1952. Factors affecting the survival of *Plasmodiophora brassicae* Wor. in the soil and its assessment by a host test. *Annals of Applied Biology* **39**: 239–256.

Marin M, Feeney DS, Brown LK, Naveed M, Ruiz S, Koebernick N, Bengough AG, Hallett PD, Roose T, Puertolas J *et al.* 2021. Significance of root hairs for plant performance under contrasting field conditions and water deficit. *Annals of Botany* **128**: 1–16.

Markell S, Khan M, Secor G, Gulya T, Lamey A. 2008. *Row crop diseases in drought years.* Fargo, ND, USA: North Dakota State University (NDSU) Extension Service. [WWW document] URL https://library.ndsu.edu/server/api/core/bitstreams/78d4b2bd-fa00-4b4c-a07a-7cc6757cdd33/content [accessed 16 May 2025].

Maron JL, Marler M, Klironomos JN, Cleveland CC. 2011. Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters* **14**: 36–41.

Masigol H, Solbach MD, Pourmoghaddam MJ, Ahadi R, Mostowfizadeh-Ghalamfarsa R, Taheri SR, Tobias-Hünefeldt SP, Bonkowski M, Grossart HP. 2025. A glimpse into Oomycota diversity in freshwater lakes and adjacent forests using a metabarcoding approach. *Scientific Reports* **15**: 19124.

Meyer ST, Ebeling A, Eisenhauer N, Hertzog L, Hillebrand H, Milcu A, Pompe S, Abbas M, Bessler H, Buchmann N *et al.* 2016. Effects of biodiversity strengthen over time as ecosystem functioning declines at low and increases at high biodiversity. *Ecosphere* **7**: e01619.

Milliken GA, Johnson DE. 1984. *Analysis of messy data. Volume 1: designed experiments.* New York, NY, USA: Van Nostrand Reinhold Co.

Mitchell RT, Deacon JW. 1986. Differential (host-specific) accumulation of zoospores of *Pythium* on roots of graminaceous and non-graminaceous plants. *New Phytologist* **102**: 113–122.

Monson RK, Trowbridge AM, Lindroth RL, Lerdau MT. 2022. Coordinated resource allocation to plant growth-defense tradeoffs. *New Phytologist* **233**: 1051–1066.

Nelson MC, Morrison HG, Benjamino J, Grim SL, Graf J. 2014. Analysis, optimization and verification of Illumina-generated 16S rRNA gene amplicon surveys. *PLoS ONE* **9**: e94249.

Neuhauser S, Bulman S, Kirchmair M. 2010. Plasmodiophorids: the challenge to understand soil-borne, obligate biotrophs with a multiphasic life cycle. In: Gherbawy Y, Voigt K, eds. *Molecular identification of fungi.* Heidelberg, Germany: Springer, 51–78.

Neuhauser S, Kirchmair M, Bulman S, Bass D. 2014. Cross-kingdom host shifts of phytomyxid parasites. *BMC Evolutionary Biology* **14**: 33.

Neupane K, Alexander L, Baysal-Gurel F. 2022. Management of *Phytophthora cinnamomi* using fungicides and host plant defense inducers under drought conditions: a case study of flowering dogwood. *Plant Disease* **106**: 475–485.

Newton AC, Fitt BD, Atkins SD, Walters DR, Daniell TJ. 2010. Pathogenesis, parasitism and mutualism in the trophic space of microbe-plant interactions. *Trends in Microbiology* **18**: 365–373.

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E *et al.* 2022. *VEGAN: community ecology package. R package v.2.6-4.* [WWW document] URL https://CRAN.R-project.org/package=vegan [accessed 16 May 2025].

Oliverio AM, Geisen S, Delgado-Baquerizo M, Maestre FT, Turner BL, Fierer N. 2020. The global-scale distributions of soil protists and their contributions to belowground systems. *Science Advances* **6**: eaax8787.

Pandaranayaka EP, Frenkel O, Elad Y, Prusky D, Harel A. 2019. Network analysis exposes core functions in major lifestyles of fungal and oomycete plant pathogens. *BMC Genomics* **20**: 1020.

Petermann JS, Fergus AJF, Turnbull LA, Schmid B. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* **89**: 2399–2406.

Pinheiro JC, Bates DM. 2000. *Mixed-effects models in S and S-PLUS.* New York, USA: Springer.

Pinheiro JC, Bates DM, R Core Team. 2022. NLME: linear and nonlinear mixed effects models. R package v.3.1-157. [WWW document] URL https://CRAN.R-project.org/package=nlme [accessed 16 May 2025].

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

Populer C. 1981. Epidemiology of downy mildews. In: Spencer DM, ed. *The downy mildews*. London, UK: Academic Press, 57–105.

R Core Team. 2022. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL https://www.R-project.org [accessed 16 May 2025].

Ramegowda V, Senthil-Kumar M. 2015. The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. *Journal of Plant Physiology* **176**: 47–54.

Rognes T, Flouri T, Nichols B, Quince C, Mahe F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**: e2584.

Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, Schmid B, Schulze E-D. 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology* **5**: 107–121.

Roscher C, Schumacher J, Gubsch M, Lipowsky A, Weigelt A, Buchmann N, Schmid B, Schulze ED. 2012. Using plant functional traits to explain diversity-productivity relationships. *PLoS ONE* **7**: e36760.

Rossmann M, Pérez-Jaramillo JE, Kavamura VN, Chiaramonte JB, Dumack K, Fiore-Donno AM, Mendes LW, Ferreira MMC, Bonkowski M, Raaijmakers JM *et al.* 2020. Multitrophic interactions in the rhizosphere microbiome of wheat: from bacteria and fungi to protists. *FEMS Microbiology Ecology* **96**: fiaa032.

Rüger L, Feng K, Dumack K, Freudenthal J, Chen Y, Sun R, Wilson M, Yu P, Sun B, Deng Y *et al.* 2021. Assembly patterns of the rhizosphere microbiome along the longitudinal root axis of maize (*Zea mays* L.). *Frontiers in Microbiology* **12**: 237.

Schaap P, Schilde C. 2018. Encystation: the most prevalent and underinvestigated differentiation pathway of eukaryotes. *Microbiology* **164**: 727–739.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ *et al.* 2009. Introducing MOTHUR: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537–7541.

Schmid B, Baruffol M, Wang Z, Niklaus PA. 2017. A guide to analyzing biodiversity experiments. *Journal of Plant Ecology* **10**: 91–110.

Schwelm A, Badstöber J, Bulman S, Desoignies N, Etemadi M, Falloon RE, Gachon CMM, Legreve A, Lukeš J, Merz U *et al.* 2018. Not in your usual Top 10: protists that infect plants and algae. *Molecular Plant Pathology* **19**: 1029–1044.

Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH, Battaglia ML. 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* **10**: 259.

Siemens DH, Duvall-Jisha J, Jacobs J, Manthey J, Haugen R, Matzner S. 2012. Water deficiency induces evolutionary tradeoff between stress tolerance and chemical defense allocation that may help explain range limits in plants. *Oikos* **121**: 790–800.

de Souza YPA, Siani R, Albracht C, Huang Y, Eisenhauer N, Vogel A, Wagg C, Schloter M, Schulz S. 2024. The effect of successive summer drought periods on bacterial diversity along a plant species richness gradient. *FEMS Microbiology Ecology* **100**: fiae096.

Spinoni J, Vogt JV, Naumann G, Barbosa P, Dosio A. 2017. Will drought events become more frequent and severe in Europe? *International Journal of Climatology* **38**: 1718–1736.

Sweeney CJ, de Vries FT, van Dongen BE, Bardgett RD. 2021. Root traits explain rhizosphere fungal community composition among temperate grassland plant species. *New Phytologist* **229**: 1492–1507.

Takahashi K. 1994. Influences of some environmental factors on the viability of resting spores of *Plasmodiophora brassicae* Wor. incubated in sterile soil. *Japanese Journal of Phytopathology* **60**: 658–666.

Thines M. 2014. Phylogeny and evolution of plant pathogenic oomycetes – a global overview. *European Journal of Plant Pathology* **138**: 431–447.

Thines M, Choi YJ. 2016. Evolution, diversity, and taxonomy of the Peronosporaceae, with focus on the genus *Peronospora*. *Phytopathology* **106**: 6–18.

Thines M, Kamoun S. 2010. Oomycete-plant coevolution: recent advances and future prospects. *Current Opinion in Plant Biology* **13**: 427–433.

Turkensteen LJ, Flier WG, Wanningen R, Mulder A. 2008. Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology* **49**: 688–696.

Uzuhashi S, Kakishima M, Tojo M. 2010. Phylogeny of the genus *Pythium* and description of new genera. *Mycoscience* **51**: 337–365.

van der Plaats-Niterink J. 1981. Monograph of the genus *Pythium*. *Studies in Mycology* **21**: 1–244.

Vogel A, Fester T, Eisenhauer N, Scherer-Lorenzen M, Schmid B, Weisser WW, Weigelt A. 2013. Separating drought effects from roof artifacts on ecosystem processes in a grassland drought experiment. *PLoS ONE* **8**: e70997.

Vogel A, Scherer-Lorenzen M, Weigelt A. 2012. Grassland resistance and resilience after drought depends on management intensity and species richness. *PLoS ONE* **7**: e36992.

de Vries F, Liiri M, Bjornlund L, Setälä H, Christensen S, Bardgett R. 2012. Legacy effects of drought on plant growth and the soil food web. *Oecologia* **170**: 821–833.

Wagg C, O'Brien MJ, Vogel A, Scherer-Lorenzen M, Eisenhauer N, Schmid B, Weigelt A. 2017. Plant diversity maintains long-term ecosystem productivity under frequent drought by increasing short-term variation. *Ecology* **98**: 2952–2961.

Wallenhammar A-C. 1996. Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. *Plant Pathology* **45**: 710–719.

Wallenhammar A-C, Omer ZS, Edin E, Jonsson A. 2021. Influence of soil-borne inoculum of *Plasmodiophora brassicae* measured by qPCR on disease severity of clubroot-resistant cultivars of winter oilseed rape (*Brassica napus* L.). *Pathogens* **10**: 433.

Wan N-F, Wang Y-Q, Fu L, Liu J, Woodcock B, Hu Y, Eskelinen A, Hector A, Loreau M, Hautier Y *et al.* 2023. Species richness promotes plant productivity by suppressing plant antagonists. Preprint (Version 1) Available at Research Square.

Wang G, Burrill HM, Podzikowski LY, Eppinga MB, Zhang F, Zhang J, Schultz PA, Bever JD. 2023. Dilution of specialist pathogens drives productivity benefits from diversity in plant mixtures. *Nature Communications* **14**: 8417.

Weber AP, Zwillenberg L, Van Der Laan P. 1952. A predacious amoeboid organism destroying larvae of the potato root eelworm and other nematodes. *Nature* **169**: 834–835.

Weiner J. 2021. *RIVERPLOT: sankey or ribbon plots. R package v.0.10.* [WWW document] URL https://CRAN.R-project.org/package=riverplot. [accessed 16 May 2025]

Weisser WW, Roscher C, Meyer ST, Ebeling A, Luo G, Allan E, Beßler H, Barnard RL, Buchmann N, Buscot F *et al.* 2017. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: patterns, mechanisms, and open questions. *Basic and Applied Ecology* **23**: 1–73.

van West P, Appiah AA, Gow NAR. 2003. Advances in research on oomycete root pathogens. *Physiological and Molecular Plant Pathology* **62**: 99–113.

Wickham H. 2016. *GGPLOT2: elegant graphics for data analysis*. Cham, Switzerland: Springer.

Williams A, de Vries FT. 2020. Plant root exudation under drought: implications for ecosystem functioning. *New Phytologist* **225**: 1899–1905.

Xi N, Chen D, Bahn M, Wu H, Chu C, Cadotte MW, Bloor JMG. 2022. Drought soil legacy alters drivers of plant diversity-productivity relationships in oldfield systems. *Science Advances* **8**: eabn3368.

Xiong W, Song Y, Yang K, Gu Y, Wei Z, Kowalchuk GA, Xu Y, Jousset A, Shen Q, Geisen S. 2020. Rhizosphere protists are key determinants of plant health. *Microbiome* **8**: 27.

Zhang C, Geisen S, Berendsen RL, van der Heijden MGA. 2024. Specialized protist communities on mycorrhizal fungal hyphae. *Mycorrhiza* **34**: 517–524.

Zhang J, Sun X, Ao N, Zou H, Shao H, Kageyama K, Feng W. 2023. Host range and loop-mediated isothermal amplification detection of *Globisporangium sylvaticum* from Guizhou, China. *Journal of Fungi* **9**: 752.

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

New Phytologist

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 List of barcode sequences used for metabarcoding.

Dataset S2 Custom Oomycota ITS1 database.

Dataset S3 Detailed statistical results of alpha and beta diversity analyses.

Fig. S1 Experimental setup.

Fig. S2 Rarefaction curves.

Fig. S3 Correlation matrix of main factors.

Fig. S4 Plots of exponential Shannon index.

Fig. S5 Plots of Pielou's evenness index.

Fig. S6 Summary of differential abundance analysis of main factors.

Fig. S7 Summary of plant species-specific differential abundance analysis.

Table S1 Plant species list of the Jena Experiment.

Table S2 Plot list of the Jena Experiment.

Please note: Wiley is not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

Disclaimer: The New Phytologist Foundation remains neutral with regard to jurisdictional claims in maps and in any institutional affiliations.

© 2025 The Author(s).
New Phytologist © 2025 New Phytologist Foundation.

New Phytologist

***New Phytologist* Supporting Information**

Article title: Legacies of consecutive summer droughts on soil-borne plant parasitic protists (Oomycota: Stramenopila & Phytomyxea: Rhizaria) and protistan consumers (Cercozoa: Rhizaria) along an experimental plant diversity gradient
Authors: Marcel Dominik Solbach, Cynthia Albracht, Kenneth Dumack, Nico Eisenhauer, Anna Maria Fiore-Donno, Nils Heck, Anja Vogel, Cameron Wagg, Michael Bonkowski
Article acceptance date: 29 October 2025

The following Supporting Information is available for this article:

Fig. S1 Experimental setup.

Fig. S2 Rarefaction curves.

Fig. S3 Correlation matrix of main factors.

Fig. S4 Plots of exponential Shannon index.

Fig. S5 Plots of Pielou's evenness index.

Fig. S6 Summary of differential abundance analysis of main factors.

Fig. S7 Summary of plant species-specific differential abundance analysis.

Table S1 Plant species list of the Jena Experiment.

Table S2 Plot list of the Jena Experiment.

Dataset S1 List of barcode sequences used for the metabarcoding (Excel file).

Dataset S2 Custom Oomycota ITS1 database (FASTA file).

Dataset S3 Detailed statistical results of alpha and beta diversity analysis (Excel file).

New Phytologist

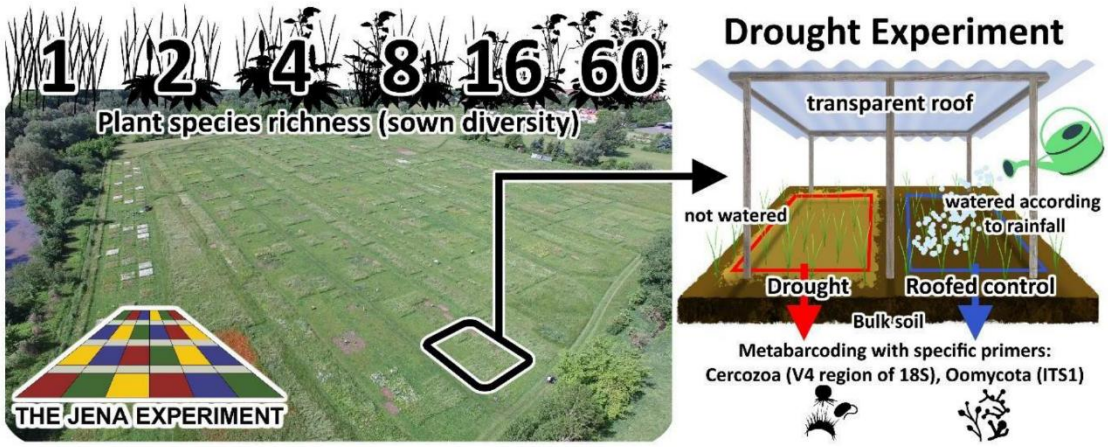


Fig. S1 Schematic overview of the experimental design. The Drought Experiment was established as a sub-plot experiment on all 80 main plots of the Jena Experiment in 2008. Plots differed in plant species richness (1, 2, 4, 8, 16, or 60 plant species) and functional group richness (1 - 4 functional groups of the functional groups "Grass", "Legume", "Small Herb", and "Tall Herb"). The roof constructions of the Drought Experiment (3 m x 2.5 m) were present from 2008 to 2016 for six weeks during each summer. Half of the area below the roof was watered with the amount of water withheld by the roof ("roofed control" subplot), while the other half was not watered ("drought" subplot). Bulk soil was collected from the central area of each subplot (1 m x 1 m, at least 0.5 m away from the outer edges) one year after the last roofing period. Metabarcoding of Oomycota and Cercozoa was performed with taxon-specific primers from DNA extracts of the collected bulk soil. Photo credit: The Jena Experiment. Illustrations: M. D. Solbach.

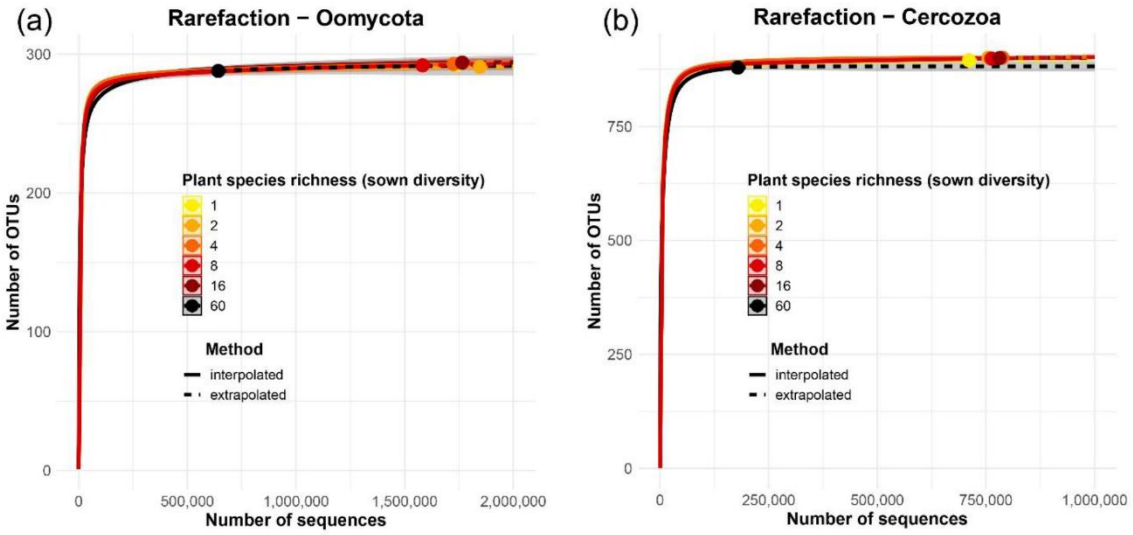


Fig. S2 Rarefaction curves from iNEXT showing the obtained number of OTUs with increasing number of sequence reads. a: Oomycota, b: Cercozoa.

New Phytologist

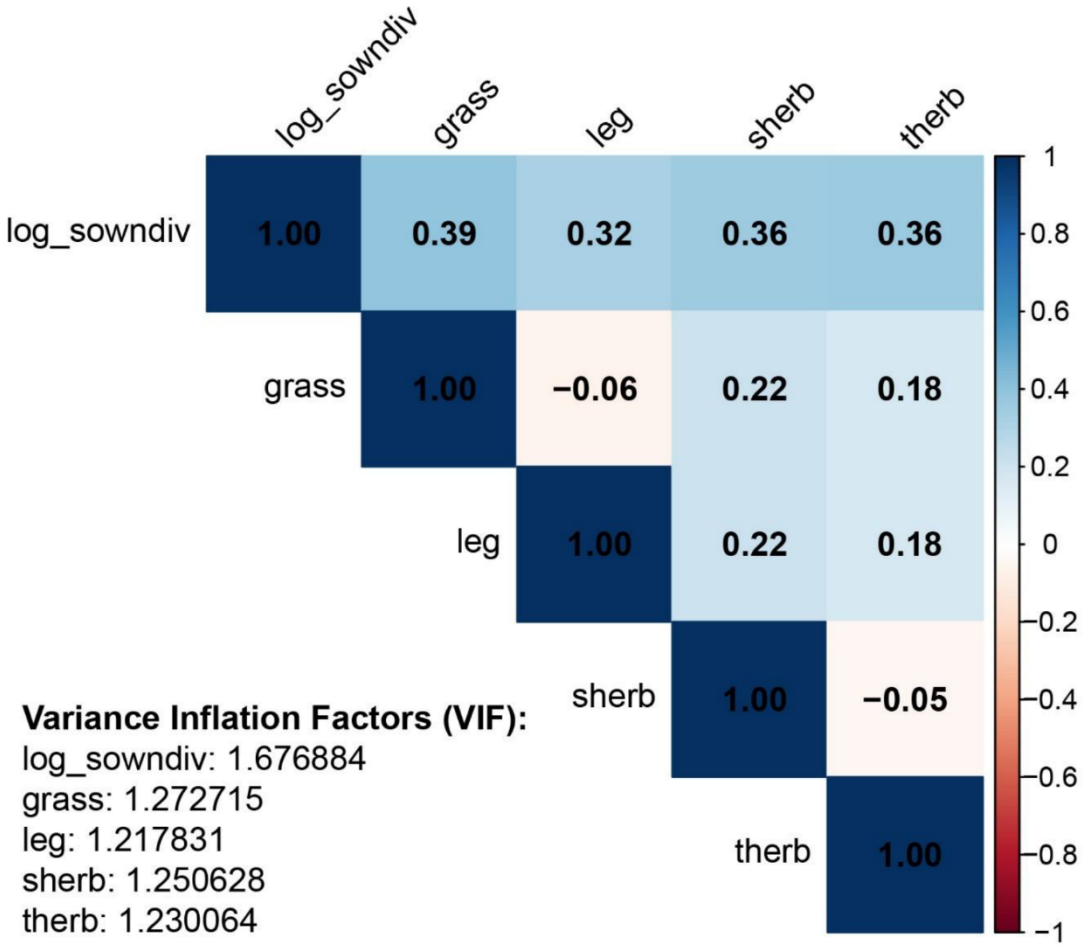


Fig. S3 Correlation matrix of main factors (plant species richness as log(sown diversity), presence/absence of functional groups (grass, legume, small herb, tall herb)) based on pairwise Spearman correlations. Factors of presence/absence of functional groups were transformed to 0/1 coding. The Variance Inflation Factors (VIFs, bottom left) measure how much a predictor is linearly explained by the others/how much the variance of a regression coefficient is inflated due to collinearity. Partial correlation of the factors occurs due to structural imbalance/impossible combinations of the experimental design. E.g., a monoculture plot can only ever contain a single plant functional group, a 2-species mixture can only contain one or two functional groups, etc. As a result, plots with higher plant diversity are automatically more likely to contain more functional groups, and vice versa. The 60-species mixtures will always contain all four functional groups, leading to empty cells in the experimental design. This makes data analysis and interpretation difficult, because many patterns cannot be unambiguously assigned to a single factor.

New Phytologist

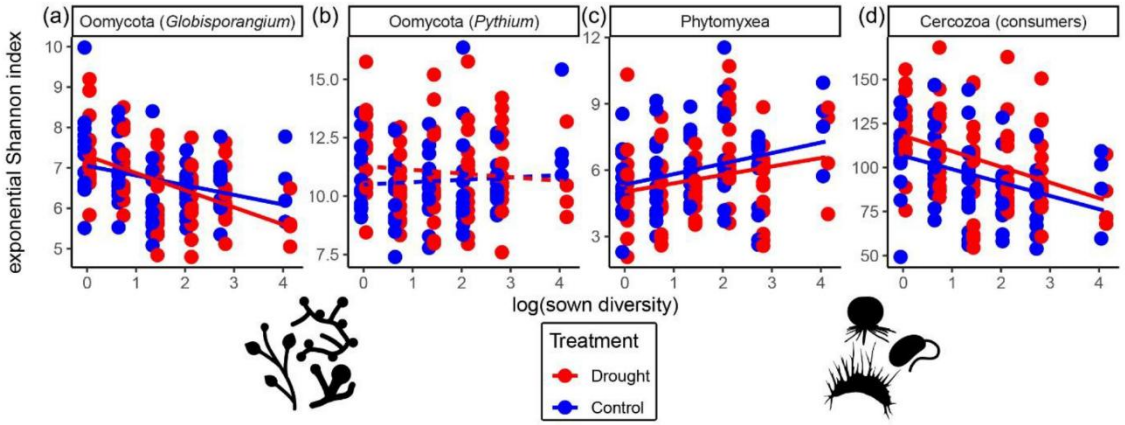


Fig. S4 Exponential Shannon index (Hill number of order q = 1; extrapolated using iNEXT::ChaoShannon) as affected by plant species richness (as log(sown diversity)) and drought treatment (drought vs. control). Results of the respective statistical analyses are given in Dataset S3. Solid lines indicate a significant effect of log(sown diversity). Two separate lines indicate a significant effect of the drought treatment. When no lines are displayed, neither log(sown diversity) nor the drought treatment had a significant effect. a: Oomycota (*Globisporangium*); b: Oomycota (*Pythium*); c: Phytomyxea; d: Cercozoa (consumers).

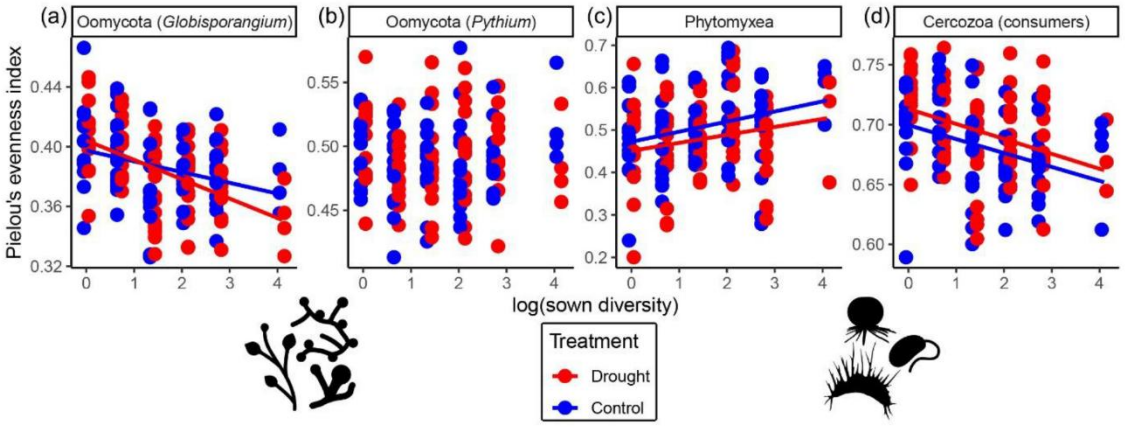


Fig. S5 Pielou's evenness index as affected by plant species richness (as log(sown diversity)) and drought treatment (drought vs. control). Results of the respective statistical analyses are given in Dataset S3. Solid lines indicate a significant effect of log(sown diversity). Two separate lines indicate a significant effect of the drought treatment. When no lines are displayed, neither log(sown diversity) nor the drought treatment had a significant effect. a: Oomycota (*Globisporangium*); b: Oomycota (*Pythium*); c: Phytomyxea; d: Cercozoa (consumers).

New Phytologist

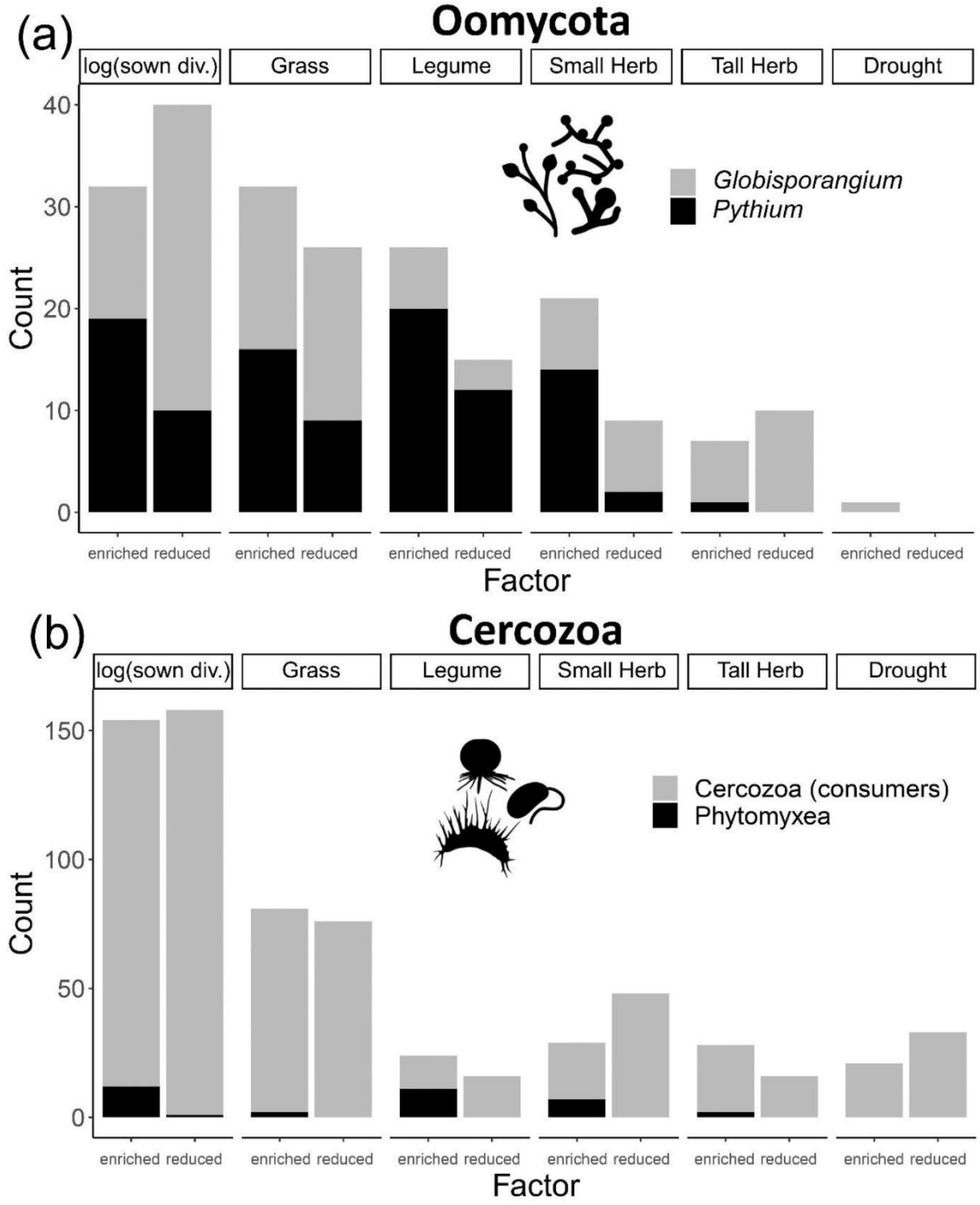


Fig. S6 Summary of differential abundance analysis of main factors with DESeq2. Bars indicate the number of differentially abundant OTUs depending on the respective main factors. "Enriched" and "reduced" refer to OTUs that were more abundant or less abundant, respectively, in plots with increasing plant species richness (log(sown div.)), in plots where the respective plant functional group (Grass, Legume, Small Herb, Tall Herb) was present, and in the drought treatment. a: Oomycota; b: Cercozoa.

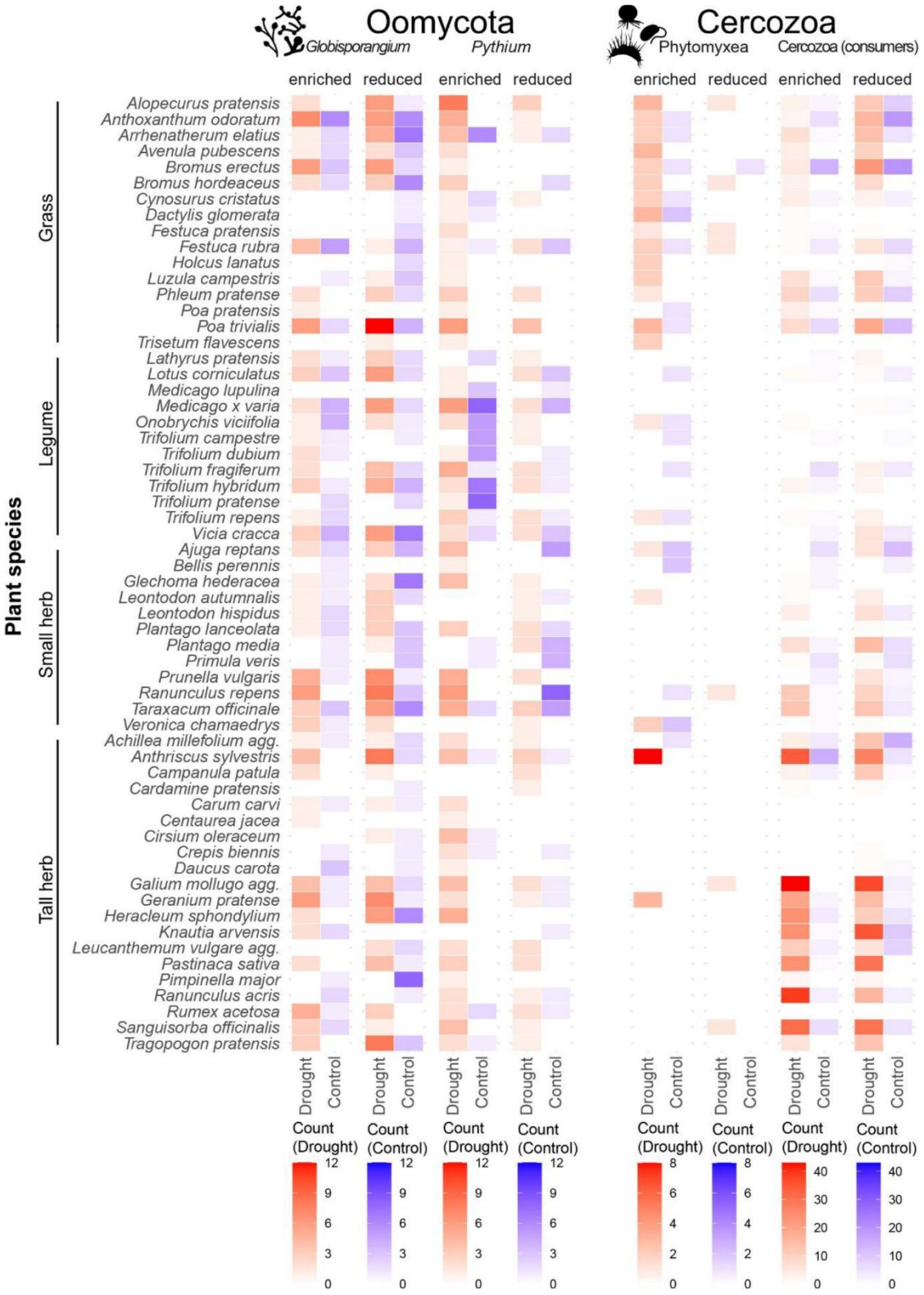


Fig. S7 Summary of differential abundance analysis in DESeq2 for all 60 plant species, differentiated between drought (red) and control (blue). Color intensity indicates the number of differentially abundant OTUs. "Enriched" OTUs were more abundant in plots with the

New Phytologist

respective plant species/less abundant in plots without the respective plant species, and "reduced" OTUs were less abundant in plots with the respective plant species/more abundant in plots without the respective plant species. The average number of differentially abundant OTUs was compared between drought and control (see main text, Fig. 4).

Table S1 Species list of the sixty plant species of the Jena Experiment, including their family and functional group (grass: grass; leg: legume; sherb: small herb; therb: tall herb). "Short name" refers to abbreviations used in Table S2.

Short name	Full name (species)	Family	Functional group
Ach.mil	*Achillea millefolium* agg.	Asteraceae	therb
Aju.rep	*Ajuga reptans*	Lamiaceae	sherb
Alo.pra	*Alopecurus pratensis*	Poaceae	grass
Ant.odo	*Anthoxanthum odoratum*	Poaceae	grass
Ant.syl	*Anthriscus sylvestris*	Apiaceae	therb
Arr.ela	*Arrhenatherum elatius*	Poaceae	grass
Ave.pub	*Avenula pubescens*	Poaceae	grass
Bel.per	*Bellis perennis*	Asteraceae	sherb
Bro.ere	*Bromus erectus*	Poaceae	grass
Bro.hor	*Bromus hordeaceus*	Poaceae	grass
Cam.pat	*Campanula patula*	Campanulaceae	therb
Car.car	*Carum carvi*	Apiaceae	therb
Car.pra	*Cardamine pratensis*	Brassicaceae	therb
Cen.jac	*Centaurea jacea*	Asteraceae	therb
Cir.ole	*Cirsium oleraceum*	Asteraceae	therb
Cre.bie	*Crepis biennis*	Asteraceae	therb
Cyn.cri	*Cynosurus cristatus*	Poaceae	grass
Dac.glo	*Dactylis glomerata*	Poaceae	grass
Dau.car	*Daucus carota*	Apiaceae	therb
Fes.pra	*Festuca pratensis*	Poaceae	grass
Fes.rub	*Festuca rubra*	Poaceae	grass
Gal.mol	*Galium mollugo* agg.	Rubiaceae	therb
Ger.pra	*Geranium pratense*	Geraniaceae	therb
Gle.hed	*Glechoma hederacea*	Lamiaceae	sherb
Her.sph	*Heracleum sphondylium*	Apiaceae	therb
Hol.lan	*Holcus lanatus*	Poaceae	grass
Kna.arv	*Knautia arvensis*	Caprifoliaceae	therb
Lat.pra	*Lathyrus pratensis*	Fabaceae	leg
Leo.aut	*Leontodon autumnalis*	Asteraceae	sherb
Leo.his	*Leontodon hispidus*	Asteraceae	sherb
Leu.vul	*Leucanthemum vulgare* agg.	Asteraceae	therb
Lot.cor	*Lotus corniculatus*	Fabaceae	leg
Luz.cam	*Luzula campestris*	Juncaceae	grass

New Phytologist

Med.lup	*Medicago lupulina*	Fabaceae	leg
Med.var	*Medicago x varia*	Fabaceae	leg
Ono.vic	*Onobrychis viciifolia*	Fabaceae	leg
Pas.sat	*Pastinaca sativa*	Apiaceae	therb
Phl.pra	*Phleum pratense*	Poaceae	grass
Pim.maj	*Pimpinella major*	Apiaceae	therb
Pla.lan	*Plantago lanceolata*	Plantaginaceae	sherb
Pla.med	*Plantago media*	Plantaginaceae	sherb
Poa.pra	*Poa pratensis*	Poaceae	grass
Poa.tri	*Poa trivialis*	Poaceae	grass
Pri.ver	*Primula veris*	Primulaceae	sherb
Pru.vul	*Prunella vulgaris*	Lamiaceae	sherb
Ran.acr	*Ranunculus acris*	Ranunculaceae	therb
Ran.rep	*Ranunculus repens*	Ranunculaceae	sherb
Rum.ace	*Rumex acetosa*	Polygonaceae	therb
San.off	*Sanguisorba officinalis*	Rosaceae	therb
Tar.off	*Taraxacum officinale*	Asteraceae	sherb
Tra.pra	*Tragopogon pratensis*	Asteraceae	therb
Tri.cam	*Trifolium campestre*	Fabaceae	leg
Tri.dub	*Trifolium dubium*	Fabaceae	leg
Tri.fla	*Trisetum flavescens*	Poaceae	grass
Tri.fra	*Trifolium fragiferum*	Fabaceae	leg
Tri.hyb	*Trifolium hybridum*	Fabaceae	leg
Tri.pra	*Trifolium pratense*	Fabaceae	leg
Tri.rep	*Trifolium repens*	Fabaceae	leg
Ver.cha	*Veronica chamaedrys*	Plantaginaceae	sherb
Vic.cra	*Vicia cracca*	Fabaceae	leg

Table S2 Plot list of the 80 plots of the main Jena Experiment. On each of these plots, a drought and roofed control subplot were erected. Plot: Plot code on the field site, Block: Experimental block on the field site with increasing distance to the river Saale (Block 1: closest to the river, Block 4: furthest away from the river); Sown div.: Plant species richness as sown diversity (1, 2, 4, 8, 16, or 60); Func. group: Functional group richness (1, 2, 3, or 4); Num. grass: Number of plant species of the functional group "grass"; Num. leg: Number of plant species of the functional group "legume"; Num. sherb: Number of plant species of the functional group "small herb"; Num. therb: Number of plant species of the functional group "tall herb"; Composition: Species composition, abbreviations refer to the "short name" in Table S1.

Plot	Block	Sown div.	Func. group	Num. grass	Num. leg	Num. sherb	Num. therb	Composition
B1A01	B1	16	4	4	4	4	4	Ant.odo, Ave.pub, Bro.hor, Poa.pra, Aju.rep, Pla.lan, Ran.rep, Tar.off, Ant.syl, Car.car,

								Ger.pra, Tra.pra, Lat.pra, Lot.cor, Tri.cam, Vic.cra
B1A02	B1	8	2	4	0	0	4	Alo.pra, Bro.ere, Fes.rub, Phl.pra, Car.pra, Her.sph, Ran.acr, San.off
B1A03	B1	8	3	3	2	3	0	Cyn.cri, Phl.pra, Tri.fla, Gle.hed, Pri.ver, Ver.cha, Lot.cor, Med.lup
B1A04	B1	4	4	1	1	1	1	Fes.pra, Pla.lan, Cam.pat, Ono.vic
B1A05	B1	2	1	0	2	0	0	Med.lup, Ono.vic
B1A06	B1	16	2	8	0	0	8	Alo.pra, Ant.odo, Ave.pub, Bro.hor, Hol.lan, Poa.pra, Poa.tri, Tri.fla, Ach.mil, Ant.syl, Cam.pat, Cen.jac, Ger.pra, Her.sph, Leu.vul, Pim.maj
B1A07	B1	2	1	0	0	0	2	Ran.acr, San.off
B1A08	B1	1	1	0	1	0	0	Med.var
B1A11	B1	16	1	0	0	0	16	Ach.mil, Ant.syl, Cam.pat, Car.pra, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Leu.vul, Pas.sat, Ran.acr, Rum.ace, San.off, Tra.pra
B1A12	B1	8	1	0	8	0	0	Lat.pra, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.hyb, Tri.pra
B1A13	B1	4	1	0	4	0	0	Lot.cor, Med.lup, Med.var, Ono.vic
B1A14	B1	8	4	2	2	2	2	Luz.cam, Tri.fla, Leo.his, Pla.lan, Ant.syl, Dau.car, Tri.cam, Tri.fra
B1A15	B1	1	1	0	0	0	1	Cre.bie
B1A16	B1	2	2	1	0	1	0	Poa.pra, Pla.lan
B1A17	B1	2	2	1	0	0	1	Alo.pra, Dau.car
B1A18	B1	1	1	0	0	1	0	Pru.vul
B1A19	B1	4	3	2	0	1	1	Arr.ela, Luz.cam, Pru.vul, Cam.pat
B1A20	B1	16	3	0	5	6	5	Aju.rep, Bel.per, Leo.aut, Leo.his, Pla.lan, Ver.cha, Ach.mil, Ger.pra, Kna.arv, Ran.acr, San.off, Lot.cor, Med.var, Ono.vic, Tri.hyb, Tri.rep
B1A21	B1	4	2	2	0	0	2	Fes.pra, Luz.cam, Ach.mil, Cre.bie
B1A22	B1	60	4	16	12	12	20	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam, Phl.pra, Poa.pra, Poa.tri, Tri.fla, Aju.rep, Bel.per, Gle.hed, Leo.aut, Leo.his, Pla.lan, Pla.med, Pri.ver, Pru.vul, Ran.rep, Tar.off, Ver.cha, Ach.mil, Ant.syl, Cam.pat, Car.pra, Car.car, Cen.jac, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Pas.sat, Pim.maj, Ran.acr, Rum.ace, San.off, Tra.pra, Lat.pra, Lot.cor, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B2A01	B2	4	4	1	1	1	1	Ant.odo, Pru.vul, Kna.arv, Tri.pra
B2A02	B2	2	1	2	0	0	0	Fes.rub, Tri.fla

B2A03	B2	60	4	16	12	12	20	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam, Phl.pra, Poa.pra, Poa.tri, Tri.fla, Aju.rep, Bel.per, Gle.hed, Leo.aut, Leo.his, Pla.lan, Pla.med, Pri.ver, Pru.vul, Ran.rep, Tar.off, Ver.cha, Ach.mil, Ant.syl, Cam.pat, Car.pra, Car.car, Cen.jac, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Pas.sat, Pim.maj, Ran.acr, Rum.ace, San.off, Tra.pra, Lat.pra, Lot.cor, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B2A04	B2	1	1	0	0	0	1	Ger.pra
B2A05	B2	1	1	1	0	0	0	Fes.pra
B2A06	B2	4	2	0	2	2	0	Pla.lan, Tar.off, Lat.pra, Med.lup
B2A08	B2	2	2	0	1	0	1	Ran.acr, Tri.cam
B2A09	B2	4	1	0	0	4	0	Aju.rep, Pla.lan, Pri.ver, Pru.vul
B2A10	B2	16	2	8	0	8	0	Alo.pra, Ant.odo, Arr.ela, Bro.ere, Fes.pra, Hol.lan, Phl.pra, Poa.pra, Bel.per, Leo.aut, Leo.his, Pla.lan, Pri.ver, Pru.vul, Ran.rep, Ver.cha
B2A12	B2	8	1	0	0	0	8	Ant.syl, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Ran.acr, San.off
B2A13	B2	1	1	0	0	1	0	Pla.lan
B2A14	B2	8	4	2	2	2	2	Luz.cam, Phl.pra, Leo.his, Ver.cha, Kna.arv, San.off, Tri.dub, Tri.hyb
B2A15	B2	1	1	0	1	0	0	Ono.vic
B2A16	B2	4	3	0	1	2	1	Leo.aut, Pla.med, Kna.arv, Vic.cra
B2A17	B2	8	2	0	4	4	0	Gle.hed, Leo.aut, Pla.med, Tar.off, Lat.pra, Tri.cam, Tri.fra, Vic.cra
B2A18	B2	16	4	4	4	4	4	Alo.pra, Bro.hor, Cyn.cri, Poa.pra, Aju.rep, Pla.med, Pri.ver, Ran.rep, Ant.syl, Cam.pat, Car.pra, Ger.pra, Med.lup, Tri.cam, Tri.dub, Tri.rep
B2A19	B2	2	1	0	0	2	0	Pla.med, Tar.off
B2A20	B2	2	2	0	1	1	0	Pla.lan, Tri.dub
B2A21	B2	8	3	0	3	2	3	Leo.his, Pla.med, Cre.bie, Gal.mol, San.off, Lot.cor, Med.lup, Ono.vic
B2A22	B2	16	3	5	6	0	5	Cyn.cri, Fes.pra, Phl.pra, Poa.tri, Tri.fla, Ach.mil, Cam.pat, Cen.jac, Rum.ace, San.off, Lat.pra, Lot.cor, Ono.vic, Tri.hyb, Tri.rep, Vic.cra
B3A01	B3	1	1	0	0	0	1	Gal.mol
B3A02	B3	2	2	1	0	0	1	Fes.pra, Car.car
B3A03	B3	4	3	1	2	1	0	Phl.pra, Pla.med, Tri.hyb, Vic.cra
B3A04	B3	8	1	8	0	0	0	Alo.pra, Arr.ela, Cyn.cri, Dac.glo, Fes.rub, Hol.lan, Poa.tri, Tri.fla

New Phytologist

B3A05	B3	8	3	3	3	0	2	Ant.odo, Bro.ere, Poa.tri, Ant.syl, Leu.vul, Lot.cor, Ono.vic, Tri.hyb
B3A06	B3	1	1	1	0	0	0	Fes.rub
B3A07	B3	8	4	2	2	2	2	Bro.hor, Hol.lan, Pri.ver, Ran.rep, Her.sph, Leu.vul, Med.lup, Ono.vic
B3A08	B3	2	1	2	0	0	0	Dac.glo, Fes.pra
B3A09	B3	16	1	16	0	0	0	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam, Phl.pra, Poa.pra, Poa.tri, Tri.fla
B3A11	B3	4	2	2	0	2	0	Bro.ere, Poa.tri, Pla.lan, Pru.vul
B3A12	B3	1	1	0	1	0	0	Lat.pra
B3A13	B3	4	1	4	0	0	0	Alo.pra, Ant.odo, Bro.ere, Poa.tri
B3A14	B3	60	4	16	12	12	20	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam, Phl.pra, Poa.pra, Poa.tri, Tri.fla, Aju.rep, Bel.per, Gle.hed, Leo.aut, Leo.his, Pla.lan, Pla.med, Pri.ver, Pru.vul, Ran.rep, Tar.off, Ver.cha, Ach.mil, Ant.syl, Cam.pat, Car.pra, Car.car, Cen.jac, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Pas.sat, Pim.maj, Ran.acr, Rum.ace, San.off, Tra.pra, Lat.pra, Lot.cor, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B3A16	B3	16	2	0	8	8	0	Aju.rep, Gle.hed, Leo.his, Pla.med, Pru.vul, Ran.rep, Tar.off, Ver.cha, Lat.pra, Med.lup, Ono.vic, Tri.cam, Tri.fra, Tri.hyb, Tri.rep, Vic.cra
B3A17	B3	1	1	0	0	1	0	Ver.cha
B3A19	B3	2	2	1	0	1	0	Tri.fla, Tar.off
B3A20	B3	8	2	0	4	0	4	Cam.pat, Car.pra, Her.sph, Kna.arv, Lot.cor, Tri.cam, Tri.fra, Tri.hyb
B3A21	B3	2	1	0	2	0	0	Lot.cor, Tri.pra
B3A22	B3	16	4	4	4	4	4	Ant.odo, Bro.ere, Fes.rub, Phl.pra, Aju.rep, Bel.per, Ran.rep, Ver.cha, Cre.bie, Gal.mol, Ger.pra, Rum.ace, Ono.vic, Tri.dub, Tri.fra, Vic.cra
B3A23	B3	4	4	1	1	1	1	Bro.hor, Ran.rep, Leu.vul, Tri.fra
B3A24	B3	16	3	6	5	5	0	Ant.odo, Arr.ela, Ave.pub, Bro.hor, Fes.pra, Poa.tri, Aju.rep, Gle.hed, Pru.vul, Ran.rep, Tar.off, Lot.cor, Med.var, Tri.pra, Tri.rep, Vic.cra
B4A01	B4	60	4	16	12	12	20	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam, Phl.pra, Poa.pra, Poa.tri, Tri.fla, Aju.rep, Bel.per, Gle.hed, Leo.aut,

New Phytologist

								Leo.his, Pla.lan, Pla.med, Pri.ver, Pru.vul, Ran.rep, Tar.off, Ver.cha, Ach.mil, Ant.syl, Cam.pat, Car.pra, Car.car, Cen.jac, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Pas.sat, Pim.maj, Ran.acr, Rum.ace, San.off, Tra.pra, Lat.pra, Lot.cor, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B4A02	B4	16	3	5	0	5	6	Arr.ela, Cyn.cri, Luz.cam, Phl.pra, Poa.pra, Gle.hed, Leo.his, Pla.med, Ran.rep, Tar.off, Ant.syl, Gal.mol, Her.sph, Kna.arv, Pas.sat, Ran.acr
B4A04	B4	4	4	1	1	1	1	Arr.ela, Pla.lan, Ant.syl, Tri.cam
B4A06	B4	8	1	0	0	8	0	Aju.rep, Bel.per, Gle.hed, Leo.aut, Pri.ver, Pru.vul, Tar.off, Ver.cha
B4A07	B4	4	2	0	2	0	2	Car.pra, Cre.bie, Med.lup, Tri.rep
B4A08	B4	8	2	4	0	4	0	Ant.odo, Ave.pub, Bro.hor, Fes.rub, Aju.rep, Pla.lan, Tar.off, Ver.cha
B4A09	B4	1	1	0	1	0	0	Tri.rep
B4A10	B4	8	3	2	0	3	3	Bro.ere, Fes.pra, Aju.rep, Pla.med, Pri.ver, Ach.mil, Car.car, Pim.maj
B4A11	B4	4	3	1	1	0	2	Tri.fla, Her.sph, Tra.pra, Med.var
B4A12	B4	1	1	1	0	0	0	Poa.pra
B4A13	B4	1	1	0	0	0	1	Cir.ole
B4A14	B4	2	1	0	0	2	0	Bel.per, Pla.lan
B4A15	B4	2	2	0	1	1	0	Ran.rep, Med.lup
B4A16	B4	8	4	2	2	2	2	Phl.pra, Poa.tri, Pri.ver, Tar.off, Ant.syl, San.off, Tri.dub, Tri.fra
B4A17	B4	2	1	0	0	0	2	Dau.car, Her.sph
B4A18	B4	16	4	4	4	4	4	Alo.pra, Bro.hor, Cyn.cri, Luz.cam, Leo.aut, Pla.med, Tar.off, Ver.cha, Car.car, Cre.bie, Her.sph, Pim.maj, Lat.pra, Ono.vic, Tri.cam, Tri.hyb
B4A20	B4	16	2	0	8	0	8	Ant.syl, Cam.pat, Car.pra, Cen.jac, Cir.ole, Ger.pra, Rum.ace, Tra.pra, Med.var, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B4A21	B4	2	2	0	1	0	1	Dau.car, Med.var
B4A22	B4	4	1	0	0	0	4	Cam.pat, Car.pra, Ger.pra, Kna.arv

New Phytologist

Dataset S1 List of barcode sequences used for the metabarcoding (Excel file). The file contains the necessary information for demultiplexing the Illumina sequencing data in mothur.

Dataset S2 Custom Oomycota ITS1 database (FASTA file). We obtained the initial reference database of 197,400 ITS sequences from Fiore-Donno and Bonkowski (2021). To update this database, we downloaded newly published Oomycota ITS1 sequences from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using an advanced search ((ITS1 OR internal transcribed spacer 1) AND 400:6000[Sequence Length] AND ("2017/08/24"[Publication Date] : "2021/01/26"[Publication Date]), species filter: "Oomycetes"), resulting in an additional 7,484 sequences. Those sequences were clustered using CD-HIT (Li & Godzik, 2006; Fu *et al.*, 2012) (current link: https://sites.google.com/view/cd-hit/home; former link: http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi?cmd=cd-hit-est) at a 96% similarity threshold, and poorly determined species were manually removed. The clustered and filtered sequences were then merged with the database from Fiore-Donno and Bonkowski (2021) and clustered again to remove duplicates. The final database comprised 41,069 sequences. The genus *Pythium* has undergone recent taxonomic revisions, resulting in its division into several new genera (Uzuhashi *et al.*, 2010). However, these changes are not consistently reflected in either the Fiore-Donno and Bonkowski (2021) database or in GenBank: Some sequences were correctly assigned to the newly established genera *Ovatisporangium*, *Pilasporangium*, *Globisporangium*, and *Elongisporangium*, while others remained under the name *Pythium*. For consistency, we reassigned all sequences from the newly established genera to *Pythium*, and thus *Pythium* in our database should be interpreted broadly (*sensu lato*), encompassing *Pythium sensu stricto* as well as the abovementioned novel genera. Accurate differentiation among these genera requires manual re-blasting of *Pythium sensu lato* sequences against GenBank and comparison across multiple sequence matches.

Dataset S3 Detailed statistical results of alpha and beta diversity analysis (Excel file). Contains models using type I SS ANOVA with different fitting sequences of the factors, and type III SS ANOVA (alpha diversity), as well as PERMANOVA results with different fitting sequences (beta diversity).

References:

Fiore-Donno AM, Bonkowski M. 2021. Different community compositions between obligate and facultative oomycete plant parasites in a landscape-scale metabarcoding survey. *Biology and Fertility of Soils* **57**(2): 245-256.

Fu L, Niu B, Zhu Z, Wu S, Li W. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* **28**(23): 3150-3152.

Li W, Godzik A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**(13): 1658-1659.

Uzuhashi S, Kakishima M, Tojo M. 2010. Phylogeny of the genus *Pythium* and description of new genera. *Mycoscience* **51**(5): 337-365.

Chapter 2: Plant-driven Soil Microbial Legacies Strengthen over Time and Include Widespread Suppression

Unsubmitted manuscript, in preparation:

Solbach MD, Hennecke J, Chen Y, Wagner K, Albracht C, Bassi L, Eisenhauer N, Fox A, Heintz-Buschart A, Lange M, Mommer L, Pinheiro Alves de Souza Y, Rai A, Weigelt A, Bonkowski M. (n.d.) Plant-driven soil microbial legacies strengthen over time and include widespread suppression. *Unsubmitted manuscript*.

Declaration of personal contributions:

I contributed to the sampling and sample preparation together with J Hennecke, C Albracht, L Bassi, A Fox, M Lange, Y Pinheiro Alves de Souza, and A Rai; the sampling campaign was supervised by A Weigelt. I performed the DNA extractions. I conducted the laboratory work for the sequencing of Cercozoa and Oomycota with help from K Wagner and performed the bioinformatic data processing. The sequencing data of fungi were provided by J Hennecke, and the sequencing data of bacteria were provided by Y Chen. I performed all statistical analyses and visualization of the results. I wrote the initial manuscript, with revisions by M Bonkowski and contributions by all other co-authors.

Plant-driven soil microbial legacies strengthen over time and include widespread suppression

(This manuscript is intended for submission to *Nature Communications* and therefore adheres to the journal's formatting, structure, and citation style.)

Authors

Marcel Dominik Solbach[1*], Justus Hennecke[2,3,4], Yan Chen[5], Katalin Wagner[1], Cynthia Albracht[6,7,8], Leonardo Bassi[2,3,9], Nico Eisenhauer[2,3], Aaron Fox[10,11], Anna Heintz-Buschart[8], Markus Lange[12], Liesje Mommer[4], Yuri Pinheiro Alves de Souza[11,13], Akanksha Rai[12], Alexandra Weigelt[2,3], Michael Bonkowski[1*]

[1] *Terrestrial Ecology, Institute of Zoology, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, 50674 Cologne, Germany*
[2] *Institute of Biology, Leipzig University, 04103 Leipzig, Germany*
[3] *German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, 04103 Leipzig, Germany*
[4] *Forest Ecology and Forest Management Group, Wageningen University and Research, 6708 PB Wageningen, The Netherlands*
[5] *Institute of Soil Science, Chinese Academy of Science, 210008 Nanjing, China*
[6] *Dept. Soil Ecology, Helmholtz-Centre for Environmental Research – UFZ Halle, 06120 Halle, Germany*
[7] *Institute for Biosafety in Plant Biotechnology, Julius Kühn-Institute, 06484 Quedlinburg, Germany*
[8] *Biosystems Data Analysis, Swammerdam Institute for Life Sciences, University of Amsterdam, 1098 XH Amsterdam, The Netherlands*
[9] *Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), 6708 PB Wageningen, The Netherlands*
[10] *Teagasc, Crops, Environment and Land-Use Programme, Johnstown Castle, Y35 TC97 Co. Wexford, Ireland*
[11] *TUM School of Life Science, Chair of Environmental Microbiology, Technische Universität München, 85354 Freising, Germany*
[12] *Max Planck Institute for Biogeochemistry, 07745 Jena, Germany*
[13] *Research Unit Comparative Microbiome Analysis, Helmholtz Zentrum München, 85764 Neuherberg, Germany*

*Correspondence:

Marcel Dominik Solbach, E-mail: marcel.solbach@uni-koeln.de

Michael Bonkowski, E-mail: m.bonkowski@uni-koeln.de

Keywords:

plant–soil feedbacks, soil legacy effects, dilution effects, plant pathogens, plant microbiome

Abstract

Plants create lasting changes in soil microbial communities that persist beyond individual plant lifespans, yet how different microbial groups contribute to soil legacy effects remains unclear. We established three treatments of predefined plant communities within a long-term grassland biodiversity experiment spanning a plant diversity gradient (1–60 species): newly sown plant communities in new soil, resown plant communities in soil conditioned by the same community composition for 14 years, and original plant communities maintained in their own conditioned soil. Metabarcoding of fungi, Oomycota, Cercozoa, and bacteria five years after treatment establishment revealed that plant species and plant diversity induced persistent shifts in soil microbial communities. These legacies were substantially stronger after 19 years of shared soil and plant history than after five years, as demonstrated by beta diversity and differential abundance analyses, and were largely independent of coevolved plants. Increasing plant diversity reduced dominance of several microbial groups, leading to higher alpha diversity. Notably, the reduction of microbial taxa by plant species and diversity was sometimes more pronounced than the enrichment, suggesting that microbial suppression is an underappreciated mechanism by which plant communities create lasting soil microbial legacies, with profound implications for understanding plant–soil feedbacks and ecosystem functioning.

(199 words)

Introduction

Plant communities fundamentally alter soil environments, creating microbial legacies that persist after individual plants die[1,2,3,4,5]. Resulting plant–soil feedbacks (PSFs) influence plant performance, community assembly, and ecosystem functioning[6,7], yet the mechanisms creating persistent plant-induced microbial soil legacies remain poorly understood. Two contrasting processes could drive legacy formation: selective enrichment of beneficial microbes[8,9] versus active suppression of potential pathogens or unwanted taxa through antimicrobial compounds[10,11]. Determining which mechanism predominates is crucial for predicting how plant diversity influences soil functioning and plant community dynamics[12].

Biodiversity–ecosystem functioning (BEF) research has consistently shown that higher plant species richness (plant diversity from hereon) enhances ecosystem productivity and stability[13,14,15]. While these relationships were long attributed to resource–use complementarity among plant species, belowground interactions are increasingly recognized as key mediators of BEF effects[12,16,17,18]. Plant diversity can influence the composition and functioning of soil microbiomes, thereby altering feedbacks that regulate plant growth and coexistence. Negative PSFs, often attributed to the accumulation of host-specific soil pathogens, may limit plant performance in monocultures, whereas positive PSFs mediated by mycorrhizal fungi or growth-promoting bacteria can enhance productivity[7,19,20]. In diverse plant communities, dilution of host-specific pathogens and a broader array of beneficial microbial associations may shift the balance from negative toward positive feedbacks, providing mechanisms through which microbial diversity promotes ecosystem functioning[21,22,23].

Strength and direction of plant–soil feedbacks have traditionally been quantified by measuring plant performance[19,24]. This plant-centered approach, however, somewhat neglects the microbial processes underlying the PSFs. Despite evidence for temporal shifts in soil microbiomes[25,26], quantifying the strength of PSFs from the microbial perspective remains a major gap in understanding how microbial legacies form in soils.

The challenge in studying plant community-induced soil legacy effects lies in separating temporal accumulation from environmental fluctuations[1]. Most studies examine contemporary plant–microbe interactions rather than the lasting imprints that past plant generations leave in soil. We addressed this gap by using the long-term Jena biodiversity

87 experiment[27,28], where we could manipulate soil history independently of direct microbe–
88 plant coevolution and of the current calendar year. In 2016, three treatments were
89 established using the plant communities of the 14-year-old Jena Experiment to isolate
90 different components of plant–soil interactions[29] (Fig. 1): "Novel interactions" (−SH −PH): new
91 soil from an adjacent field sown with commercially sourced seeds, representing initial
92 colonization without a shared growth history of the plant communities in the soil; "Soil
93 history" (+SH −PH): 14-year-old experimental soil conditioned by preexisting plant
94 communities of the same composition, resown with commercially sourced seeds, isolating
95 effects of soil history including potential microbial legacies; and "Coupled history" (+SH +PH):
96 unchanged plant communities with both soil and plant histories from 14 years of coevolution.
97 This design allowed us to separate potential soil microbial legacy effects from direct plant–
98 microbe coevolution across fixed plant communities in a plant diversity gradient (1, 2, 4, 8, 16,
99 and 60 species; Supplementary Tables 1 & 2). After additional five years of growth (2016–
100 2021), we analyzed bulk soil microbial communities using metabarcoding of fungi (ITS2),
101 oomycetes (ITS1), cercozoan protists (18S V4), and bacteria (16S V4) to capture the lasting
102 legacy effects that persist in soil after multiple plant generations. These groups represent key
103 functional guilds in soil: fungi include both plant-pathogenic and non-pathogenic saprotrophic
104 taxa[30]; most Oomycota are saprotrophs or facultative/opportunistic plant pathogens such as
105 *Pythium* or *Phytophthora*[31]; Cercozoa comprise mainly microbial consumers alongside the
106 plant-parasitic Phytomyxea[32,33]; and soil bacteria encompass diverse plant-associated taxa
107 with key roles in nutrient cycling and plant growth promotion, including decomposition and
108 nutrient mineralization[34].

109 We hypothesize that long-term plant–soil interactions lead to increasing microbial legacy
110 effects characterized by both the enrichment and suppression of specific microbial taxa, with
111 especially pronounced responses in parasitic groups (pathogenic fungi, Oomycota, and
112 Phytomyxea) due to the accumulation of long-lived propagules (e.g., chlamydospores/conidia,
113 oospores, and resting spores) in the soil. We therefore expect plant-driven soil legacies to
114 persist after plant communities are resown and to be stronger in soils with 19 years of shared
115 history than in soils with only 5 years of shared history.

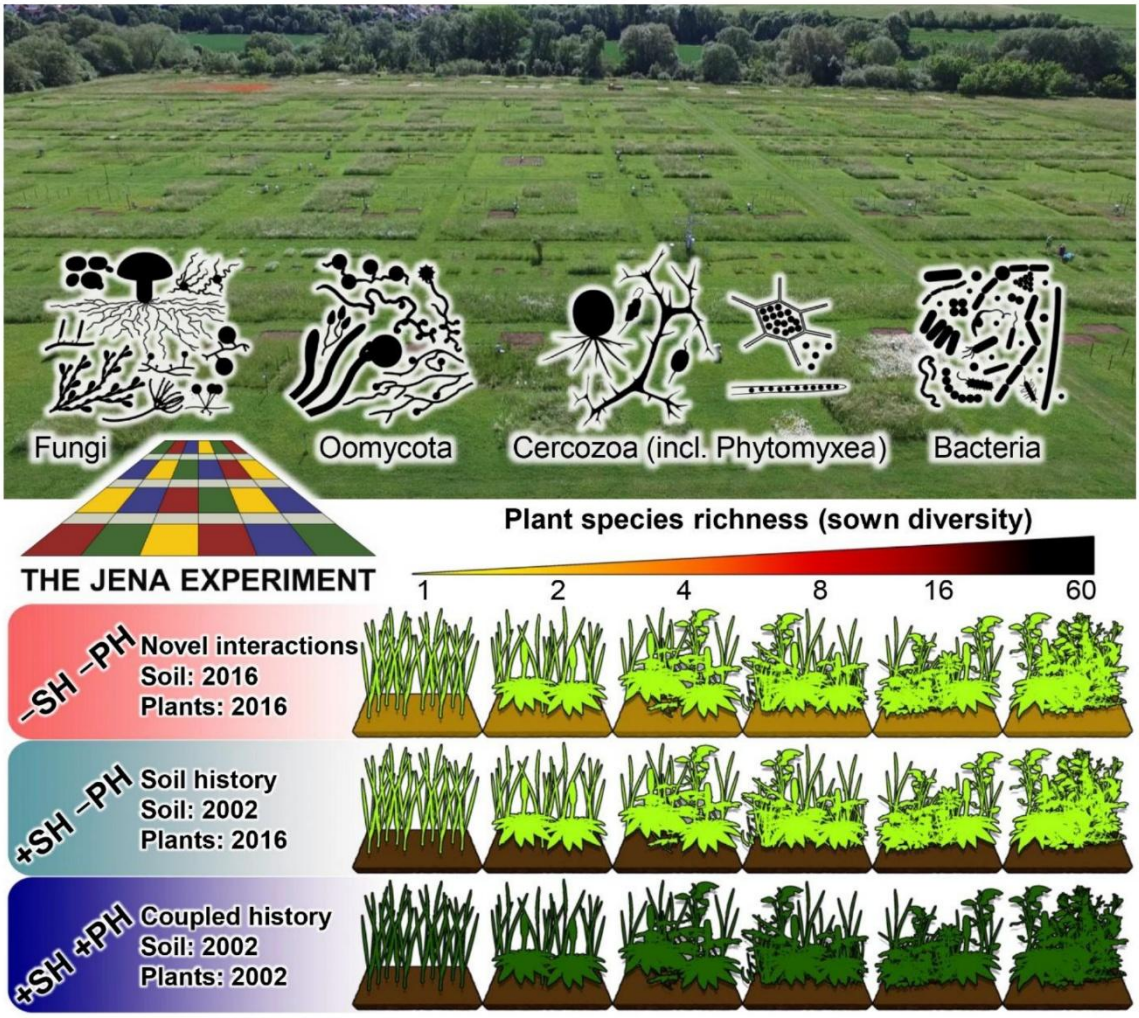


Fig. 1 | Schematic overview of the experimental design. The main Jena Experiment consists of 80 plots with differing predefined plant communities across a plant diversity gradient ranging from 1 to 60 plant species per plot. Each plot was subdivided into three subplots differing in their soil and plant history (–SH –PH: "Novel interactions"; +SH –PH: "Soil legacy"; +SH +PH: "Coupled legacy"). Bulk soil samples were collected in 2021, i.e., 19 years after the establishment of the original plots of the Jena Experiment, and 5 years after treatment establishment. DNA extracts were subjected to sequencing of fungi, Oomycota, Cercozoa (including plant-parasitic Phytomyxea), and bacteria. Photo credit and logo: The Jena Experiment; Illustrations: M. D. Solbach.

Results and Discussion

Composition of fungal, oomycete, cercozoan, and bacterial communities

Fungal communities were dominated by Ascomycota (mainly Sordariomycetes, Dothideomycetes, and Leotiomycetes), followed by Basidiomycota (Agaricomycetes, Tremellomycetes) and Mortierellomycota (Fig. 2a, Supplementary Fig. 1). Oomycota were almost exclusively represented by Peronosporomycetes, with *Globisporangium* and *Pythium*

sensu stricto as the predominant genera (Fig. 2b, Supplementary Fig. 2). Within Cercozoa, approximately 5% of reads corresponded to plant-parasitic Plasmodiophorida (class Phytomyxea), while the remainder was dominated by consumers such as Vampyrellida (naked amoebae), Cercomonadida and Glissomonadida (naked flagellates), and Cryomonadida (mostly thecate amoebae) (Fig. 2c, Supplementary Fig. 3). Bacterial communities were dominated by Actinobacteriota (e.g., Thermoleophilia and Actinobacteria), Acidobacteriota (e.g., Vicinamibacteria), Proteobacteria (e.g., Alphaproteobacteria and Gammaproteobacteria), Planctomycetota, and Chloroflexi (e.g., Chloroflexia and KD4-96), as well as several less abundant and unclassified taxa (Fig. 2d, Supplementary Fig. 4).

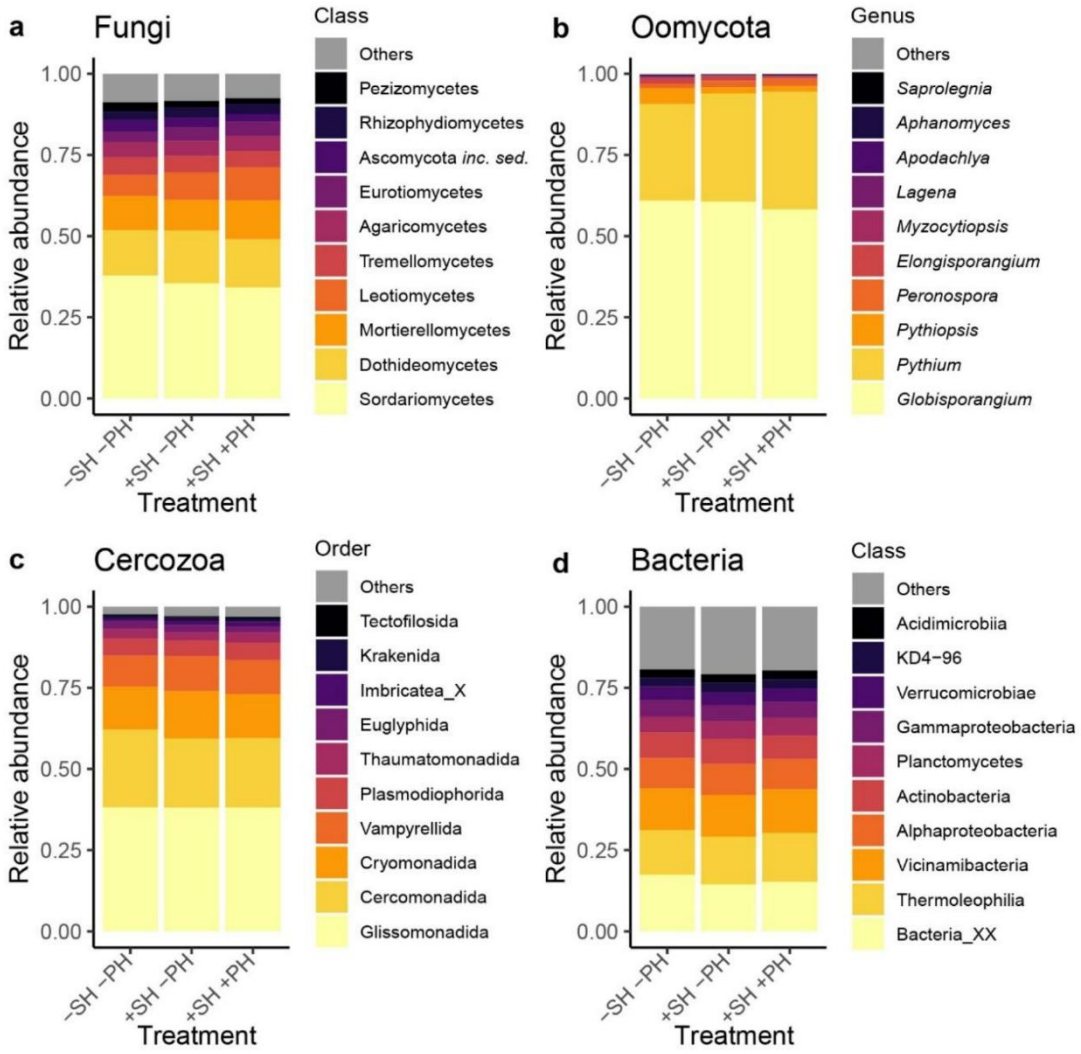


Fig. 2 | Relative microbial abundances. Stacked bar plots displaying the overall community compositions of the investigated microbial taxa as relative abundances based on sequence reads across the three history treatments. Only the 10 most abundant classes, orders, or genera, respectively, are displayed; the rest is summarized in "Others". a: Fungi; b: Oomycota; c: Cercozoa; d: Bacteria. For overviews with more taxonomic ranks, please refer to Supplementary Figs. 1–4.

Plant species create distinct microbial legacies that strengthen with time

Differential abundance analysis across all 60 plant species revealed that plant species influence microbial taxa in different ways, with some taxa predominantly being reduced while others predominantly being enriched (Fig. 3, Supplementary Fig. 5). For plant-pathogenic fungi, each plant species reduced more operational taxonomic units (OTUs) than it enriched (Fig. 3a). This suppressive signature was also evident in *Globisporangium* oomycetes (Fig. 3b), and notably also in cercozoan consumers with coupled history (+SH +PH; Fig. 3c) and bacteria with soil history and coupled history (+SH −PH and +SH +PH; Fig. 3d). Non-plant-pathogenic fungi (Fig. 3a), Oomycota of the genus *Pythium* (Fig. 3b), as well as Phytomyxea (Fig. 3c), and bacteria in novel interactions (−SH −PH) (Fig. 3d) showed the opposite trend with more OTUs being enriched than reduced.

The strength of these legacy effects increased dramatically with soil history. Novel interactions (−SH −PH) showed relatively weak plant-specific effects, while soil history (+SH −PH) and especially coupled history (+SH +PH) treatments exhibited stronger patterns (Fig. 3), demonstrating that microbial legacies accumulate over time in soil, but the direction of the effect (mostly facilitative or suppressive) depends on the microbial group.

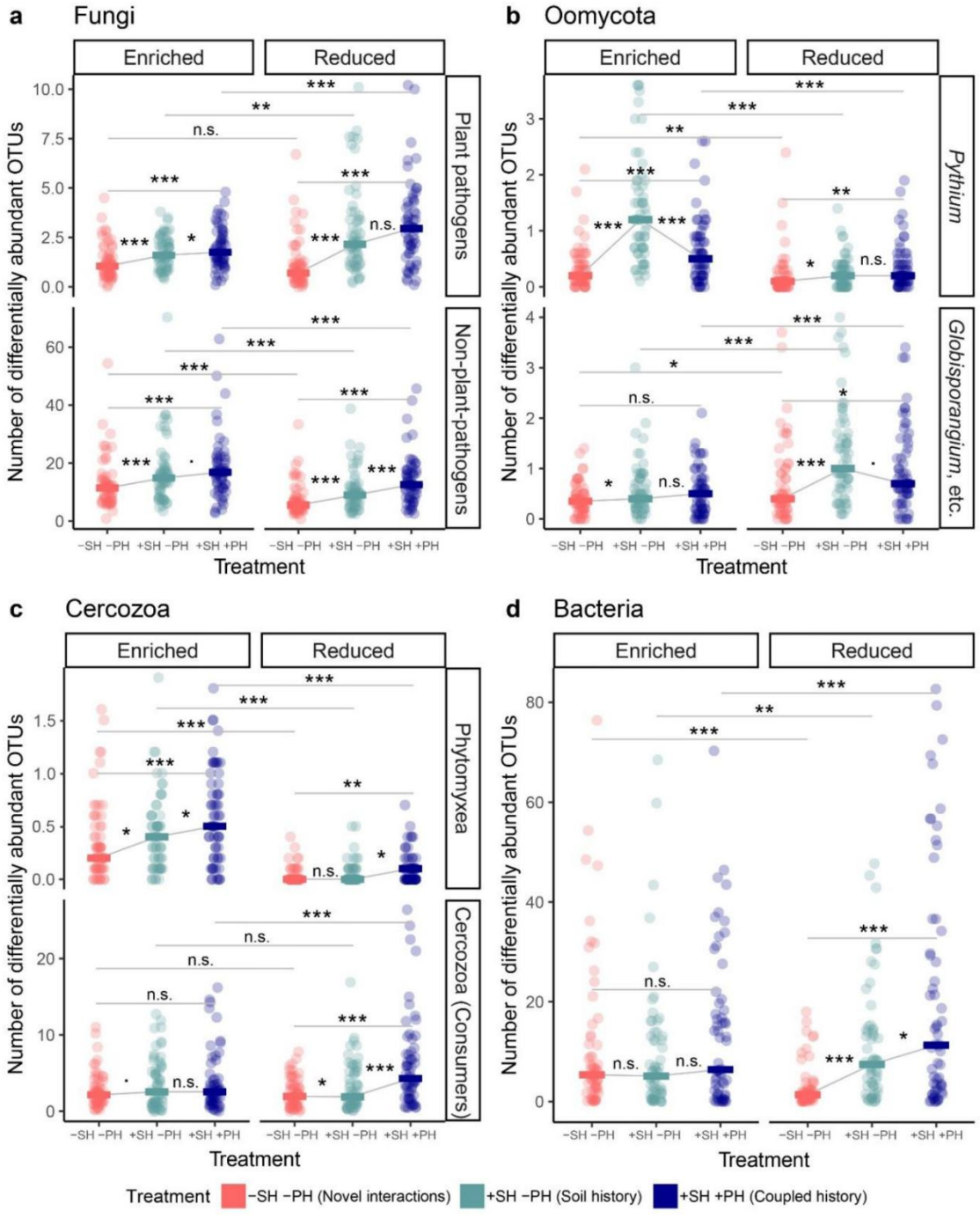


Fig. 3 | Summary of plant species-specific differential abundance analysis. Dots represent the number of differentially abundant OTUs in dependence of the 60 plant species from DESeq2 analyses (Supplementary Fig. 5), differentiated by the three treatments (red: −SH −PH (Novel interactions); teal: +SH −PH (Soil history); blue: +SH +PH (Coupled history)). Horizontal bars indicate group medians. "Enriched" OTUs were more abundant in plots with the respective plant species/less abundant in plots without the respective plant species, and "reduced" OTUs were less abundant in plots with the respective plant species/more abundant in plots without the respective plant species. Statistical significances were obtained from Wilcoxon signed-rank tests for paired samples (Supplementary Table 3). a: Fungi; b: Oomycota; c: Cercozoa; d: Bacteria. Significance code: $p \leq 0.001$: "***"; $p \leq 0.01$: "**"; $p \leq 0.05$: "*"; $p \leq 0.1$: "."; $p > 0.1$: "n.s." (not significant).

Soil history effects exceed direct coevolution effects

Multivariate analyses revealed that all microbial communities were influenced by the history treatments (Supplementary Table 4), with soil history effects vastly exceeding direct plant–microbe coevolution effects (Fig. 4). For all microbial groups, communities in novel interactions (−SH −PH) were clearly distinct from those in soil history (+SH −PH) and coupled history (+SH +PH) treatments (PERMANOVA, all $p \leq 0.001$), while differences between +SH −PH and +SH +PH were generally smaller (Supplementary Table 5). The stark differences between soil histories may partly reflect soil-origin effects rather than legacies driven by the plant communities of the Jena Experiment alone, as the new soil originated from a separately managed, fertilized agricultural field. While this confounds plant-induced microbial legacies with effects of prior land use and soil properties, it mirrors the starting conditions of the Jena Experiment and thus represents a realistic transition from cropland to semi-natural grassland.

This pattern indicates that the major component of legacy effects resides in soil-borne microbial propagules rather than in direct short-term coevolutionary adaptations between plants and microbes, and the loss or gain of plant-driven soil legacies requires multiple plant generations. Consistent with this view, the influence of the plant diversity gradient on microbial communities was generally strongest after 19 years of coupled history (+SH +PH; Fig. 4h). Accordingly, the number of OTUs differentially expressed along the plant diversity gradient increased with soil history, with coupled history (+SH +PH) showing the highest numbers (Fig. 5), mostly mirroring plant species-specific patterns (Fig. 3). The soil mixing and/or removal of old roots during +SH −PH treatment establishment disrupted some microbial communities, revealing that certain microbial legacies depend on intact root-associated reservoirs, but the majority of legacy effects remained in place, even after community reestablishment.

A notable exception was the strong enrichment of *Pythium* (Oomycota) OTUs in the treatment with soil history and new plants (+SH −PH; Fig. 3b and 5b). Given that *Pythium* species are facultative pathogens[35], this enrichment likely indicates lower defense of naïve plants, enabling increased opportunistic infection from accumulated oospores in the older soil. Interestingly, no such pattern was observed for putatively plant-pathogenic fungi or parasitic Phytomyxea.

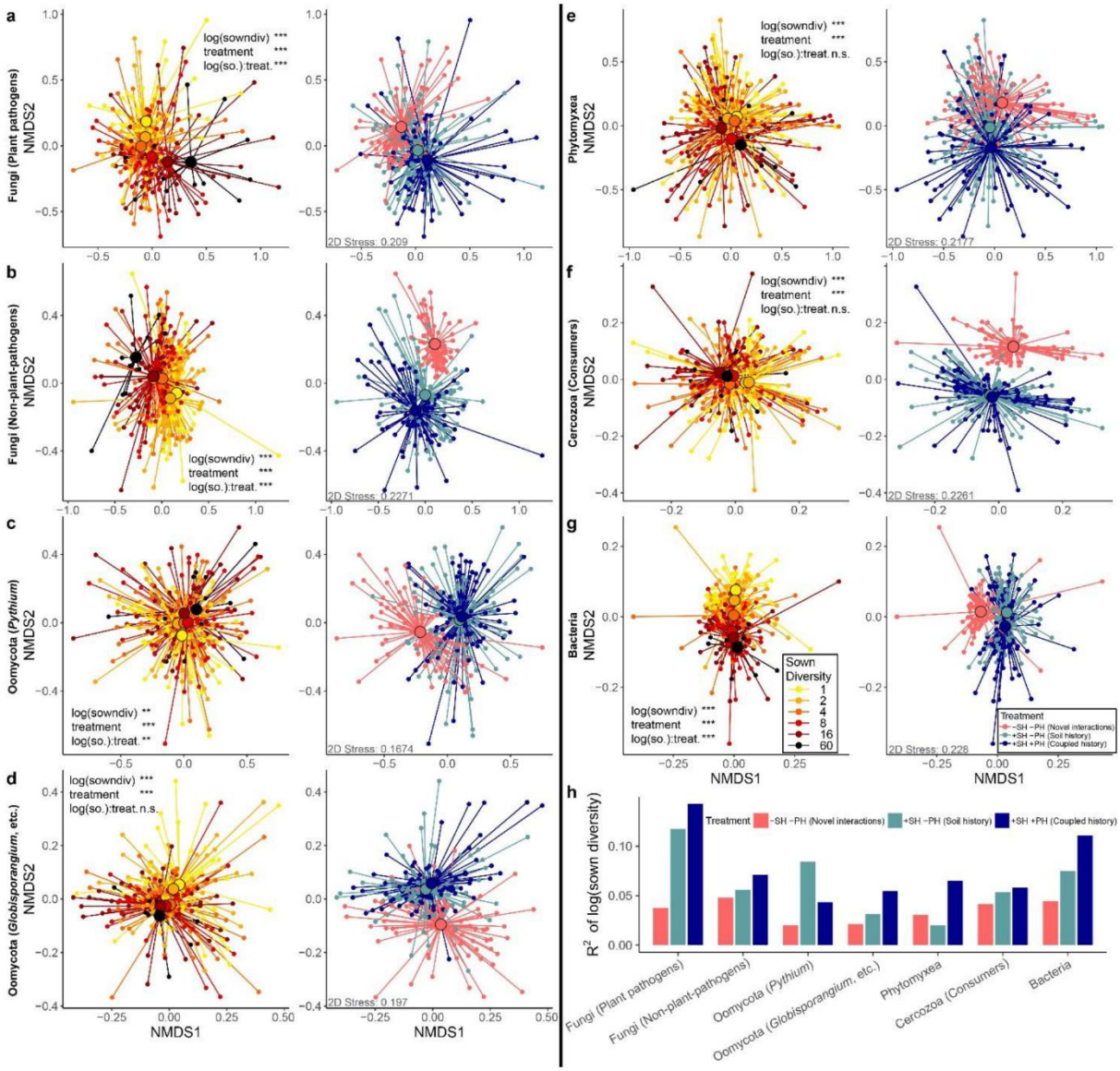
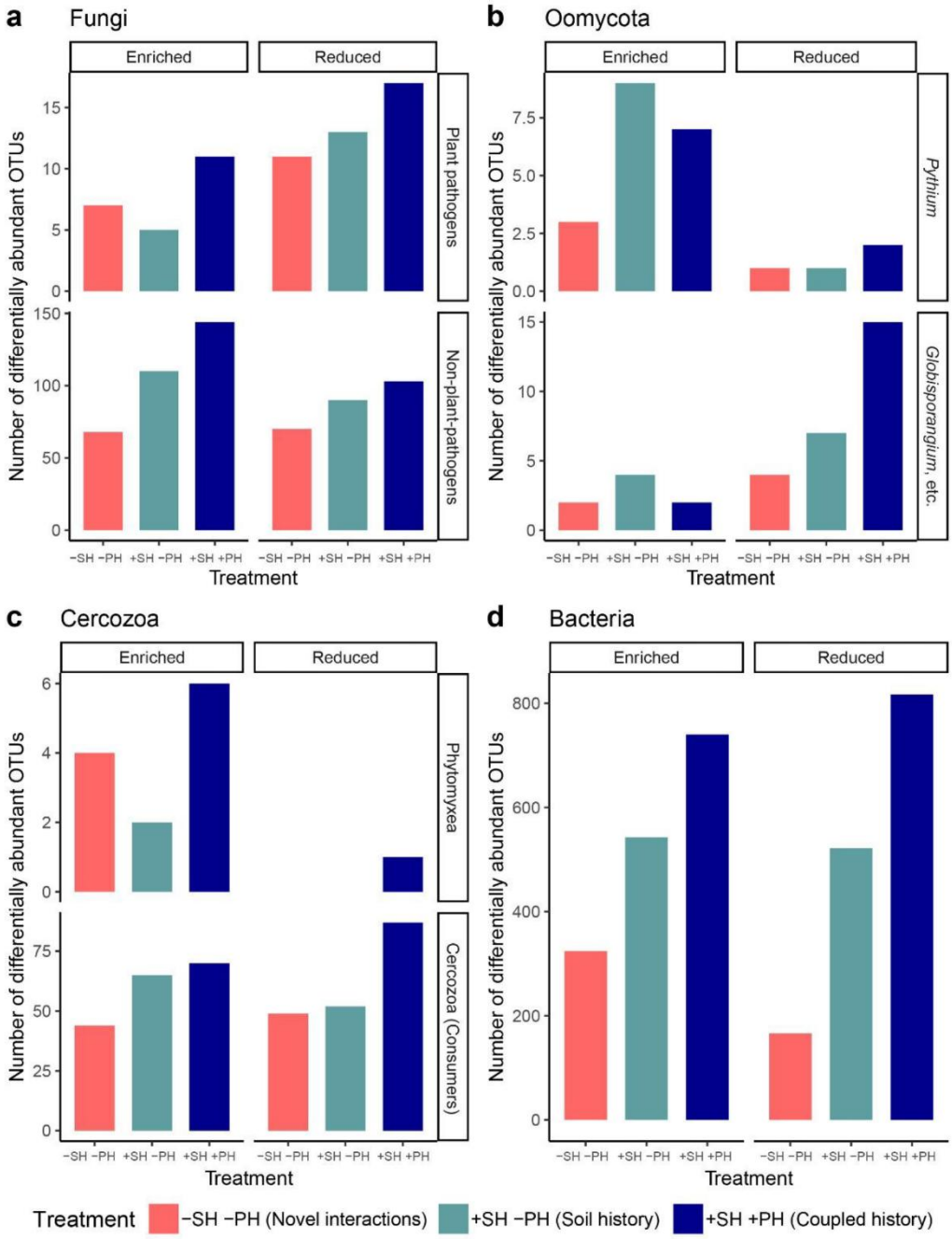


Fig. 4 | Beta diversity analysis. a–g: Non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarities. Statistical significances were obtained by PERMANOVA (Supplementary Table 4). h: Summary of R^2 values of log-transformed sown diversity obtained by PERMANOVA in the individual treatments (Supplementary Table 6). log(sowndiv): Plant diversity as log-transformed sown diversity, linear; log(so.):treat.: interaction of log-transformed sown diversity and treatment. Significance code: $p \leq 0.001$: "***"; $p \leq 0.01$: "**"; $p \leq 0.05$: "*"; $p \leq 0.1$: "."; $p > 0.1$: "n.s." (not significant).



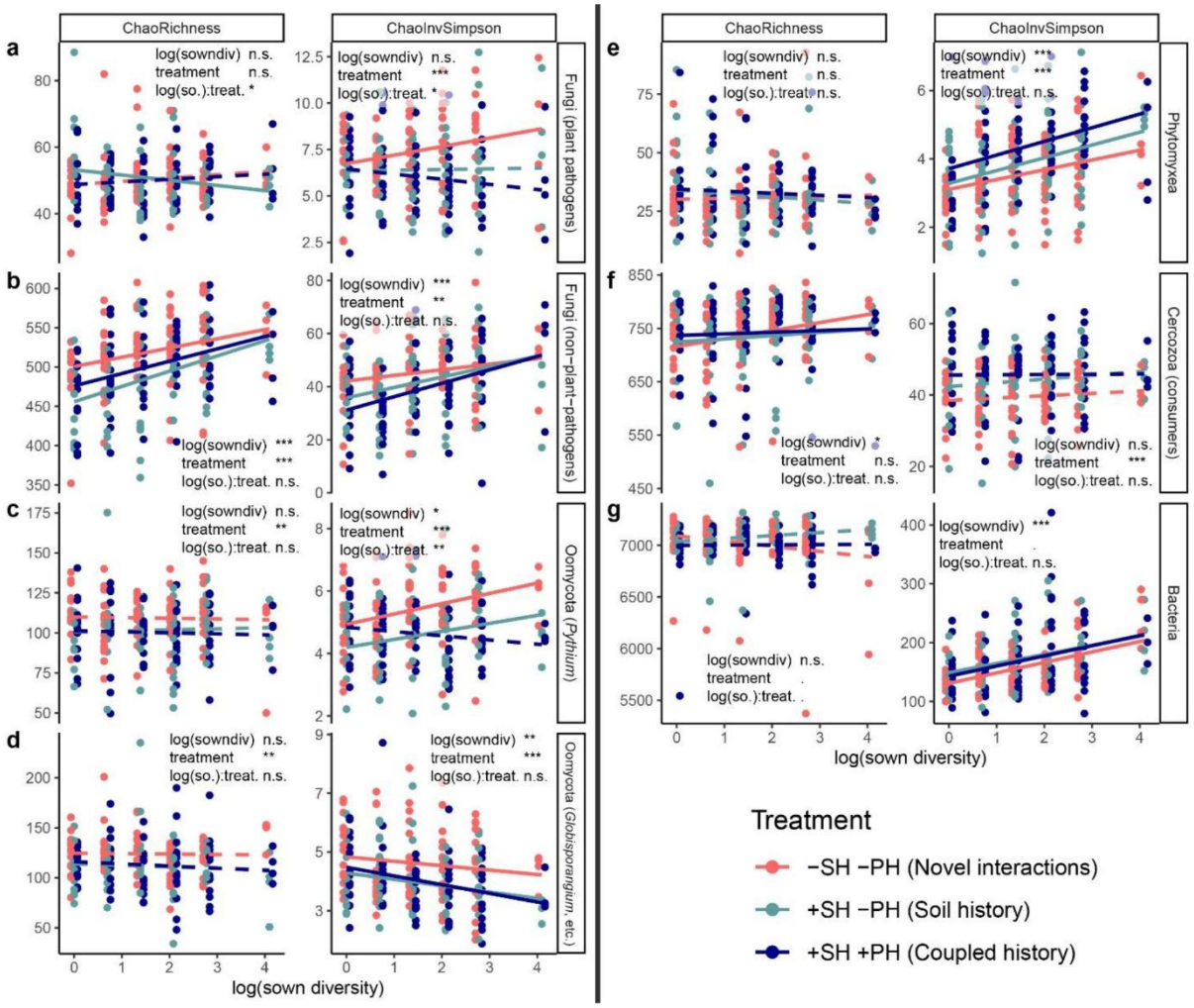
211

212 **Fig. 5 | Summary of differential abundance analysis of plant diversity.** Bars represent the number of
213 differentially abundant OTUs with increasing plant species richness (as log(sown diversity), linear) from
214 DESeq2 analyses, differentiated by the three treatments (red: −SH −PH (Novel interactions); teal: +SH
215 −PH (Soil history); blue: +SH +PH (Coupled history)). “Enriched” OTUs were more abundant in plots
216 with high plant species richness/less abundant in plots with low plant species richness, and “reduced”
217 OTUs were less abundant in plots with high plant species/more abundant in plots with low plant
218 species richness. a: Fungi; b: Oomycota; c: Cercozoa; d: Bacteria.

Plant diversity dilutes dominance

Plant diversity decreased dominance (increased inverse Simpson index) within plant-pathogenic fungi (−SH −PH, Fig. 6a), *Pythium* oomycetes (−SH −PH and +SH −PH, Fig. 6c), and plant-parasitic Phytomyxea (Fig. 6e), potentially supporting the dilution effect/pathogen dilution hypothesis[23,36,37]: In species-poor plant communities, host-specific taxa dominated microbial communities, while diverse plant communities showed more even pathogen distributions. Notably, the same pattern was observed for non-plant-pathogenic fungi (Fig. 6b), as well as bacteria (Fig. 6g), indicating that dilution effects are not exclusive to parasites or pathogenic taxa, but are a general consequence of increased plant diversity for many microbial groups with presumably different ecological functions.

Conversely, plant diversity decreased the inverse Simpson index in *Globisporangium* oomycetes[38] (Fig. 6d) as a result of strong reduction of many subdominant taxa (Figs. 3b, 5b), suggesting that different parasite groups respond differently to plant diversity. Plant diversity also increased OTU richness of non-plant-pathogenic fungi and cercozoan consumers (Fig. 6b,f), suggesting that diverse plant communities foster more complex microbial associations through increased niche availability and competitive interactions.



235

236 **Fig. 6 | Alpha diversity analysis.** Displayed are OTU richness (Hill number q = 0) and inverse Simpson
237 index (Hill number q = 2). Statistical significances were obtained from ANOVA (type I SS) based on the
238 linear mixed effects models (Supplementary Table 7). Trends represent predictions from the models.
239 Solid lines represent a significant effect of plant diversity (as log(sown diversity)), while dashed lines
240 represent a non-significant effect; in case of significant interactions, significance of the slopes of the
241 individual treatments was assessed based on estimated marginal means of linear trends. Significance
242 code: $p \leq 0.001$: "***"; $p \leq 0.01$: "**"; $p \leq 0.05$: "*"; $p \leq 0.1$: "."; $p > 0.1$: "n.s." (not significant).

243

244 **Temporal strengthening of legacy effects**

245 The temporal dimension of our experiment revealed how legacy effects develop. Weak plant-
246 specific effects in novel interactions (−SH −PH) contrasted sharply with strong effects in
247 treatments with longer soil history (+SH −PH, +SH +PH), demonstrating that legacy effects
248 require multiple plant generations to develop. Consistent with this view, bacterial responses
249 showed that overall enrichment and reduction of taxa both increased from −SH −PH to +SH
250 +PH with increasing plant diversity (Fig. 5d). While no general difference in plant species-

specific enrichment was observed between treatments, our results revealed that in novel soil (−SH −PH), plants preferentially enriched bacterial taxa, whereas in treatments with longer soil history (+SH −PH, +SH +PH), suppression of bacterial taxa became the dominant pattern (Fig 3d). This finding explains why short-term studies may miss the dominant role of suppression in plant–soil interactions as was also observed for pathogenic fungi and *Globisporangium* oomycetes.

The accumulation of effects over 19 years, coupled with strong persistence after plant removal (+SH −PH treatment), indicates that plants create chemical or biological changes in soil that outlast individual plant lifespans[39]. These could include antimicrobial compounds[40,41], altered soil chemistry[42,43], or establishment of antagonistic microbial networks[44].

Implications for plant–soil feedback theory

Our findings integrate two previously distinct concepts of plant–soil feedback theory: the recruitment of beneficial microorganisms in plant-specific microbiomes[9,45] and the development of suppressive soils, where continuous monoculture leads to inhibition of specific microbial pathogens[44,46]. By linking these perspectives, we show that for some microbial groups, suppression rather than purely beneficial recruitment, constitutes the dominant and most persistent mechanism underlying soil legacy effects.

These mechanisms provide a foundation for understanding how plant diversity enhances ecosystem-level disease suppression[23,36,47,48]. The temporal strengthening of suppressive effects also explains why emerging plant communities may require time to achieve maximum disease suppression benefits. However, the specific compounds and mechanisms underlying both enrichment and suppression of microbes remain to be identified. Future research should focus on characterizing the chemical and functional signatures of legacies[4,49] as well as key microbial taxa, and determining how they vary among plant species and environmental conditions. Such a mechanistic understanding could inform targeted approaches to enhance ecosystem services in managed systems.

278 ## Methods

279 **Experimental design**

280 The experiment was conducted within the Jena Experiment[27], established in 2002 with
281 different plant communities (Supplementary Table 2) varying in species richness (1, 2, 4, 8, 16,
282 or 60 species) and functional group composition (grasses, legumes, small herbs, tall herbs; 1–
283 4 plant functional groups). In 2016, three 1.5 m × 3 m subplots were established on each of
284 the 80 plots[29]:

285 "Novel interactions" (−SH −PH; "without soil history, without plant history"): Soil was removed
286 to 30 cm depth and replaced with soil from an adjacent agricultural field, equivalent to the
287 original soil from 2002. Sown with commercial seeds matching original plot composition; "Soil
288 history" (+SH −PH; "with soil history, without plant history"): Plant sod removed, soil
289 homogenized to a depth of 30 cm to remove large roots, but soil was retained on subplots.
290 Sown with commercial seeds; "Coupled history" (+SH +PH; "with soil history, with plant
291 history"): Unmodified subplots from the original experiment with continuous plant and soil
292 history since 2002 (Fig. 1).

293

294 **Soil sampling and DNA extraction**

295 Bulk soil was collected in May–June 2021 (19 years after the original establishment of the Jena
296 Experiment, 5 years after treatment establishment). Four soil cores (4 cm diameter, 5 cm
297 depth) were collected per subplot, pooled, and stored at -20°C. DNA was extracted from 0.25–
298 0.30 g of homogenized soil using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo
299 Research).

300

301 **Microbial metabarcoding and bioinformatic processing**

302 Community compositions of fungi, Oomycota, Cercozoa, and bacteria were assessed by
303 metabarcoding (Illumina high-throughput sequencing; Illumina Inc., San Diego, CA, USA) using
304 standard protocols for each microbial group (details in Supplementary Methods). The internal
305 transcribed spacer 2 (ITS2) of fungi was amplified using the primers ITS4 and ITS7:ITS7o[50,51,52].
306 Fungal sequences were partially published by Hennecke et al.[53] (treatment +SH +PH), but all

307 samples were sequenced following the same laboratory protocol. Sequencing was performed
308 with a MiSeq Reagent Kit v.3 (2 x 300 bp) at the Department of Soil Ecology of the Helmholtz-
309 Centre for Environmental Research (UFZ, Halle/Saale, Germany). Oomycota (ITS1) were
310 amplified with a semi-nested approach using the primer pairs S1777F/58SOomR and
311 consecutively S1786StraF/58SOomR[35]. Cercozoa (V4 region; 18S SSU rDNA) were amplified
312 with a semi-nested approach using the primer pairs S615F_Cerco and S615F_Phyt (50%
313 each)/S963R_Phyt and consecutively S615F_Cer/S947R_Cer[54]. Oomycota and Cercozoa were
314 sequenced using MiSeq v.3 (2x 300 bp) at the Cologne Center for Genomics (Cologne,
315 Germany). Bacteria (V4 region; 16S SSU rDNA) were amplified using the primer pair 515F[55]
316 and 806R[56]. Sequencing was performed on an Illumina NovaSeq 6000 (2 x 250 bp) by OE
317 biotech Co., Ltd. (Shanghai, China).

318 Sequences were processed in mothur[57] (fungi, Oomycota, Cercozoa), or Trimmomatic[58],
319 FLASH[59], and QIIME[60] (bacteria). Sequences of all groups were quality-filtered, and clustered
320 to operational taxonomic units (OTUs; 97% similarity threshold) by abundance-based greedy
321 clustering (agc) using VSEARCH v.2.7.1[61].

322 Taxonomic classification was performed using UNITE[62] for fungi, a curated ITS1 database[38] for
323 Oomycota, the PR^2 database[63] for Cercozoa, and SILVA[64] for bacteria. Functional traits were
324 assigned using FungalTraits[30], the Oomycota trait table by Fiore-Donno and Bonkowski[35], or
325 the Cercozoa trait table by Dumack et al.[32].

326

327 **Statistical analyses**

328 Data was statistically analyzed in R (version 4.2.1)[65] and plots were mostly visualized with
329 ggplot2[66] if not stated otherwise. Sankey diagrams showing relative abundances on different
330 taxonomic levels were created with the riverplot package[67] and a function from Freudenthal
331 et al.[68].

332 The fungal dataset was split into “plant pathogens” and non-“plant pathogens” based on the
333 primary lifestyle assigned by the FungalTraits database[30]. The Oomycota dataset was split into
334 *Pythium sensu stricto* (referred to as “*Pythium*”) and remaining Oomycota (dominated by the
335 genus *Globisporangium*; referred to as “*Globisporangium*, etc.”). The Cercozoa dataset was

split into plant-parasitic Plasmodiophorida (class Phytomyxea) and remaining Cercozoa (mostly phagotrophs/consumers). The bacterial dataset was not split.

Differential abundance analysis was performed in DESeq2[69] using the poscounts parameter for size factor estimation to account for zeros in the datasets. The number of differentially abundant OTUs (adjusted p-value ≤0.05) was calculated for each of the 60 plant species and summarized, always comparing all plots with the respective plant species vs. a subset of an equal number of plots without the respective plant species. Random subsampling and subsequent DESeq2 analyses were performed ten times and the average taken per plant species. This analysis was performed independently for each of the three history treatments, then the number of differentially abundant OTUs was counted, summarized, and statistical differences between the treatments were determined by Wilcoxon signed-rank tests for paired samples. Additionally, the number of differentially abundant OTUs as affected by plant species richness (as log(sown diversity), linear) was determined for each of the three treatment levels individually.

Differences in community compositions between the samples (beta diversity) were investigated by multivariate statistical analyses. Total abundances of OTUs were converted to relative abundances, and log(+1)-transformed. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities was performed using vegan::metaMDS[70]. Statistical testing was performed by Permutational Multivariate Analysis of Variance (PERMANOVA, 999 permutations, vegan::adonis2). The PERMANOVA models for beta diversity analysis were designed to reflect the linear mixed models from alpha diversity analysis: “Block” was fitted before the tested factors to remove the effects from the subsequent terms. Plant species richness (log(sown diversity)) was tested using “plot” as the error term, and permutations were only allowed between plots. The history treatment effects and interactions were tested using the residuals as the error term and permutations were only allowed within plots. These procedures were performed to correctly account for the nested experimental design[71]. Additionally, the effect of plant species richness (log(sown diversity)) was tested for the three treatment levels individually using PERMANOVA, and the respective R^2 values of log(sown diversity) were summarized as an effect size estimate.

Alpha diversity indices were estimated from the OTU tables as Hill numbers (effective number of species) using the extrapolation methods from iNEXT[72,73]: OTU richness (Hill number q = 0)

was estimated with iNEXT::ChaoRichness and inverse Simpson index (Hill number q = 2) with iNEXT::ChaoSimpson. For each of these diversity metrics, we analyzed the interactive effect of plant species richness (sown diversity, log-transformed, linear) and the three history treatments using linear mixed models (nlme::lme[74,75]). "Block" was included as a random term to account for the edaphic variation and block-wise weeding, as well as "plot" to account for the nested split-plot design. Statistical analyses of all models were performed with Type I SS (sequential sums of squares) ANOVA.

Data availability

Fungal sequencing data were submitted to the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) as BioProject PRJNA1074103. The raw fastq files of the Illumina sequencing runs of Oomycota and Cercozoa were submitted to the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home) under the project accession number PRJEB77807. This work is based on data elaborated by Subproject 2 — Protist Plant Pathogens of the Jena Experiment, which is funded by the Deutsche Forschungsgemeinschaft (FOR 5000). The datasets are publicly available in the Jena Experiment database (https://jexis.idiv.de/) under the following dataset IDs: 90 (plot info), 233 (plant species info), 793 (fungi, OTU table), 794 (fungi, taxonomic and functional assignment), 524 (Oomycota, OTU table), 518 (Oomycota, taxonomic and functional assignment), 520 (Cercozoa, OTU table), 517 (Cercozoa, taxonomic and functional assignment), 599 (bacteria, OTU table), 603 (bacteria, taxonomic assignment), 809 (R code).

References

1. Kardol P, Cornips NJ, van Kempen MML, Bakx-Schotman JMT, van der Putten WH. Microbe-Mediated Plant–Soil Feedback Causes Historical Contingency Effects in Plant Community Assembly. *Ecol. Monogr.* **77**, 147-162 (2007).
2. Bever JD. Feedback between plants and their soil communities in an old field community. *Ecology* **75**, 1965-1977 (1994).
3. Wubs ERJ*, et al.* Single introductions of soil biota and plants generate long-term legacies in soil and plant community assembly. *Ecol. Lett.* **22**, 1145-1151 (2019).

4. Nannipieri P, Hannula SE, Pietramellara G, Schloter M, Sizmur T, Pathan SI. Legacy effects of rhizodeposits on soil microbiomes: A perspective. *Soil Biol. Biochem.* **184**, 109107 (2023).
5. Frouz J. Plant-soil feedback across spatiotemporal scales from immediate effects to legacy. *Soil Biol. Biochem.* **189**, 109289 (2024).
6. Lekberg Y*, et al.* Relative importance of competition and plant–soil feedback, their synergy, context dependency and implications for coexistence. *Ecol. Lett.* **21**, 1268-1281 (2018).
7. van der Putten WH*, et al.* Plant–soil feedbacks: the past, the present and future challenges. *J. Ecol.* **101**, 265-276 (2013).
8. Badri DV, Vivanco JM. Regulation and function of root exudates. *Plant, Cell Environ.* **32**, 666-681 (2009).
9. Hu L*, et al.* Root exudate metabolites drive plant–soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nat Commun* **9**, 2738 (2018).
10. Weller DM, Raaijmakers JM, Gardener BB, Thomashow LS. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.* **40**, 309-348 (2002).
11. Chapelle E, Mendes R, Bakker PA, Raaijmakers JM. Fungal invasion of the rhizosphere microbiome. *ISME J.* **10**, 265-268 (2015).
12. Eisenhauer N*, et al.* The multiple-mechanisms hypothesis of biodiversity–stability relationships. *Basic Appl. Ecol.* **79**, 153-166 (2024).
13. Cardinale BJ*, et al.* Biodiversity loss and its impact on humanity. *Nature* **486**, 59-67 (2012).
14. Isbell F*, et al.* Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature* **526**, 574-577 (2015).
15. Tilman D. An evolutionary approach to ecosystem functioning. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 10979-10980 (2001).
16. Eisenhauer N, Reich PB, Scheu S. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic Appl. Ecol.* **13**, 571-578 (2012).
17. Schnitzer SA, Klironomos J. Soil microbes regulate ecosystem productivity and maintain species diversity. *Plant Signal Behav.* **6**, 1240-1243 (2011).
18. Thakur MP*, et al.* Plant–Soil Feedbacks and Temporal Dynamics of Plant Diversity–Productivity Relationships. *Trends Ecol. Evol.* **36**, 651-661 (2021).
19. Bever JD, Westover KM, Antonovics J. Incorporating the Soil Community into Plant Population Dynamics: The Utility of the Feedback Approach. *J. Ecol.* **85**, 561-573 (1997).
20. Hannula SE*, et al.* Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. *Nat Commun* **12**, 5686 (2021).

21. Mommer L*, et al.* Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. *New Phytol.* **218**, 542-553 (2018).
22. Maron JL, Marler M, Klironomos JN, Cleveland CC. Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecol. Lett.* **14**, 36-41 (2011).
23. Wang G*, et al.* Dilution of specialist pathogens drives productivity benefits from diversity in plant mixtures. *Nat Commun* **14**, 8417 (2023).
24. Kulmatiski A, Beard KH, Stevens JR, Cobbold SM. Plant-soil feedbacks: a meta-analytical review. *Ecol. Lett.* **11**, 980-992 (2008).
25. Schmid MW*, et al.* Effects of plant community history, soil legacy and plant diversity on soil microbial communities. *J. Ecol.* **109**, 3007-3023 (2021).
26. Albracht C*, et al.* Common soil history is more important than plant history for arbuscular mycorrhizal community assembly in an experimental grassland diversity gradient. *Biol. Fertility Soils* **60**, 547–562 (2024).
27. Roscher C*, et al.* The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic Appl. Ecol.* **5**, 107-121 (2004).
28. Weisser WW*, et al.* Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic Appl. Ecol.* **23**, 1-73 (2017).
29. Vogel A*, et al.* A new experimental approach to test why biodiversity effects strengthen as ecosystems age. In: *Mechanisms underlying the relationship between biodiversity and ecosystem function* (eds Eisenhauer N, Bohan DA, Dumbrell AJ). Academic Press (2019).
30. Põlme S*, et al.* FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Divers.* **105**, 1-16 (2021).
31. Thines M, Kamoun S. Oomycete-plant coevolution: recent advances and future prospects. *Curr. Opin. Plant Biol.* **13**, 427-433 (2010).
32. Dumack K, Fiore-Donno AM, Bass D, Bonkowski M. Making sense of environmental sequencing data: Ecologically important functional traits of the protistan groups Cercozoa and Endomyxa (Rhizaria). *Mol. Ecol. Resour.* **20**, 398-403 (2020).
33. Bulman S, Neuhauser S. Phytomyxea. In: *Handbook of the Protists* (eds Archibald JM, Simpson AGB, Slamovits CH). Springer (2017).
34. Fierer N. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* **15**, 579-590 (2017).
35. Fiore-Donno AM, Bonkowski M. Different community compositions between obligate and facultative oomycete plant parasites in a landscape-scale metabarcoding survey. *Biol. Fertility Soils* **57**, 245-256 (2021).

36. Civitello DJ*, et al.* Biodiversity inhibits parasites: Broad evidence for the dilution effect. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8667-8671 (2015).
37. Schmidt KA, Ostfeld RS. Biodiversity and the Dilution Effect in Disease Ecology. *Ecology* **82**, 609–619 (2001).
38. Solbach MD*, et al.* Legacies of consecutive summer droughts on soil-borne plant parasitic protists (Oomycota: Stramenopila and Phytomyxea: Rhizaria) and protistan consumers (Cercozoa: Rhizaria) along an experimental plant diversity gradient. *New Phytol.* **Early view** (2025).
39. Guerrero-Ramírez NR, Reich PB, Wagg C, Ciobanu M, Eisenhauer N. Diversity-dependent plant–soil feedbacks underlie long-term plant diversity effects on primary productivity. *Ecosphere* **10**, e02704 (2019).
40. Oburger E, Jones DL. Sampling root exudates – Mission impossible? *Rhizosphere* **6**, 116-133 (2018).
41. Raaijmakers JM, Weller DM. Natural Plant Protection by 2,4-Diacetylphloroglucinol-Producing *Pseudomonas* spp. in Take-All Decline Soils. *Mol. Plant-Microbe Interact.* **11**, 144–152 (1998).
42. Wardle DA, Bardgett RD, Walker LR, Bonner KI. Among- and within-species variation in plant litter decomposition in contrasting long-term chronosequences. *Funct. Ecol.* **23**, 442-453 (2009).
43. Bever JD*, et al.* Rooting theories of plant community ecology in microbial interactions. *Trends Ecol. Evol.* **25**, 468-478 (2010).
44. Mendes R*, et al.* Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science* **332**, 1097-1100 (2011).
45. Badri DV, Weir TL, van der Lelie D, Vivanco JM. Rhizosphere chemical dialogues: plant-microbe interactions. *Curr. Opin. Biotechnol.* **20**, 642-650 (2009).
46. Latz E*, et al.* Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. *J. Ecol.* **100**, 597-604 (2012).
47. Latz E, Eisenhauer N, Rall BC, Scheu S, Jousset A. Unravelling Linkages between Plant Community Composition and the Pathogen-Suppressive Potential of Soils. *Sci. Rep.* **6**, 23584 (2016).
48. Berendsen RL, Pieterse CM, Bakker PA. The rhizosphere microbiome and plant health. *Trends Plant Sci.* **17**, 478-486 (2012).
49. Delory BM, Callaway RM, Semchenko M. A trait-based framework linking the soil metabolome to plant–soil feedbacks. *New Phytol.* **241**, 1910-1921 (2024).

50. White TJ, Bruns T, Lee S, Taylor J. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: *PCR protocols: A Guide to Methods and Applications* (eds Innis MA, Gelfand DH, Sninsky JJ, White TJ). Academic Press (1990).

51. Ihrmark K*, et al.* New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol. Ecol.* **82**, 666-677 (2012).

52. Kohout P*, et al.* Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: Is there a universal solution? *Soil Biol. Biochem.* **68**, 482-493 (2014).

53. Hennecke J*, et al.* Plant Species Richness and the Root Economics Space Drive Soil Fungal Communities. *Ecol. Lett.* **28**, e70032 (2025).

54. Fiore-Donno AM, Richter-Heitmann T, Bonkowski M. Contrasting Responses of Protistan Plant Parasites and Phagotrophs to Ecosystems, Land Management and Soil Properties. *Front. Microbiol.* **11**, 1823 (2020).

55. Caporaso JG*, et al.* Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A* **108**, 4516-4522 (2011).

56. Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* **75**, 129-137 (2015).

57. Schloss PD*, et al.* Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537-7541 (2009).

58. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120 (2014).

59. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**, 2957-2963 (2011).

60. Caporaso JG*, et al.* QIIME allows analysis of highthroughput community sequencing data. *Nat. Methods* **7**, 335–336 (2010).

61. Rognes T, Flouri T, Nichols B, Quince C, Mahe F. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**, e2584 (2016).

62. Abarenkov K*, et al.* The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. *Nucleic Acids Res.* **52**, D791-D797 (2023).

63. Guillou L*, et al.* The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Res.* **41**, D597-D604 (2013).

64. Pruesse E, *et al.* SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **35**, 7188-7196 (2007).
65. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org. (2022).
66. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. Springer Cham (2016).
67. Weiner J. riverplot: Sankey or Ribbon Plots. R package version 0.10, <https://CRAN.R-project.org/package=riverplot>. (2021).
68. Freudenthal J, Dumack K, Schaffer S, Schlegel M, Bonkowski M. Algae-fungi symbioses and bacteria-fungi co-exclusion drive tree species-specific differences in canopy bark microbiomes. *ISME J.* **18**, wrae206 (2024).
69. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome. Biol.* **15**, 550 (2014).
70. Oksanen J, *et al.* vegan: Community Ecology Package. R package version 2.6-4, <https://CRAN.R-project.org/package=vegan>. (2022).
71. Bakker JD. Restricting Permutations. In: *Applied Multivariate Statistics in R*. University of Washington (2024).
72. Hsieh TC, Ma KH, Chao A, McInerny G. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods Ecol. Evol.* **7**, 1451-1456 (2016).
73. Chao A, *et al.* Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecol. Monogr.* **84**, 45-67 (2014).
74. Pinheiro JC, Bates DM, R Core Team. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-157, <https://CRAN.R-project.org/package=nlme>. (2022).
75. Pinheiro JC, Bates DM. *Mixed-Effects Models in S and S-PLUS*, 1 edn. Springer New York (2000).

Acknowledgements

We thank the technical staff Steffen Eismann, Ute Köber, Gerlinde Kratzsch, Katja Kunze, and Heike Scheffler for their work in maintaining the field site and also many student helpers for weeding of the experimental plots, as well as for their help during the sampling campaign. Further, we thank the central coordination team Anne Ebeling and Annette Jesch, and the data management team, especially Yuanyuan Huang. The Jena Experiment is funded by the Deutsche Forschungsgemeinschaft (DFG, FOR 5000), with additional support by the Friedrich Schiller University Jena, the Max Planck Institute for Biogeochemistry, and the German Centre

567 for Integrative Biodiversity Research (iDiv) Halle–Jena–Leipzig (DFG FZT 118). The current
568 study was funded by the DFG project number 432642583 (Subproject 2 — Protist Plant
569 Pathogens of the Jena Experiment; FOR 5000-1).
570

571 ## Author contributions

572 M.B. supervised the current study. M.D.S, J.H., L.B., C.A., A.F., Y.P.A.d.S, A.R., M.L, and A.W.
573 contributed to the sampling. M.D.S. extracted DNA. M.D.S. and K.W. prepared the sequencing
574 of Oomycota and Cercozoa, supervised by M.B.; J.H. prepared the sequencing of fungi,
575 supervised by A.H.-B. and A.W.; Y.C. prepared the sequencing of bacteria. M.D.S. performed
576 the data analysis. M.D.S. wrote the initial draft with help from M.B.; all authors contributed to
577 the writing of the manuscript.

Supplementary Information

Plant-driven soil microbial legacies strengthen over time and include widespread suppression

Marcel Dominik Solbach[1*], Justus Hennecke[2,3,4], Yan Chen[5], Katalin Wagner[1], Cynthia Albracht[6,7,8], Leonardo Bassi[2,3,9], Nico Eisenhauer[2,3], Aaron Fox[10,11], Anna Heintz-Buschart[8], Markus Lange[12], Liesje Mommer[4], Yuri Pinheiro Alves de Souza[11,13], Akanksha Rai[12], Alexandra Weigelt[2,3], Michael Bonkowski[1*]

[1] Terrestrial Ecology, Institute of Zoology, Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, 50674 Cologne, Germany
[2] Institute of Biology, Leipzig University, 04103 Leipzig, Germany
[3] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, 04103 Leipzig, Germany
[4] Forest Ecology and Forest Management Group, Wageningen University and Research, 6708 PB Wageningen, The Netherlands
[5] Institute of Soil Science, Chinese Academy of Science, 210008 Nanjing, China
[6] Dept. Soil Ecology, Helmholtz-Centre for Environmental Research – UFZ Halle, 06120 Halle, Germany
[7] Institute for Biosafety in Plant Biotechnology, Julius Kühn-Institute, 06484 Quedlinburg, Germany
[8] Biosystems Data Analysis, Swammerdam Institute for Life Sciences, University of Amsterdam, 1098 XH Amsterdam, The Netherlands
[9] Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), 6708 PB Wageningen, The Netherlands
[10] Teagasc, Crops, Environment and Land-Use Programme, Johnstown Castle, Y35 TC97 Co. Wexford, Ireland
[11] TUM School of Life Science, Chair of Environmental Microbiology, Technische Universität München, 85354 Freising, Germany
[12] Max Planck Institute for Biogeochemistry, 07745 Jena, Germany
[13] Research Unit Comparative Microbiome Analysis, Helmholtz Zentrum München, 85764 Neuherberg, Germany

*Correspondence:

Marcel Dominik Solbach, E-mail: marcel.solbach@uni-koeln.de

Michael Bonkowski, E-mail: m.bonkowski@uni-koeln.de

Supplementary Methods

Metabarcoding and bioinformatic processing of Fungi

The internal transcribed spacer 2 (ITS2) of Fungi was amplified using the primers ITS4 and ITS7:ITS7o[1,2,3]. The sequencing data were partially published by Hennecke et al.[4] (treatment +SH +PH), but all samples were sequenced together following the same laboratory protocol. Each sample was amplified using 7.5 µl KAPA HiFi HotStart ReadyMix Polymerase (Roche) and 0.3 µl of each primer and 1 µl of DNA template (concentration adjusted to 10 ng μl^{-1}) in 15 µl reactions. The following PCR conditions were used: Initial denaturation at 95°C for 3 min, 30 cycles (denaturation at 95°C for 20 s, annealing at 56°C for 20 s, elongation at 72°C for 20 s), terminal extension at 72°C for 5 min. Amplification success was verified by gel electrophoresis, and three PCR products of each sample were pooled. 30 µl of amplicons per sample were purified with AMPure XP beads (Beckman Coulter, Krefeld, Germany) and the Illumina index and sequencing adapters were added by PCR using a Nextera XT Illumina Index Kit (Illumina Inc., San Diego, CA, USA). Finally, libraries were quantified using a Nanodrop spectrophotometer and samples were pooled to equal molarity. Paired-end sequencing of 2 x 300 bp was performed with a MiSeq Reagent Kit v.3 on an Illumina MiSeq System at the Department of Soil Ecology of the Helmholtz-Centre for Environmental Research (UFZ, Halle/Saale, Germany).

Quality of the raw reads in FASTQ format was checked using FASTX-toolkit (Version 0.0.13, http://hannonlab.cshl.edu/fastx_toolkit/). Fungal sequences were trimmed to a length of 250 bp due to poor sequence quality towards the sequence termini. Reads were then assembled to sequence contigs in mothur (v.1.45.3)[5]. Quality filtering parameters were based on the assembly reports. A maximum of seven mismatches with up to two ambiguities in the overlap were allowed and merged sequences < 200 bp, or with an overlap < 90 bp, were discarded. Contigs were clustered into operational taxonomic units (OTUs) by abundance-based greedy clustering (agc) with a similarity threshold of 97% using VSEARCH (v.2.7.1)[6]. OTUs representing less than 0.005% of all reads were removed as they likely represent amplification or sequencing errors[7,8]. Fungal sequences were searched against the UNITE database (version 10)[9] with an E-value cutoff of $1e^{-50}$ using the blastn algorithm in BLAST+[10]. OTUs with a non-target taxon as their top hit, or OTUs that failed to return a hit under the E-value cutoff, were

discarded. Afterwards, chimeric sequences were detected with de novo chimera detection (self-reference) using UCHIME[11] as implemented in mothur. Functional traits of fungi were then assigned to the OTUs on genus-level based on the FungalTraits database[12].

Metabarcoding and bioinformatic processing of Oomycota

Oomycota were amplified with a semi-nested metabarcoding approach based on the protocol from Fiore-Donno and Bonkowski[13] targeting the ITS1 region. The first PCR was conducted with the primer pair S1777F and 58SOomR; the second PCR was conducted with barcoded primers S1786StraF and 58SOomR[13]. The first PCR step was conducted with 1 µl of extracted DNA as the template and 10 µl of PCR master mix containing 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), 1 µl Thermo Scientific DreamTaq Green Buffer, 0.2 µl dNTPs (10 mM), 0.1 µl Thermo Scientific DreamTaq Polymerase, and 6.7 µl nuclease-free water. The second, semi-nested PCR was conducted with 1 µl of the first PCR as the template, and 17 µl of master mix containing 1.7 µl of barcoded forward primer, 1.7 µl of barcoded reverse primer (dual-indexing; for the sample list and their respective barcode combinations, see Supplementary Table 8), 1.7 µl Thermo Scientific DreamTaq Green Buffer, 0.34 µl dNTPs (10 mM), 0.17 µl Thermo Scientific DreamTaq Polymerase, and 11.4 µl nuclease-free water. The following PCR conditions were used: Initial denaturation at 95°C for 2 min, 24 cycles (denaturation at 95°C for 30 s, annealing at 58°C for 30 s, elongation at 72°C for 30 s), terminal extension at 72°C for 5 min, and cooling at 15°C. PCRs were conducted in duplicates (2 x 10 µl for the first and 2 x 17 µl for the second PCR) to reduce the potential artificial dominance of a few amplicons by PCR competition. 12.5 µl of the two duplicates of the second (barcoded) PCR were pooled, and then purified and normalized using the SequalPrep Normalization Plate Kit (Invitrogen, Thermo Fisher Scientific, USA). The purified amplicons were pooled, and sequencing was performed with a MiSeq v3 Reagent kit of 600 cycles on a MiSeq Desktop Sequencer (Illumina Inc., San Diego, CA, USA) at the Cologne Center for Genomics (Germany).

Quality of the raw reads in FASTQ format was checked using FASTX-toolkit. Reads were then assembled to sequence contigs in mothur. Quality filtering parameters were based on the assembly reports. No mismatches and no ambiguities in the overlap were permitted, and merged sequences < 170 bp, or with an overlap < 70 bp, were discarded. Contigs were clustered into operational taxonomic units (OTUs) by abundance-based greedy clustering (agc)

3

with a similarity threshold of 97% using VSEARCH. OTUs representing less than 0.005% of all reads were removed. Oomycete sequences were searched against a custom Oomycota ITS1 database[14] with an E-value cutoff of $1e^{-10}$. OTUs with a non-target taxon as their top hit, or OTUs that failed to return a hit under the specific E-value cutoffs, were discarded. Because reference-based as well as de novo chimera detection with UCHIME frequently produced false positives and negatives for the Oomycota dataset, we instead discarded Oomycota sequences with a query coverage <70% based on BLAST alignment results[15]. The resulting OTU database was manually curated and corrected because the genus *Pythium* has undergone taxonomic changes during the last few years and was split into at least five genera[16], which is not well reflected by the NCBI database, and therefore also not implemented in the reference database from Solbach et al.[14]. After correction, functional traits of Oomycota were assigned to the OTUs on genus level based on the trait table by Fiore-Donno and Bonkowski[13].

Metabarcoding and bioinformatic processing of Cercozoa

Cercozoa (including the subclade Endomyxa) were amplified with a semi-nested metabarcoding approach based on the protocol from Fiore-Donno et al.[17], targeting the hypervariable V4 region of the 18S ribosomal RNA gene (SSU rDNA). The first PCR was conducted with a mixture of the forward primers S615F_Cerco and S615F_Phyt, 50% each, and reverse primer S963R_Phyt; the second PCR was conducted with the barcoded primers S615F_Cer and S947R_Cer[17]. The PCR mixtures contained the same ingredients as described for Oomycota. The following PCR conditions were used: Initial denaturation at 95°C for 2 min, 24 cycles (denaturation at 95°C for 30 s, annealing at 52°C for 30 s, elongation at 72°C for 30 s), terminal extension at 72°C for 5 min, and cooling at 15°C. PCRs were conducted in duplicates (2 x 10 µl for the first and 2 x 17 µl for the second PCR) to reduce the potential artificial dominance of a few amplicons by PCR competition. 12.5 µl of the two duplicates of the second (barcoded) PCR were pooled, and then purified and normalized using the SequalPrep Normalization Plate Kit (Invitrogen, Thermo Fisher Scientific, USA). The purified amplicons were pooled, and sequencing was performed with a MiSeq v3 Reagent kit of 600 cycles on a MiSeq Desktop Sequencer (Illumina Inc., San Diego, CA, USA) at the Cologne Center for Genomics (Germany).

4

Quality of the raw reads in FASTQ checked using FASTX-toolkit. Reads were then assembled to sequence contigs in mothur. Quality filtering parameters were based on the assembly reports. A maximum of one mismatch with no ambiguities in the overlap were allowed and sequences < 300 bp, or with an overlap < 200 bp, were discarded. Contigs were clustered into operational taxonomic units (OTUs) by abundance-based greedy clustering (agc) with a similarity threshold of 97% using VSEARCH. OTUs representing less than 0.005% of all reads were removed. Cercozoan sequences were searched against the PR^2 database[18] with an E-value cutoff of $1e^{-50}$ using the blastn algorithm in BLAST+. OTUs with a non-target taxon as their top hit, or OTUs that failed to return a hit under the specific E-value cutoffs, were discarded. Cercozoan OTU sequences were aligned against a curated template alignment of 78 representative cercozoan V4 sequences spanning all major cercozoan clades[19] using the align.seqs command in mothur with a penalty for opening gaps of −5. Query sequences with a similarity of less than 50% to the template sequences were discarded. Sequences that started after the position that 90% of the sequences do, ended before the position that 90% of the sequences do, or had gaps of more than five nucleotides, were also removed. Reference-based chimera detection was performed using UCHIME with the same 78 representative cercozoan sequences from the template alignment[19] serving as the reference database. Functional traits of Cercozoa were assigned to the OTUs on genus level based on the trait table by Dumack et al.[20].

Metabarcoding and bioinformatic processing of Bacteria

Bacteria were sequenced using the primers 515F[21] and 806R[22], targeting a circa 390 bp long fragment of the V4 region of the 16S ribosomal rRNA gene (SSU rDNA). The PCRs were conducted with 2 µl of template DNA, 12.5 µl of TaKaRa Premix TaqTM (TaKaRa Bio Group) and 10 mM of forward and reverse primer each. The following thermal program was used: Initial denaturation at 95°C for 5 min, 30 cycles (denaturation at 94°C for 30 s, annealing at 56°C for 30 s, elongation at 72°C for 40 s), final extension step at 72°C for 5 min, and cooling at 15°C. The PCR products were purified with AMPure XP beads (Agencourt) and amplified again in a new round of PCRs. After another purification with AMPure XP beads, the final amplicons were quantified using a Qubit dsDNA Assay Kit (Thermo Fisher Scientific, USA), and the concentrations were equalized accordingly. Sequencing was performed on an Illumina

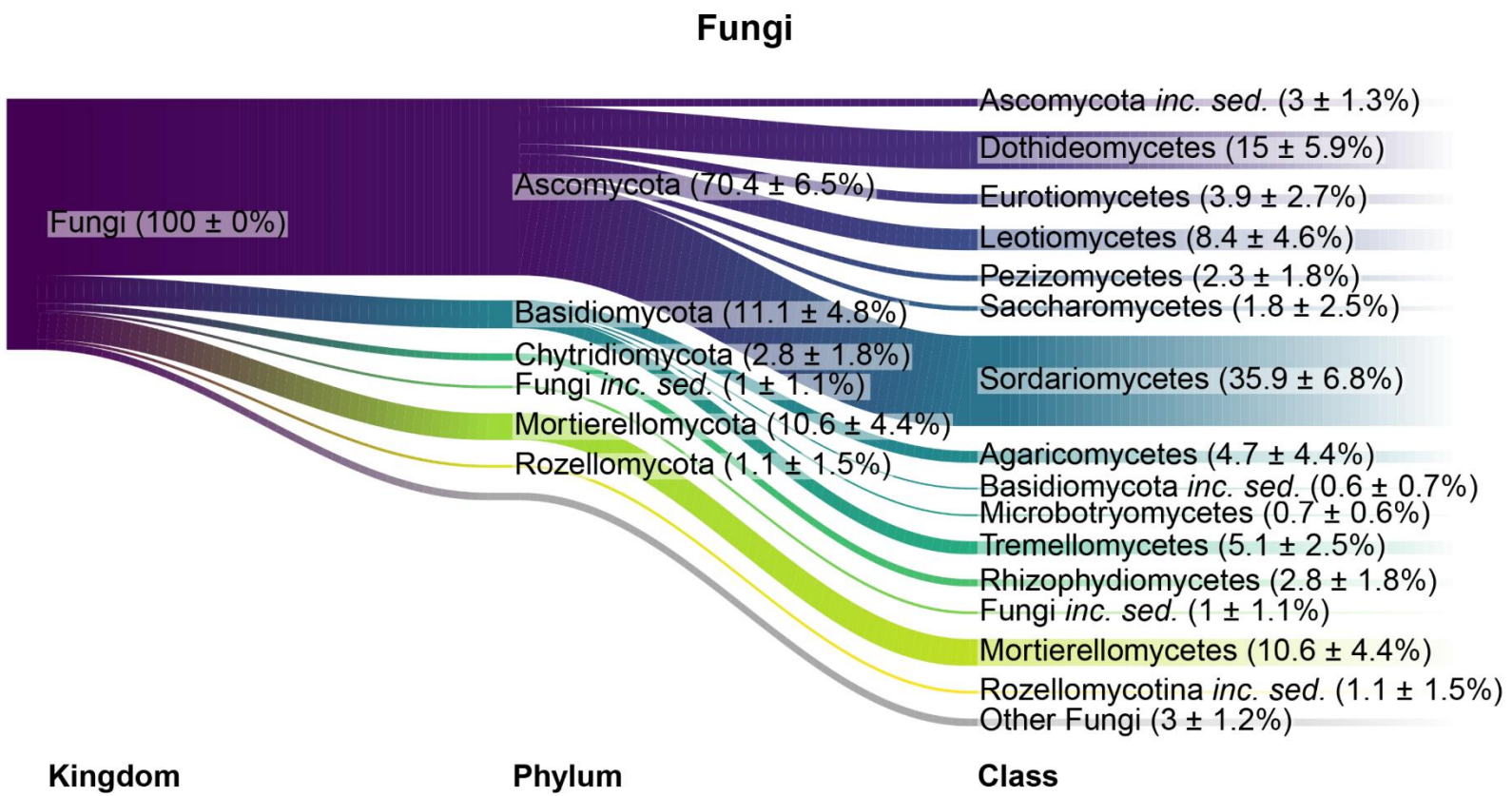
5

NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) with 250 bp paired-end reads by OE biotech Co., Ltd. (Shanghai, China).

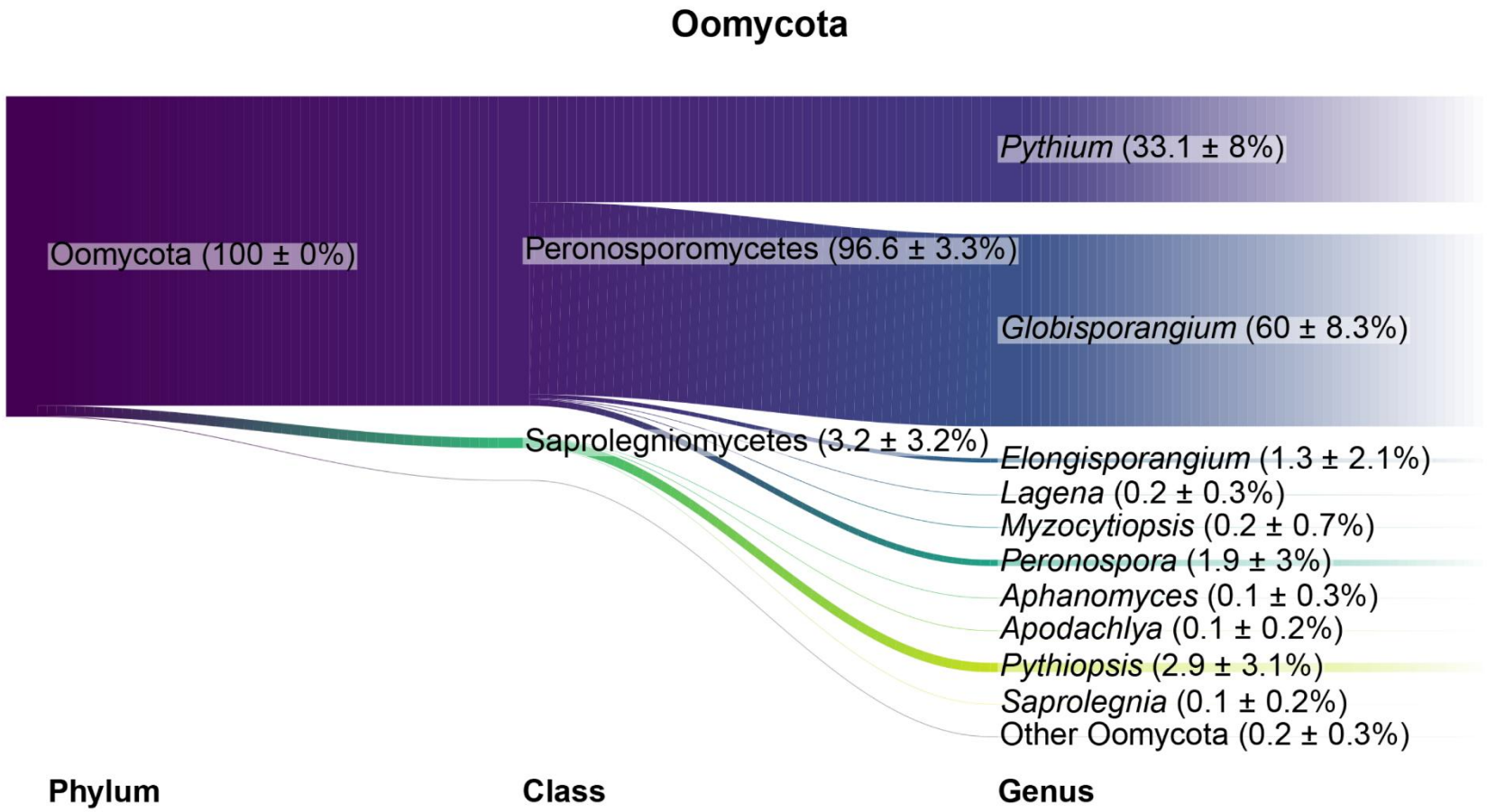
Paired-end reads in FASTQ format were preprocessed using Trimmomatic[23] to detect and cut off ambiguous bases (N) and low quality sequences with average quality score below 20 using sliding window trimming approach. After trimming, paired-end reads were assembled using FLASH[24]. Parameters of assembly were: 10 bp of minimal overlapping, 200 bp of maximum overlapping and 20% of maximum mismatch rate. Sequences were further denoised as follows: reads with ambiguous, homologous sequences or below 200 bp were discarded. Reads with 75% of bases above Q20 were retained, and chimeric sequences were detected and removed using QIIME (version 1.8.0)[25]. Clean reads were subjected to primer sequence removal and clustering to generate operational taxonomic units (OTUs) using VSEARCH with a 97% similarity cutoff. The representative read of each OTU was selected using QIIME. All representative reads were annotated with the Silva database (version 138)[26,27,28] using RDP classifier (confidence threshold 70%)[29]. OTUs representing less than 0.001% of all sequence reads were removed.

6

Supplementary Figures



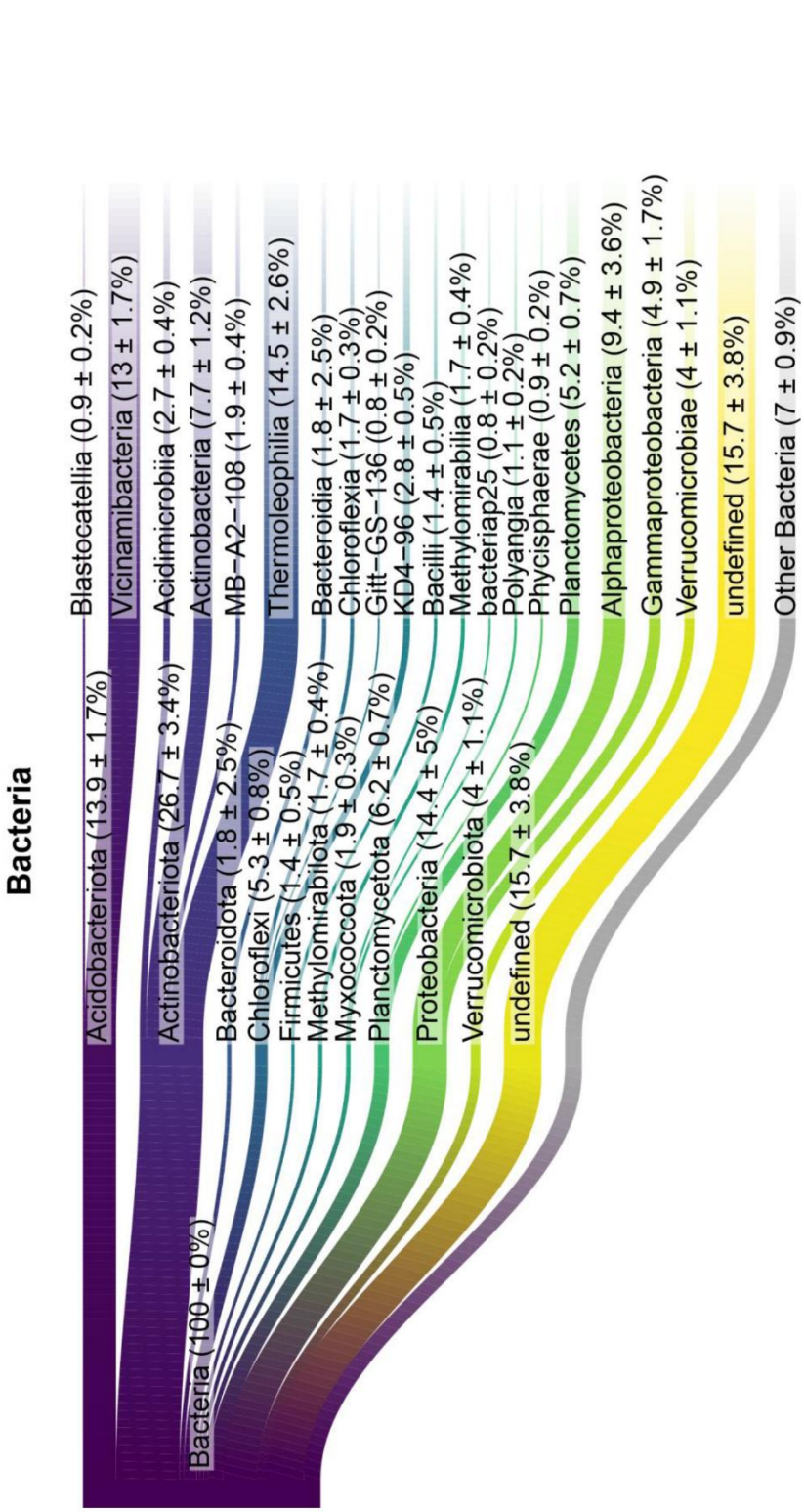
Supplementary Fig. 1 | Sankey diagram displaying relative abundances of fungi. Only the 10 most abundant classes are shown. The remaining classes are summarized in "Other Fungi". Numbers in parentheses refer to the average relative abundances based on sequence reads ± standard deviation in percent, rounded to one decimal place.



Supplementary Fig. 2 | Sankey diagram displaying relative abundances of Oomycota. Only the 10 most abundant genera are shown. The remaining genera are summarized in "Other Oomycota". Numbers in parentheses refer to the average relative abundances based on sequence reads ± standard deviation in percent, rounded to one decimal place.

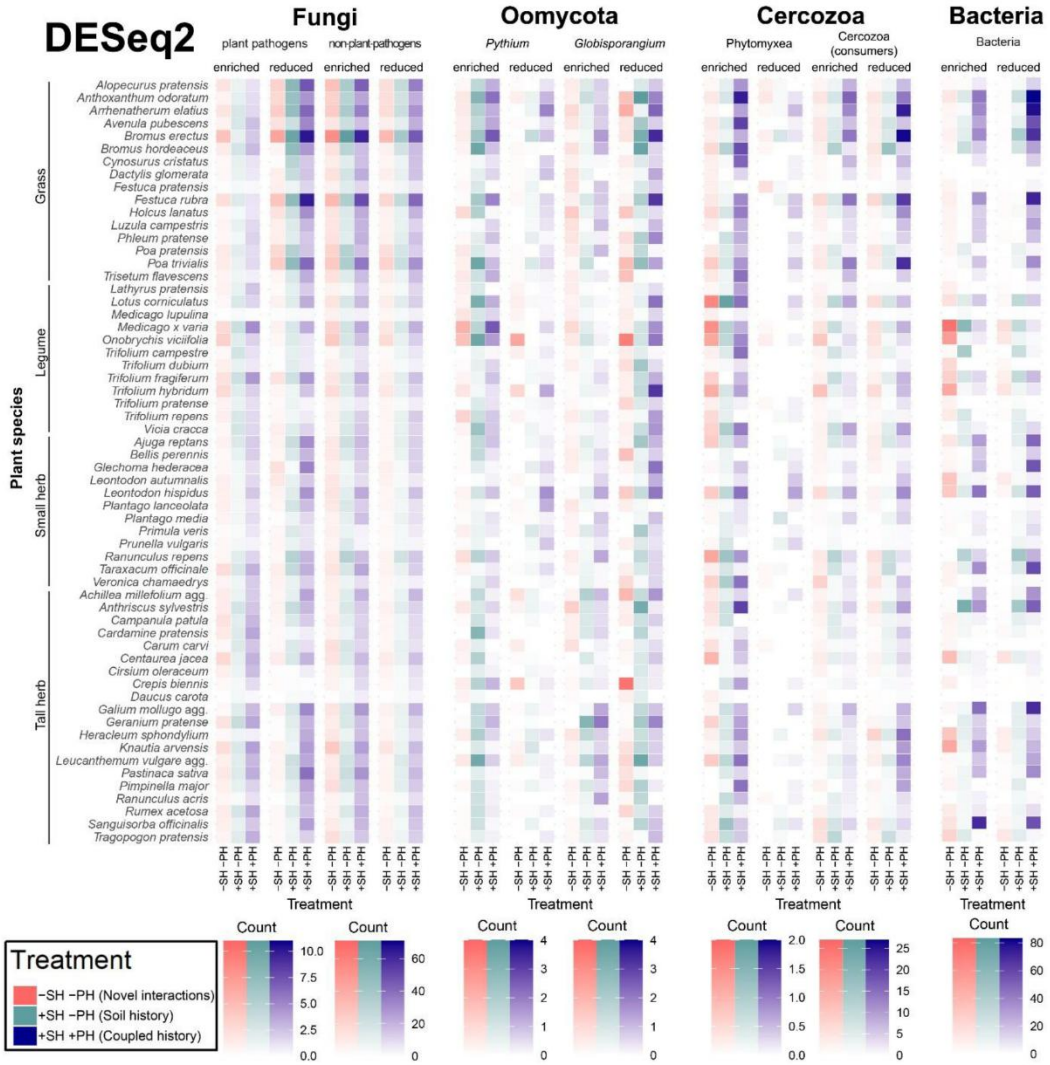
Supplementary Fig. 3 | Sankey diagram displaying relative abundances of Cercozoa. Only the 10 most abundant orders are shown. The remaining orders are summarized in "Other Cercozoa". The order Plasmodiophorida (plant parasites) is further expanded to genus-level. Numbers in parentheses refer to the average relative abundances based on sequence reads ± standard deviation in percent, rounded to one decimal place.

9



Supplementary Fig. 4 | Sankey diagram displaying relative abundances of bacteria. Only the 20 most abundant classes are shown. The remaining classes are summarized in "Other Bacteria". Numbers in parentheses refer to the average relative abundances based on sequence reads ± standard deviation in percent, rounded to one decimal place.

10



Supplementary Fig. 5 | Number of plant species-specific differentially abundant OTUs identified by DESeq2. Differential abundance analyses were conducted for each of the 60 plant species present in the experimental design. For each plant species, all plots containing that species were compared against an equal number of randomly selected plots without the respective species, ensuring a balanced design for differential abundance analysis. This random selection and DESeq2 analysis were repeated 10 times per species, the number of differentially abundant OTUs was counted, and results were averaged across iterations. DESEq2 analyses were performed independently for each treatment and for each microbial dataset (Fungi, Oomycota, Cercozoa, Bacteria). “Enriched” OTUs were more abundant in plots with the respective plant species/less abundant in plots without the respective plant species, and “reduced” OTUs were less abundant in plots with the respective plant species/more abundant in plots without the respective plant species. Comparisons of the numbers of affected OTUs are shown in Fig. 3 (main text), and detailed statistical results from Wilcoxon signed-rank tests for paired samples are provided in Supplementary Table 3.

Supplementary Tables

Supplementary Table 1 | Species list. Plant species list of the sixty plant species used in the Jena Experiment, including family and plant functional group. Information derived from JEXIS dataset 233 (https://doi.org/10.25829/CJMH-AW38).

Short name	Full scientific species name	Family	Functional group
Ach.mil	*Achillea millefolium* agg.	Asteraceae	Tall herb
Aju.rep	*Ajuga reptans*	Lamiaceae	Small herb
Alo.pra	*Alopecurus pratensis*	Poaceae	Grass
Ant.odo	*Anthoxanthum odoratum*	Poaceae	Grass
Ant.syl	*Anthriscus sylvestris*	Apiaceae	Tall herb
Arr.ela	*Arrhenatherum elatius*	Poaceae	Grass
Ave.pub	*Avenula pubescens*	Poaceae	Grass
Bel.per	*Bellis perennis*	Asteraceae	Small herb
Bro.ere	*Bromus erectus*	Poaceae	Grass
Bro.hor	*Bromus hordeaceus*	Poaceae	Grass
Cam.pat	*Campanula patula*	Campanulaceae	Tall herb
Car.car	*Carum carvi*	Apiaceae	Tall herb
Car.pra	*Cardamine pratensis*	Brassicaceae	Tall herb
Cen.jac	*Centaurea jacea*	Asteraceae	Tall herb
Cir.ole	*Cirsium oleraceum*	Asteraceae	Tall herb
Cre.bie	*Crepis biennis*	Asteraceae	Tall herb
Cyn.cri	*Cynosurus cristatus*	Poaceae	Grass
Dac.glo	*Dactylis glomerata*	Poaceae	Grass
Dau.car	*Daucus carota*	Apiaceae	Tall herb
Fes.pra	*Festuca pratensis*	Poaceae	Grass
Fes.rub	*Festuca rubra*	Poaceae	Grass
Gal.mol	*Galium mollugo* agg.	Rubiaceae	Tall herb
Ger.pra	*Geranium pratense*	Geraniaceae	Tall herb
Gle.hed	*Glechoma hederacea*	Lamiaceae	Small herb

Her.sph	*Heracleum sphondylium*	Apiaceae	Tall herb
Hol.lan	*Holcus lanatus*	Poaceae	Grass
Kna.arv	*Knautia arvensis*	Caprifoliaceae	Tall herb
Lat.pra	*Lathyrus pratensis*	Fabaceae	Legume
Leo.aut	*Leontodon autumnalis*	Asteraceae	Small herb
Leo.his	*Leontodon hispidus*	Asteraceae	Small herb
Leu.vul	*Leucanthemum vulgare* agg.	Asteraceae	Tall herb
Lot.cor	*Lotus corniculatus*	Fabaceae	Legume
Luz.cam	*Luzula campestris*	Juncaceae	Grass
Med.lup	*Medicago lupulina*	Fabaceae	Legume
Med.var	*Medicago x varia*	Fabaceae	Legume
Ono.vic	*Onobrychis viciifolia*	Fabaceae	Legume
Pas.sat	*Pastinaca sativa*	Apiaceae	Tall herb
Phl.pra	*Phleum pratense*	Poaceae	Grass
Pim.maj	*Pimpinella major*	Apiaceae	Tall herb
Pla.lan	*Plantago lanceolata*	Plantaginaceae	Small herb
Pla.med	*Plantago media*	Plantaginaceae	Small herb
Poa.pra	*Poa pratensis*	Poaceae	Grass
Poa.tri	*Poa trivialis*	Poaceae	Grass
Pri.ver	*Primula veris*	Primulaceae	Small herb
Pru.vul	*Prunella vulgaris*	Lamiaceae	Small herb
Ran.acr	*Ranunculus acris*	Ranunculaceae	Tall herb
Ran.rep	*Ranunculus repens*	Ranunculaceae	Small herb
Rum.ace	*Rumex acetosa*	Polygonaceae	Tall herb
San.off	*Sanguisorba officinalis*	Rosaceae	Tall herb
Tar.off	*Taraxacum officinale*	Asteraceae	Small herb
Tra.pra	*Tragopogon pratensis*	Asteraceae	Tall herb
Tri.cam	*Trifolium campestre*	Fabaceae	Legume
Tri.dub	*Trifolium dubium*	Fabaceae	Legume

13

Tri.fla	*Trisetum flavescens*	Poaceae	Grass
Tri.fra	*Trifolium fragiferum*	Fabaceae	Legume
Tri.hyb	*Trifolium hybridum*	Fabaceae	Legume
Tri.pra	*Trifolium pratense*	Fabaceae	Legume
Tri.rep	*Trifolium repens*	Fabaceae	Legume
Ver.cha	*Veronica chamaedrys*	Plantaginaceae	Small herb
Vic.cra	*Vicia cracca*	Fabaceae	Legume

Supplementary Table 2 | Plot list of the 80 plots of the Jena Experiment. Plot: Plot code on the experimental field site, Block: Block code on the experimental field site with increasing distance to the river Saale (B1 - B4); Sown diversity: Plant species richness (1, 2, 4, 8, 16, or 60 plant species); Functional groups: Functional group richness (1, 2, 3, or 4 functional groups); Num. Grass: Number of plant species of the functional group "Grass"; Num. Legume: Number of plant species of the functional group "Legume"; Num. Small herb: Number of plant species of the functional group "Small herb"; Num. Tall herb: Number of plant species of the functional group "Tall herb"; Composition: Sown plant species composition, abbreviations refer to the "short name" in Supplementary Table 1. Information derived from JEXIS dataset 90 (https://doi.org/10.25829/XFG6-WD77). On each plot, three subplots representing the soil and plant history treatments (−SH −PH: novel interactions, +SH −PH: soil history, +SH +PH: coupled history) were established.

Plot	Block	Sown diversity	Functional groups	Num. Grass	Num. Legume	Num. Small herb	Num. Tall herb	Composition
B1A01	B1	16	4	4	4	4	4	Ant.odo, Ave.pub, Bro.hor, Poa.pra, Aju.rep, Pla.lan, Ran.rep, Tar.off, Ant.syl, Car.car, Ger.pra, Tra.pra, Lat.pra, Lot.cor, Tri.cam, Vic.cra
B1A02	B1	8	2	4	0	0	4	Alo.pra, Bro.ere, Fes.rub, Phl.pra, Car.pra, Her.sph, Ran.acr, San.off
B1A03	B1	8	3	3	2	3	0	Cyn.cri, Phl.pra, Tri.fla, Gle.hed, Pri.ver, Ver.cha, Lot.cor, Med.lup
B1A04	B1	4	4	1	1	1	1	Fes.pra, Pla.lan, Cam.pat, Ono.vic
B1A05	B1	2	1	0	2	0	0	Med.lup, Ono.vic

B1A06	B1	16	2	8	0	0	8	Alo.pra, Ant.odo, Ave.pub, Bro.hor, Hol.lan, Poa.pra, Poa.tri, Tri.fla, Ach.mil, Ant.syl, Cam.pat, Cen.jac, Ger.pra, Her.sph, Leu.vul, Pim.maj
B1A07	B1	2	1	0	0	0	2	Ran.acr, San.off
B1A08	B1	1	1	0	1	0	0	Med.var
B1A11	B1	16	1	0	0	0	16	Ach.mil, Ant.syl, Cam.pat, Car.pra, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Leu.vul, Pas.sat, Ran.acr, Rum.ace, San.off, Tra.pra
B1A12	B1	8	1	0	8	0	0	Lat.pra, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.hyb, Tri.pra
B1A13	B1	4	1	0	4	0	0	Lot.cor, Med.lup, Med.var, Ono.vic
B1A14	B1	8	4	2	2	2	2	Luz.cam, Tri.fla, Leo.his, Pla.lan, Ant.syl, Dau.car, Tri.cam, Tri.fra
B1A15	B1	1	1	0	0	0	1	Cre.bie
B1A16	B1	2	2	1	0	1	0	Poa.pra, Pla.lan
B1A17	B1	2	2	1	0	0	1	Alo.pra, Dau.car
B1A18	B1	1	1	0	0	1	0	Pru.vul
B1A19	B1	4	3	2	0	1	1	Arr.ela, Luz.cam, Pru.vul, Cam.pat
B1A20	B1	16	3	0	5	6	5	Aju.rep, Bel.per, Leo.aut, Leo.his, Pla.lan, Ver.cha, Ach.mil, Ger.pra, Kna.arv, Ran.acr, San.off, Lot.cor, Med.var, Ono.vic, Tri.hyb, Tri.rep
B1A21	B1	4	2	2	0	0	2	Fes.pra, Luz.cam, Ach.mil, Cre.bie
B1A22	B1	60	4	16	12	12	20	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam, Phl.pra, Poa.pra, Poa.tri, Tri.fla, Aju.rep, Bel.per, Gle.hed, Leo.aut, Leo.his, Pla.lan, Pla.med, Pri.ver, Pru.vul, Ran.rep, Tar.off, Ver.cha, Ach.mil, Ant.syl, Cam.pat, Car.pra, Car.car, Cen.jac, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Pas.sat, Pim.maj, Ran.acr, Rum.ace, San.off, Tra.pra, Lat.pra, Lot.cor, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B2A01	B2	4	4	1	1	1	1	Ant.odo, Pru.vul, Kna.arv, Tri.pra
B2A02	B2	2	1	2	0	0	0	Fes.rub, Tri.fla
B2A03	B2	60	4	16	12	12	20	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam,

15

								Phl.pra, Poa.pra, Poa.tri, Tri.fla, Aju.rep, Bel.per, Gle.hed, Leo.aut, Leo.his, Pla.lan, Pla.med, Pri.ver, Pru.vul, Ran.rep, Tar.off, Ver.cha, Ach.mil, Ant.syl, Cam.pat, Car.pra, Car.car, Cen.jac, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Pas.sat, Pim.maj, Ran.acr, Rum.ace, San.off, Tra.pra, Lat.pra, Lot.cor, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B2A04	B2	1	1	0	0	0	1	Ger.pra
B2A05	B2	1	1	1	0	0	0	Fes.pra
B2A06	B2	4	2	0	2	2	0	Pla.lan, Tar.off, Lat.pra, Med.lup
B2A08	B2	2	2	0	1	0	1	Ran.acr, Tri.cam
B2A09	B2	4	1	0	0	4	0	Aju.rep, Pla.lan, Pri.ver, Pru.vul
B2A10	B2	16	2	8	0	8	0	Alo.pra, Ant.odo, Arr.ela, Bro.ere, Fes.pra, Hol.lan, Phl.pra, Poa.pra, Bel.per, Leo.aut, Leo.his, Pla.lan, Pri.ver, Pru.vul, Ran.rep, Ver.cha
B2A12	B2	8	1	0	0	0	8	Ant.syl, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Ran.acr, San.off
B2A13	B2	1	1	0	0	1	0	Pla.lan
B2A14	B2	8	4	2	2	2	2	Luz.cam, Phl.pra, Leo.his, Ver.cha, Kna.arv, San.off, Tri.dub, Tri.hyb
B2A15	B2	1	1	0	1	0	0	Ono.vic
B2A16	B2	4	3	0	1	2	1	Leo.aut, Pla.med, Kna.arv, Vic.cra
B2A17	B2	8	2	0	4	4	0	Gle.hed, Leo.aut, Pla.med, Tar.off, Lat.pra, Tri.cam, Tri.fra, Vic.cra
B2A18	B2	16	4	4	4	4	4	Alo.pra, Bro.hor, Cyn.cri, Poa.pra, Aju.rep, Pla.med, Pri.ver, Ran.rep, Ant.syl, Cam.pat, Car.pra, Ger.pra, Med.lup, Tri.cam, Tri.dub, Tri.rep
B2A19	B2	2	1	0	0	2	0	Pla.med, Tar.off
B2A20	B2	2	2	0	1	1	0	Pla.lan, Tri.dub
B2A21	B2	8	3	0	3	2	3	Leo.his, Pla.med, Cre.bie, Gal.mol, San.off, Lot.cor, Med.lup, Ono.vic
B2A22	B2	16	3	5	6	0	5	Cyn.cri, Fes.pra, Phl.pra, Poa.tri, Tri.fla, Ach.mil, Cam.pat, Cen.jac, Rum.ace, San.off, Lat.pra, Lot.cor, Ono.vic, Tri.hyb, Tri.rep, Vic.cra

16

B3A01	B3	1	1	0	0	0	1	Gal.mol
B3A02	B3	2	2	1	0	0	1	Fes.pra, Car.car
B3A03	B3	4	3	1	2	1	0	Phl.pra, Pla.med, Tri.hyb, Vic.cra
B3A04	B3	8	1	8	0	0	0	Alo.pra, Arr.ela, Cyn.cri, Dac.glo, Fes.rub, Hol.lan, Poa.tri, Tri.fla
B3A05	B3	8	3	3	3	0	2	Ant.odo, Bro.ere, Poa.tri, Ant.syl, Leu.vul, Lot.cor, Ono.vic, Tri.hyb
B3A06	B3	1	1	1	0	0	0	Fes.rub
B3A07	B3	8	4	2	2	2	2	Bro.hor, Hol.lan, Pri.ver, Ran.rep, Her.sph, Leu.vul, Med.lup, Ono.vic
B3A08	B3	2	1	2	0	0	0	Dac.glo, Fes.pra
B3A09	B3	16	1	16	0	0	0	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam, Phl.pra, Poa.pra, Poa.tri, Tri.fla
B3A11	B3	4	2	2	0	2	0	Bro.ere, Poa.tri, Pla.lan, Pru.vul
B3A12	B3	1	1	0	1	0	0	Lat.pra
B3A13	B3	4	1	4	0	0	0	Alo.pra, Ant.odo, Bro.ere, Poa.tri
B3A14	B3	60	4	16	12	12	20	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam, Phl.pra, Poa.pra, Poa.tri, Tri.fla, Aju.rep, Bel.per, Gle.hed, Leo.aut, Leo.his, Pla.lan, Pla.med, Pri.ver, Pru.vul, Ran.rep, Tar.off, Ver.cha, Ach.mil, Ant.syl, Cam.pat, Car.pra, Car.car, Cen.jac, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Pas.sat, Pim.maj, Ran.acr, Rum.ace, San.off, Tra.pra, Lat.pra, Lot.cor, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B3A16	B3	16	2	0	8	8	0	Aju.rep, Gle.hed, Leo.his, Pla.med, Pru.vul, Ran.rep, Tar.off, Ver.cha, Lat.pra, Med.lup, Ono.vic, Tri.cam, Tri.fra, Tri.hyb, Tri.rep, Vic.cra
B3A17	B3	1	1	0	0	1	0	Ver.cha
B3A19	B3	2	2	1	0	1	0	Tri.fla, Tar.off
B3A20	B3	8	2	0	4	0	4	Cam.pat, Car.pra, Her.sph, Kna.arv, Lot.cor, Tri.cam, Tri.fra, Tri.hyb
B3A21	B3	2	1	0	2	0	0	Lot.cor, Tri.pra

17

B3A22	B3	16	4	4	4	4	4	Ant.odo, Bro.ere, Fes.rub, Phl.pra, Aju.rep, Bel.per, Ran.rep, Ver.cha, Cre.bie, Gal.mol, Ger.pra, Rum.ace, Ono.vic, Tri.dub, Tri.fra, Vic.cra
B3A23	B3	4	4	1	1	1	1	Bro.hor, Ran.rep, Leu.vul, Tri.fra
B3A24	B3	16	3	6	5	5	0	Ant.odo, Arr.ela, Ave.pub, Bro.hor, Fes.pra, Poa.tri, Aju.rep, Gle.hed, Pru.vul, Ran.rep, Tar.off, Lot.cor, Med.var, Tri.pra, Tri.rep, Vic.cra
B4A01	B4	60	4	16	12	12	20	Alo.pra, Ant.odo, Arr.ela, Ave.pub, Bro.ere, Bro.hor, Cyn.cri, Dac.glo, Fes.pra, Fes.rub, Hol.lan, Luz.cam, Phl.pra, Poa.pra, Poa.tri, Tri.fla, Aju.rep, Bel.per, Gle.hed, Leo.aut, Leo.his, Pla.lan, Pla.med, Pri.ver, Pru.vul, Ran.rep, Tar.off, Ver.cha, Ach.mil, Ant.syl, Cam.pat, Car.pra, Car.car, Cen.jac, Cir.ole, Cre.bie, Dau.car, Gal.mol, Ger.pra, Her.sph, Kna.arv, Leu.vul, Pas.sat, Pim.maj, Ran.acr, Rum.ace, San.off, Tra.pra, Lat.pra, Lot.cor, Med.lup, Med.var, Ono.vic, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B4A02	B4	16	3	5	0	5	6	Arr.ela, Cyn.cri, Luz.cam, Phl.pra, Poa.pra, Gle.hed, Leo.his, Pla.med, Ran.rep, Tar.off, Ant.syl, Gal.mol, Her.sph, Kna.arv, Pas.sat, Ran.acr
B4A04	B4	4	4	1	1	1	1	Arr.ela, Pla.lan, Ant.syl, Tri.cam
B4A06	B4	8	1	0	0	8	0	Aju.rep, Bel.per, Gle.hed, Leo.aut, Pri.ver, Pru.vul, Tar.off, Ver.cha
B4A07	B4	4	2	0	2	0	2	Car.pra, Cre.bie, Med.lup, Tri.rep
B4A08	B4	8	2	4	0	4	0	Ant.odo, Ave.pub, Bro.hor, Fes.rub, Aju.rep, Pla.lan, Tar.off, Ver.cha
B4A09	B4	1	1	0	1	0	0	Tri.rep
B4A10	B4	8	3	2	0	3	3	Bro.ere, Fes.pra, Aju.rep, Pla.med, Pri.ver, Ach.mil, Car.car, Pim.maj
B4A11	B4	4	3	1	1	0	2	Tri.fla, Her.sph, Tra.pra, Med.var
B4A12	B4	1	1	1	0	0	0	Poa.pra
B4A13	B4	1	1	0	0	0	1	Cir.ole
B4A14	B4	2	1	0	0	2	0	Bel.per, Pla.lan
B4A15	B4	2	2	0	1	1	0	Ran.rep, Med.lup
B4A16	B4	8	4	2	2	2	2	Phl.pra, Poa.tri, Pri.ver, Tar.off, Ant.syl, San.off, Tri.dub, Tri.fra

18

B4A17	B4	2	1	0	0	0	2	Dau.car, Her.sph
B4A18	B4	16	4	4	4	4	4	Alo.pra, Bro.hor, Cyn.cri, Luz.cam, Leo.aut, Pla.med, Tar.off, Ver.cha, Car.car, Cre.bie, Her.sph, Pim.maj, Lat.pra, Ono.vic, Tri.cam, Tri.hyb
B4A20	B4	16	2	0	8	0	8	Ant.syl, Cam.pat, Car.pra, Cen.jac, Cir.ole, Ger.pra, Rum.ace, Tra.pra, Med.var, Tri.cam, Tri.dub, Tri.fra, Tri.hyb, Tri.pra, Tri.rep, Vic.cra
B4A21	B4	2	2	0	1	0	1	Dau.car, Med.var
B4A22	B4	4	1	0	0	0	4	Cam.pat, Car.pra, Ger.pra, Kna.arv

19

Supplementary Table 3 | Results of Wilcoxon signed-rank tests for paired samples. The results correspond to the plant species-specific differential abundance analyses using DESeq2 shown in Fig. 3 and Supplementary Fig. 5. Tests compare the numbers of enriched and reduced OTUs associated with each plant species across treatments, as well as the number of enriched vs. reduced OTUs within each treatment. Significance code: p ≤ 0.001: "***"; p ≤ 0.01: "**"; p ≤ 0.05: "*"; p ≤ 0.1: "."; p > 0.1: "n.s." (not significant).

	Fungi (Plant pathogens)			**Fungi (Non-plant-pathogens)**			**Oomycota (*Pythium*)**			**Oomycota (*Globisporangium*, etc.)**		
	V	p		V	p		V	p		V	p	
	enriched/upregulated			enriched/upregulated			enriched/upregulated			enriched/upregulated		
−SH −PH vs +SH −PH	305.5	2.08E-05	***	311.5	9.03E-06	***	34	1.36E-10	***	588	3.85E-02	*
−SH −PH vs +SH +PH	251	1.73E-06	***	181	6.67E-08	***	290	5.82E-05	***	612	1.87E-01	n.s.
+SH −PH vs +SH +PH	561	0.03519	*	679.5	0.08363	.	1484	2.23E-08	***	998	0.174	n.s.
	reduced/downregulated			reduced/downregulated			reduced/downregulated			reduced/downregulated		
−SH −PH vs +SH −PH	42	4.68E-10	***	224	3.71E-07	***	406.5	4.06E-02	*	388.5	1.81E-04	***
−SH −PH vs +SH +PH	12.5	4.63E-11	***	37	2.40E-10	***	393.5	4.36E-03	**	570.5	1.78E-02	*
+SH −PH vs +SH +PH	677.5	0.1182	n.s.	183	1.96E-07	***	570.5	0.2815	n.s.	1156.5	0.076	.
	enriched vs. reduced			enriched vs. reduced			enriched vs. reduced			enriched vs. reduced		
−SH −PH	1030	0.2753	n.s.	1828	1.85E-11	***	880	0.002702	**	532.5	0.03058	*
+SH −PH	437	0.001209	**	1830	1.67E-11	***	1748	7.45E-11	***	154.5	9.48E-08	***
+SH +PH	366.5	0.0001553	***	1752	7.35E-10	***	1289.5	0.0007831	***	333.5	0.000258	***

	Phytomyxea			**Cercozoa (Consumers)**			**Bacteria**		
	V	p		V	p		V	p	
	enriched/upregulated			enriched/upregulated			enriched/upregulated		
−SH −PH vs +SH −PH	482.5	3.93E-02	*	639.5	6.44E-02	.	1008.5	2.38E-01	n.s.
−SH −PH vs +SH +PH	397.5	6.56E-04	***	680.5	1.24E-01	n.s.	749	2.23E-01	n.s.
+SH −PH vs +SH +PH	443.5	0.01012	*	833	0.6975	n.s.	670.5	0.07245	n.s.
	reduced/downregulated			reduced/downregulated			reduced/downregulated		
−SH −PH vs +SH −PH	102	2.69E-01	n.s.	527.5	1.77E-02	*	226	4.01E-07	***
−SH −PH vs +SH +PH	121.5	1.25E-03	**	225	6.43E-07	***	241.5	7.25E-07	***
+SH −PH vs +SH +PH	204.5	0.01392	*	343.5	7.48E-05	***	567	0.01052	*
	enriched vs. reduced			enriched vs. reduced			enriched vs. reduced		
−SH −PH	1194	6.86E-09	***	1027	0.112	n.s.	1804.5	5.96E-11	***
+SH −PH	1389	2.44E-09	***	938	0.1604	n.s.	472.5	0.004974	**
+SH +PH	1446	1.42E-09	***	265	2.92E-06	***	232	3.97E-06	***

Supplementary Table 4 | PERMANOVA results for beta diversity analysis, simple models. Results of permutational multivariate analysis of variance (PERMANOVA; Bray-Curtis dissimilarities, 999 permutations) testing differences in microbial community composition (beta diversity) across the plant diversity gradient and among treatments. The factor "block" was fitted first to account for spatial effects. To properly reflect the nested experimental design, plant diversity (as log(sown diversity)) was tested using "plot" as the error term, with permutations restricted among plots. Treatment effects and the interaction between log(sown diversity) and treatment were tested using the residuals as the error term, with permutations restricted within plots. The corresponding significance levels are displayed in Fig. 4a-g. The models tested the factor "treatment" as a whole with three levels (−SH −PH, +SH −PH, +SH +PH). More complex models with planned contrasts are provided in Supplementary Table 5.

	Fungi (Plant pathogens)						**Fungi (Non-plant-pathogens)**						**Oomycota (*Pythium*)**						**Oomycota (*Globisporangium*, etc.)**					
	Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p	
block	3	1.2574	0.04774	2.815	0.001	***	3	3.126	0.07784	5.377	0.001	***	3	0.518	0.03441	2.311	0.008	**	3	0.274	0.03634	2.883	0.001	***
log(sowndiv)	1	1.8373	0.06975	12.34	0.001	***	1	1.674	0.04168	8.637	0.001	***	1	0.4008	0.02663	5.365	0.003	**	1	0.1907	0.02529	6.019	0.001	***
plot	75	11.1665	0.42394	2.4936	0.001	***	75	14.536	0.36194	1.9693	0.001	***	75	5.6028	0.37224	1.8835	0.001	***	75	2.3762	0.31514	1.2187	0.001	***
treatment	2	2.0877	0.07926	17.4826	0.001	***	2	5.06	0.126	25.7086	0.001	***	2	2.0915	0.13896	26.3665	0.001	***	2	0.5909	0.07836	11.3643	0.001	***
log(so.):treat.	2	0.6763	0.02567	5.663	0.001	***	2	0.411	0.01024	2.0899	0.001	***	2	0.2511	0.01668	3.1651	0.003	**	2	0.053	0.00703	1.0198	0.406	n.s.
Residual	156	9.3146	0.35363				156	15.353	0.38229				156	6.1873	0.41108				156	4.0554	0.53784			
Total	239	26.3397	1				239	40.161	1				239	15.0516	1				239	7.5402	1			

	Phytomyxea						**Cercozoa (Consumers)**						**Bacteria**					
	Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p	
block	3	1.8463	0.06324	4.868	0.001	***	3	0.725	0.06309	4.936	0.001	***	3	0.6848	0.09631	7.358	0.001	***
log(sowndiv)	1	0.9152	0.03135	7.239	0.001	***	1	0.4312	0.03753	8.808	0.001	***	1	0.392	0.05513	12.636	0.001	***
plot	75	9.4821	0.32477	1.3052	0.001	***	72	3.5249	0.30676	1.4012	0.001	***	65	2.0165	0.28358	1.3576	0.001	***
treatment	2	1.6711	0.05723	8.6255	0.001	***	2	1.4804	0.12883	21.1853	0.001	***	2	0.7986	0.11231	17.475	0.001	***
log(so.):treat.	2	0.1706	0.00584	0.8807	0.631	n.s.	2	0.0885	0.0077	1.2667	0.104	n.s.	2	0.1111	0.01563	2.4315	0.001	***
Residual	156	15.1113	0.51757				150	5.2407	0.45609				136	3.1077	0.43704			
Total	239	29.1966	1				230	11.4906	1				209	7.1109	1			

Supplementary Table 5 | PERMANOVA results for beta diversity analysis, models with planned contrasts. Results of permutational multivariate analysis of variance (PERMANOVA; Bray-Curtis dissimilarities, 999 permutations) testing differences in microbial community composition (beta diversity) across the plant diversity gradient and among treatments. In these models, the factor "treatment" was split into two planned contrasts: −SH vs. +SH (new soil from 2016 (−SH) vs. old soil from 2002 (+SH)), and subsequently −PH vs. +PH (new plants from 2016 (−PH) vs. old plants from 2002 (+PH)). Simpler models are provided in Supplementary Table 4. Significance code: p ≤ 0.001: "***"; p ≤ 0.01: "**"; p ≤ 0.05: "*"; p ≤ 0.1: "."; p > 0.1: "n.s." (not significant).

	Fungi (Plant pathogens)						**Fungi (Non-plant-pathogens)**						**Oomycota (*Pythium*)**						**Oomycota (*Globisporangium*, etc.)**					
	Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p	
block	3	1.2574	0.04774	2.815	0.001	***	3	3.126	0.07784	5.377	0.001	***	3	0.518	0.03441	2.311	0.008	**	3	0.274	0.03634	2.883	0.001	***
log(sowndiv)	1	1.8373	0.06975	12.34	0.001	***	1	1.674	0.04168	8.637	0.001	***	1	0.4008	0.02663	5.365	0.003	**	1	0.1907	0.02529	6.019	0.001	***
plot	75	11.1665	0.42394	2.4936	0.001	***	75	14.536	0.36194	1.9693	0.001	***	75	5.6028	0.37224	1.8835	0.001	***	75	2.3762	0.31514	1.2187	0.001	***
−SH vs. +SH	1	1.7426	0.06616	29.1853	0.001	***	1	4.197	0.1045	42.6413	0.001	***	1	2.0147	0.13385	50.7966	0.001	***	1	0.5345	0.07088	20.559	0.001	***
−PH vs. +PH	1	0.3451	0.0131	5.7799	0.001	***	1	0.864	0.02151	8.7759	0.001	***	1	0.0768	0.0051	1.9364	0.088	.	1	0.0564	0.00748	2.1696	0.036	*
log(so.):−SH vs. +SH	1	0.3773	0.01432	6.3191	0.001	***	1	0.245	0.0061	2.4892	0.005	**	1	0.1367	0.00908	3.4476	0.018	*	1	0.0354	0.0047	1.3634	0.185	n.s.
log(so.):−PH vs. +PH	1	0.299	0.01135	5.007	0.002	**	1	0.166	0.00414	1.6906	0.055	.	1	0.1143	0.0076	2.8827	0.024	*	1	0.0176	0.00233	0.6762	0.71	n.s.
Residual	156	9.3146	0.35363				156	15.353	0.38229				156	6.1873	0.41108				156	4.0554	0.53784			
Total	239	26.3397	1				239	40.161	1				239	15.0516	1				239	7.5402	1			

	Phytomyxea						**Cercozoa (Consumers)**						**Bacteria**					
	Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p		Df	SumSq	R^2	F	p	
block	3	1.8463	0.06324	4.868	0.001	***	3	0.725	0.06309	4.936	0.001	***	3	0.6848	0.09631	7.358	0.001	***
log(sowndiv)	1	0.9152	0.03135	7.239	0.001	***	1	0.4312	0.03753	8.808	0.001	***	1	0.392	0.05513	12.636	0.001	***
plot	75	9.4821	0.32477	1.3052	0.001	***	72	3.5249	0.30676	1.4012	0.001	***	65	2.0165	0.28358	1.3576	0.001	***
−SH vs. +SH	1	1.1972	0.041	12.3591	0.001	***	1	1.3356	0.11623	38.2264	0.001	***	1	0.5997	0.08433	26.2435	0.001	***
−PH vs. +PH	1	0.4739	0.01623	4.8919	0.001	***	1	0.1448	0.0126	4.1442	0.001	***	1	0.199	0.02798	8.7065	0.001	***
log(so.):−SH vs. +SH	1	0.0873	0.00299	0.9009	0.519	n.s.	1	0.0421	0.00367	1.2062	0.169	n.s.	1	0.0785	0.01104	3.437	0.001	***
log(so.):−PH vs. +PH	1	0.0834	0.00285	0.8605	0.586	n.s.	1	0.0464	0.00404	1.3271	0.117	n.s.	1	0.0326	0.00458	1.426	0.095	.
Residual	156	15.1113	0.51757				150	5.2407	0.45609				136	3.1077	0.43704			
Total	239	29.1966	1				230	11.4906	1				209	7.1109	1			

Supplementary Table 6 | PERMANOVA results for the effect of plant species richness within treatments. To quantify the effect strength of plant diversity (as log(sown diversity)) within each treatment, datasets were analyzed separately per treatment using PERMANOVA (Bray-Curtis dissimilarities, 999 permutations). Corresponding R^2 values of log(sown diversity) are visualized in Fig. 4h. Significance code: p ≤ 0.001: "***"; p ≤ 0.01: "**"; p ≤ 0.05: "*"; p ≤ 0.1: "."; p > 0.1: "n.s." (not significant).

	Fungi (Plant pathogens)													
	−SH −PH (novel interactions)					**+SH −PH (soil history)**					**+SH +PH (coupled history)**			
	Df	SumSq	R^2	F	p	SumSq	R^2	F	p		SumSq	R^2	F	p	
block	3	0.5598	0.0788	2.2289	0.002	**	0.616	0.0738	2.2801	0.001	***	0.5363	0.061	1.914	0.008	**
log(sowndiv)	1	0.2673	0.0376	3.193	0.002	**	0.9797	0.1173	10.88	0.001	***	1.2553	0.1427	13.439	0.001	***
Residual	75	6.2787	0.8836				6.7535	0.8089				7.0054	0.7963			
Total	79	7.1058	1				8.3492	1				8.7971	1			
	Fungi (Non-plant-pathogens)													
	−SH −PH (novel interactions)					**+SH −PH (soil history)**					**+SH +PH (coupled history)**			
	Df	SumSq	R^2	F	p	SumSq	R^2	F	p		SumSq	R^2	F	p	
block	3	1.3054	0.1451	4.4955	0.001	***	1.7057	0.1304	4.0068	0.001	***	1.4504	0.1113	3.4043	0.001	***
log(sowndiv)	1	0.4313	0.0479	4.4558	0.001	***	0.7282	0.0557	5.1319	0.001	***	0.9259	0.0711	6.5194	0.001	***
Residual	75	7.2593	0.807				10.6426	0.8139				10.6515	0.8176			
Total	79	8.9959	1				13.0766	1				13.0278	1			
	Oomycota (*Pythium*)													
	−SH −PH (novel interactions)					**+SH −PH (soil history)**					**+SH +PH (coupled history)**			
	Df	SumSq	R^2	F	p	SumSq	R^2	F	p		SumSq	R^2	F	p	
block	3	0.3301	0.075	2.0725	0.015	*	0.3254	0.0667	1.9657	0.024	*	0.256	0.0695	1.9575	0.023	*
log(sowndiv)	1	0.0884	0.0201	1.6654	0.126	n.s.	0.4117	0.0845	7.4622	0.001	***	0.1596	0.0433	3.6621	0.004	**
Residual	75	3.9814	0.9049				4.1379	0.8488				3.2695	0.88721			
Total	79	4.3998	1				4.875	1				3.6852	1			
	Oomycota (*Globisporangium*, etc.)													
	−SH −PH (novel interactions)					**+SH −PH (soil history)**					**+SH +PH (coupled history)**			
	Df	SumSq	R^2	F	p	SumSq	R^2	F	p		SumSq	R^2	F	p	
block	3	0.2269	0.0901	2.5341	0.001	***	0.1618	0.0724	2.019	0.007	**	0.1614	0.0735	2.1091	0.004	**
log(sowndiv)	1	0.0534	0.0212	1.79	0.07	.	0.0701	0.0314	2.6239	0.015	*	0.1200	0.0547	4.7047	0.001	***
Residual	75	2.2382	0.8887				2.0039	0.8963				1.9136	0.8718			
Total	79	2.5185	1				2.2358	1				2.1951	1			
	Phytomyxea													
	−SH −PH (novel interactions)					**+SH −PH (soil history)**					**+SH +PH (coupled history)**			
	Df	SumSq	R^2	F	p	SumSq	R^2	F	p		SumSq	R^2	F	p	
block	3	0.4805	0.0624	1.7204	0.081	.	0.9787	0.1010	2.8732	0.001	***	1.4712	0.1451	4.5913	0.001	***
log(sowndiv)	1	0.2339	0.0304	2.5129	0.065	.	0.1939	0.02	1.708	0.12	n.s.	0.6591	0.065	6.171	0.001	***
Residual	75	6.9816	0.9072				8.5159	0.879				8.0108	0.7899			
Total	79	7.6959	1				9.6885	1				10.1411	1			
	Cercozoa (Consumers)													
	−SH −PH (novel interactions)					**+SH −PH (soil history)**					**+SH +PH (coupled history)**			
	Df	SumSq	R^2	F	p	SumSq	R^2	F	p		SumSq	R^2	F	p	
block	3	0.3053	0.1104	3.1248	0.001	***	0.3713	0.1032	2.9371	0.001	***	0.3734	0.1024	2.9269	0.001	***
log(sowndiv)	1	0.1151	0.0416	3.533	0.001	***	0.1923	0.0535	4.5633	0.001	***	0.2127	0.0583	5.0016	0.001	***
Residual	72	2.3449	0.848				3.0337	0.84334				3.0617	0.8393			
Total	76	2.76529	1				3.5972	1				3.6478	1			
	Bacteria													
	−SH −PH (novel interactions)					**+SH −PH (soil history)**					**+SH +PH (coupled history)**			
	Df	SumSq	R^2	F	p	SumSq	R^2	F	p		SumSq	R^2	F	p	
block	3	0.3189	0.1691	4.6608	0.001	***	0.3422	0.1616	4.5863	0.001	***	0.3454	0.1496	4.3818	0.001	***
log(sowndiv)	1	0.0841	0.0446	3.6883	0.001	***	0.1586	0.0749	6.3745	0.001	***	0.2558	0.1108	9.7362	0.001	***
Residual	65	1.4826	0.7863				1.6167	0.7635				1.7078	0.7396			
Total	69	1.8857	1				2.1175	1				2.3090	1			

Supplementary Table 7 | ANOVA results for alpha diversity analysis. Results of linear mixed-effects models tested with analysis of variance (ANOVA, type I SS) assessing differences in alpha diversity (Hill numbers) across the plant diversity gradient and among treatments. "Block" and "plot" were included as random effects to account for spatial variation and to appropriately reflect the nested experimental design. Trends based on model predictions and the respective significance levels from the ANOVAs are displayed in Fig. 6. Significance code: p ≤ 0.001: "***"; p ≤ 0.01: "**"; p ≤ 0.05: "*"; p ≤ 0.1: "."; p > 0.1: "n.s." (not significant).

	Fungi (Plant pathogens)								**Fungi (Non-plant-pathogens)**							
			ChaoRichness			**ChaoInvSimpson**					**ChaoRichness**			**ChaoInvSimpson**		
	numDF	denDF	F	p		F	p		numDF	denDF	F	p		F	p	
(Intercept)	1	156	2723	<.0001	***	1093	<.0001	***	1	156	4712.919	<.0001	***	583.196	<.0001	***
log(sowndiv)	1	75	0.02	0.888	n.s.	0.26	0.6098	n.s.	1	75	23.733	<.0001	***	20.0523	<.0001	***
treatment	2	156	0.29	0.752	n.s.	15	<.0001	***	2	156	14.835	<.0001	***	5.795	0	**
log(so.):treat.	2	156	3.65	0.028	*	4.65	0.011	*	2	156	0.96	0.39	n.s.	1.4557	0.24	n.s.
	Oomycota (*Pythium*)								**Oomycota (*Globisporangium*, etc.)**							
			ChaoRichness			**ChaoInvSimpson**					**ChaoRichness**			**ChaoInvSimpson**		
	numDF	denDF	F	p		F	p		numDF	denDF	F	p		F	p	
(Intercept)	1	156	7736	<.0001	***	4509	<.0001	***	1	156	5614.434	<.0001	***	772.335	<.0001	***
log(sowndiv)	1	75	0.02	0.902	n.s.	5.18	0.0257	*	1	75	0.925	0.34	n.s.	9.5488	0	**
treatment	2	156	5.65	0.004	**	15.9	<.0001	***	2	156	6.419	0	**	9.435	0	***
log(so.):treat.	2	156	0.14	0.868	n.s.	5.25	0.0062	**	2	156	0.13	0.88	n.s.	0.4814	0.62	n.s.
	Phytomyxea								**Cercozoa (Consumers)**							
			ChaoRichness			**ChaoInvSimpson**					**ChaoRichness**			**ChaoInvSimpson**		
	numDF	denDF	F	p		F	p		numDF	denDF	F	p		F	p	
(Intercept)	1	156	1293	<.0001	***	487	<.0001	***	1	153	29739.96	<.0001	***	1331.3	<.0001	***
log(sowndiv)	1	75	0.62	0.435	n.s.	25.6	<.0001	***	1	75	4.323	0.04	*	1.1545	0.29	n.s.
treatment	2	156	0.59	0.556	n.s.	10.8	<.0001	***	2	153	0.294	0.75	n.s.	11.7829	<.0001	***
log(so.):treat.	2	156	0.49	0.616	n.s.	0.31	0.7346	n.s.	2	153	0.858	0.43	n.s.	0.3253	0.72	n.s.

	Bacteria							
			ChaoRichness			**ChaoInvSimpson**		
	numDF	denDF	F	p		F	p	
(Intercept)	1	146	168811	<.0001	***	307	<.0001	***
log(sowndiv)	1	75	0.17	0.684	n.s.	36.8	<.0001	***
treatment	2	146	2.67	0.073	.	2.68	0.072	.
log(so.):treat.	2	146	3.04	0.051	.	0.08	0.9195	n.s.

Supplementary Table 8 | Barcodes and primers for Oomycota and Cercozoa. The table was formatted as an oligos file for the mothur pipeline and can be used for demultiplexing the raw FASTQ files of Oomycota and Cercozoa (ENA project accession number PRJEB77807). The last column contains the sample names composed of block code (B1-B4), plot code (A01-A24), and treatment/subplot (D1: "Novel interactions" (−SH −PH); D2: "Soil history" (+SH −PH); D3: "Coupled history" (+SH +PH)). An additional artificial sample composed of DNA extracts from laboratory cultures ("Mock") was added to the sequencing batches for quality filtering.

Oomycota			
Primer	GCGGAAGGATCATTACCAC	TCTTCATCGDTGTGCGAGC	
barcode	GCTTCTAG	GATACTGC	B4A06D1
barcode	GCTTCTAG	TGACAGGT	B4A20D2
barcode	GCTTCTAG	GCTGTGAT	B2A20D1
barcode	GCTTCTAG	TGGAGCTT	B3A04D2
barcode	GCTTCTAG	TTACGCGA	B4A22D2
barcode	GCTTCTAG	CGATAAGG	B4A13D1
barcode	GCTTCTAG	TCGATCTC	B3A16D2
barcode	GCTTCTAG	TGTCATGC	B4A10D3
barcode	GCTTCTAG	CACATGCT	B4A12D2
barcode	GCTTCTAG	GACTCTTA	B4A11D2
barcode	GCTTCTAG	CCTTAATG	B3A17D3
barcode	GCTTCTAG	ATAGCTTG	B1A01D1
barcode	GCTTCTAG	TTACTGCA	B3A08D1
barcode	TAGCTCTG	GATACTGC	B3A23D3
barcode	TAGCTCTG	TGACAGGT	B3A07D3
barcode	TAGCTCTG	GCTGTGAT	B4A09D3
barcode	TAGCTCTG	AGGCTGTA	B3A14D2
barcode	TAGCTCTG	TCGCTGAA	B4A16D2
barcode	TAGCTCTG	CTAGGCTT	B1A13D2
barcode	TAGCTCTG	TCGATCTC	B3A04D3
barcode	TAGCTCTG	TGTCATGC	B1A02D3
barcode	TAGCTCTG	AACGTGGA	B3A19D2
barcode	TAGCTCTG	GACTCTTA	B3A07D1
barcode	TAGCTCTG	ACTGTGTA	B4A21D2
barcode	TAGCTCTG	TTACTGCA	B3A04D1
barcode	GGTACTCA	GATACTGC	B4A11D3
barcode	GGTACTCA	TGACAGGT	B4A12D1
barcode	GGTACTCA	GCTGTGAT	B4A09D2
barcode	GGTACTCA	AGGCTGTA	B1A03D1
barcode	GGTACTCA	TGGAGCTT	B1A16D2
barcode	GGTACTCA	TTACGCGA	B2A18D2
barcode	GGTACTCA	CGATAAGG	B1A14D2
barcode	GGTACTCA	TGTCATGC	B4A01D3
barcode	GGTACTCA	AACGTGGA	B3A24D3
barcode	GGTACTCA	CACATGCT	B3A14D3
barcode	GGTACTCA	CCTTAATG	B4A12D3
barcode	GGTACTCA	ATAGCTTG	B3A09D1
barcode	TGTAGGTC	GATACTGC	B4A17D2

25

barcode	TGTAGGTC	TGACAGGT	B1A08D3
barcode	TGTAGGTC	GCTGTGAT	B4A17D1
barcode	TGTAGGTC	AGGCTGTA	B1A08D1
barcode	TGTAGGTC	TGGAGCTT	B4A10D1
barcode	TGTAGGTC	TCGCTGAA	B4A06D3
barcode	TGTAGGTC	CTAGGCTT	B2A04D2
barcode	TGTAGGTC	TCGATCTC	B4A09D1
barcode	TGTAGGTC	AACGTGGA	B4A07D3
barcode	TGTAGGTC	CACATGCT	B4A20D3
barcode	TGTAGGTC	GACTCTTA	B4A17D3
barcode	TGTAGGTC	ACTGTGTA	B4A02D2
barcode	TGTAGGTC	TTACTGCA	B2A06D3
barcode	TGCAACCA	GATACTGC	B4A02D1
barcode	TGCAACCA	TGACAGGT	B4A02D3
barcode	TGCAACCA	GCTGTGAT	B4A10D2
barcode	TGCAACCA	AGGCTGTA	B2A13D2
barcode	TGCAACCA	TGGAGCTT	B3A03D1
barcode	TGCAACCA	TTACGCGA	B3A13D3
barcode	TGCAACCA	CGATAAGG	B1A20D3
barcode	TGCAACCA	TGTCATGC	B1A19D1
barcode	TGCAACCA	CACATGCT	B2A06D2
barcode	TGCAACCA	GACTCTTA	B1A11D2
barcode	TGCAACCA	CCTTAATG	B1A07D1
barcode	TGCAACCA	ATAGCTTG	B3A20D3
barcode	TGCAACCA	TTACTGCA	B2A12D2
barcode	CAATGCGA	GATACTGC	B1A20D2
barcode	CAATGCGA	TGACAGGT	B4A13D3
barcode	CAATGCGA	GCTGTGAT	B2A18D1
barcode	CAATGCGA	AGGCTGTA	B2A17D1
barcode	CAATGCGA	TGGAGCTT	B1A03D2
barcode	CAATGCGA	TCGCTGAA	B1A15D3
barcode	CAATGCGA	TTACGCGA	B1A05D2
barcode	CAATGCGA	CTAGGCTT	B1A21D1
barcode	CAATGCGA	TCGATCTC	B1A04D1
barcode	CAATGCGA	AACGTGGA	B1A02D1
barcode	CAATGCGA	GACTCTTA	B1A14D3
barcode	CAATGCGA	CCTTAATG	B3A12D1
barcode	CAATGCGA	ACTGTGTA	B1A11D3
barcode	CAATGCGA	TTACTGCA	B1A16D3
barcode	GCTATTGC	GATACTGC	B4A22D3
barcode	GCTATTGC	TGACAGGT	B4A14D3
barcode	GCTATTGC	GCTGTGAT	B3A24D1
barcode	GCTATTGC	AGGCTGTA	B1A06D2
barcode	GCTATTGC	TGGAGCTT	B1A01D2
barcode	GCTATTGC	TCGCTGAA	B2A09D1
barcode	GCTATTGC	TTACGCGA	B2A19D2
barcode	GCTATTGC	CTAGGCTT	B4A16D3
barcode	GCTATTGC	CGATAAGG	B2A20D3
barcode	GCTATTGC	TGTCATGC	B1A17D1

26

barcode	GCTATTGC	CACATGCT	B1A21D2
barcode	GCTATTGC	CCTTAATG	B4A06D2
barcode	GCTATTGC	ACTGTGTA	B1A15D2
barcode	GCTATTGC	ATAGCTTG	B1A18D3
barcode	AGTAACGG	GATACTGC	B1A14D1
barcode	AGTAACGG	TGACAGGT	B2A13D3
barcode	AGTAACGG	GCTGTGAT	B4A14D1
barcode	AGTAACGG	AGGCTGTA	B1A18D1
barcode	AGTAACGG	TGGAGCTT	B1A06D3
barcode	AGTAACGG	TCGCTGAA	B3A05D3
barcode	AGTAACGG	TTACGCGA	B2A09D3
barcode	AGTAACGG	CTAGGCTT	B3A05D2
barcode	AGTAACGG	AACGTGGA	B4A18D2
barcode	AGTAACGG	GACTCTTA	B3A07D2
barcode	AGTAACGG	ACTGTGTA	B4A13D2
barcode	AGTAACGG	ATAGCTTG	B4A15D2
barcode	AGTAACGG	TTACTGCA	B1A12D3
barcode	ACTTCTGA	GATACTGC	B4A14D2
barcode	ACTTCTGA	TGACAGGT	B2A04D1
barcode	ACTTCTGA	GCTGTGAT	B4A08D2
barcode	ACTTCTGA	AGGCTGTA	B3A05D1
barcode	ACTTCTGA	TGGAGCTT	B4A21D1
barcode	ACTTCTGA	TCGCTGAA	B1A13D3
barcode	ACTTCTGA	TTACGCGA	B3A23D2
barcode	ACTTCTGA	CTAGGCTT	B3A02D1
barcode	ACTTCTGA	CGATAAGG	B2A04D3
barcode	ACTTCTGA	TCGATCTC	B3A06D2
barcode	ACTTCTGA	CACATGCT	B1A04D3
barcode	ACTTCTGA	CCTTAATG	B2A01D1
barcode	ACTTCTGA	ATAGCTTG	B4A15D3
barcode	ACTTAGCA	TGACAGGT	B2A10D1
barcode	ACTTAGCA	GCTGTGAT	B3A08D2
barcode	ACTTAGCA	AGGCTGTA	B2A05D1
barcode	ACTTAGCA	TGGAGCTT	B3A03D2
barcode	ACTTAGCA	TCGCTGAA	B2A17D3
barcode	ACTTAGCA	TTACGCGA	B1A12D1
barcode	ACTTAGCA	CTAGGCTT	B2A02D3
barcode	ACTTAGCA	CGATAAGG	B2A17D2
barcode	ACTTAGCA	TCGATCTC	B1A08D2
barcode	ACTTAGCA	TGTCATGC	B1A05D3
barcode	ACTTAGCA	AACGTGGA	B4A07D2
barcode	ACTTAGCA	GACTCTTA	B1A21D3
barcode	ACTTAGCA	ACTGTGTA	B2A18D3
barcode	ACTTAGCA	TTACTGCA	B3A22D1
barcode	AACTGTTC	GATACTGC	B4A04D2
barcode	AACTGTTC	GCTGTGAT	B4A18D3
barcode	AACTGTTC	AGGCTGTA	B3A22D3
barcode	AACTGTTC	TGGAGCTT	B3A22D2
barcode	AACTGTTC	TCGCTGAA	B4A04D3

27

barcode	AACTGTTC	TTACGCGA	B1A02D2
barcode	AACTGTTC	CTAGGCTT	B3A21D1
barcode	AACTGTTC	CGATAAGG	B2A22D2
barcode	AACTGTTC	TCGATCTC	B2A20D2
barcode	AACTGTTC	TGTCATGC	B3A21D3
barcode	AACTGTTC	AACGTGGA	B4A01D2
barcode	AACTGTTC	CACATGCT	B4A22D1
barcode	AACTGTTC	CCTTAATG	B3A11D3
barcode	AACTGTTC	ATAGCTTG	B3A24D2
barcode	CATCTTGA	TGACAGGT	B3A11D1
barcode	CATCTTGA	AGGCTGTA	B4A20D1
barcode	CATCTTGA	TGGAGCTT	B3A19D3
barcode	CATCTTGA	TCGCTGAA	B4A07D1
barcode	CATCTTGA	TTACGCGA	B1A16D1
barcode	CATCTTGA	CTAGGCTT	B2A14D1
barcode	CATCTTGA	CGATAAGG	B2A05D2
barcode	CATCTTGA	TCGATCTC	B2A16D1
barcode	CATCTTGA	TGTCATGC	B1A06D1
barcode	CATCTTGA	AACGTGGA	B1A05D1
barcode	CATCTTGA	CACATGCT	B3A06D1
barcode	CATCTTGA	GACTCTTA	B1A11D1
barcode	CATCTTGA	ACTGTGTA	B3A16D1
barcode	CATCTTGA	TTACTGCA	B4A08D3
barcode	AGCTAGTA	GATACTGC	B4A16D1
barcode	AGCTAGTA	GCTGTGAT	B2A16D3
barcode	AGCTAGTA	TGGAGCTT	B4A04D1
barcode	AGCTAGTA	TCGCTGAA	B3A14D1
barcode	AGCTAGTA	TTACGCGA	B3A09D2
barcode	AGCTAGTA	CTAGGCTT	B2A08D2
barcode	AGCTAGTA	CGATAAGG	B2A08D3
barcode	AGCTAGTA	TCGATCTC	B1A13D1
barcode	AGCTAGTA	TGTCATGC	B2A12D3
barcode	AGCTAGTA	AACGTGGA	B2A13D1
barcode	AGCTAGTA	CACATGCT	B2A02D2
barcode	AGCTAGTA	GACTCTTA	B3A20D2
barcode	AGCTAGTA	CCTTAATG	B2A03D1
barcode	AGCTAGTA	ATAGCTTG	B3A13D2
barcode	TTACCTGA	TGACAGGT	B2A21D3
barcode	TTACCTGA	AGGCTGTA	B2A09D2
barcode	TTACCTGA	TCGCTGAA	B2A14D2
barcode	TTACCTGA	TTACGCGA	B1A12D2
barcode	TTACCTGA	CTAGGCTT	B1A17D3
barcode	TTACCTGA	CGATAAGG	B2A21D2
barcode	TTACCTGA	TCGATCTC	B1A07D3
barcode	TTACCTGA	TGTCATGC	B3A12D3
barcode	TTACCTGA	AACGTGGA	B2A03D2
barcode	TTACCTGA	CACATGCT	B3A09D3
barcode	TTACCTGA	GACTCTTA	B2A14D3
barcode	TTACCTGA	CCTTAATG	B4A08D1

28

barcode	TTACCTGA	ACTGTGTA	B2A22D3
barcode	TTACCTGA	TTACTGCA	B3A16D3
barcode	TATACGAG	GATACTGC	B2A12D1
barcode	TATACGAG	GCTGTGAT	B2A15D1
barcode	TATACGAG	TGGAGCTT	B3A01D3
barcode	TATACGAG	TTACGCGA	B3A13D1
barcode	TATACGAG	CTAGGCTT	B4A11D1
barcode	TATACGAG	CGATAAGG	B3A12D2
barcode	TATACGAG	TCGATCTC	B1A04D2
barcode	TATACGAG	TGTCATGC	B2A02D1
barcode	TATACGAG	AACGTGGA	B1A18D2
barcode	TATACGAG	CACATGCT	B3A02D3
barcode	TATACGAG	GACTCTTA	B2A05D3
barcode	TATACGAG	CCTTAATG	B1A19D2
barcode	TATACGAG	ACTGTGTA	B4A21D3
barcode	TATACGAG	ATAGCTTG	B4A18D1
barcode	TATCAGTC	TGACAGGT	B4A15D1
barcode	TATCAGTC	AGGCTGTA	B2A22D1
barcode	TATCAGTC	TCGCTGAA	B1A22D2
barcode	TATCAGTC	CTAGGCTT	B3A23D1
barcode	TATCAGTC	CGATAAGG	B2A15D2
barcode	TATCAGTC	TCGATCTC	B2A10D2
barcode	TATCAGTC	TGTCATGC	B1A22D1
barcode	TATCAGTC	AACGTGGA	B2A10D3
barcode	TATCAGTC	CACATGCT	B2A21D1
barcode	TATCAGTC	GACTCTTA	B2A15D3
barcode	TATCAGTC	CCTTAATG	B2A16D2
barcode	TATCAGTC	ACTGTGTA	B1A07D2
barcode	TATCAGTC	ATAGCTTG	B2A19D3
barcode	TATCAGTC	TTACTGCA	B2A19D1
barcode	TGACTTAG	GATACTGC	B3A01D2
barcode	TGACTTAG	GCTGTGAT	B4A01D1
barcode	TGACTTAG	TGGAGCTT	B2A06D1
barcode	TGACTTAG	TTACGCGA	B3A08D3
barcode	TGACTTAG	CGATAAGG	B2A01D2
barcode	TGACTTAG	TCGATCTC	B1A01D3
barcode	TGACTTAG	TGTCATGC	B1A20D1
barcode	TGACTTAG	AACGTGGA	B1A15D1
barcode	TGACTTAG	CACATGCT	B3A17D1
barcode	TGACTTAG	GACTCTTA	B3A02D2
barcode	TGACTTAG	CCTTAATG	B1A22D3
barcode	TGACTTAG	ACTGTGTA	B2A01D3
barcode	TGACTTAG	ATAGCTTG	B1A03D3
barcode	TGACTTAG	TTACTGCA	B3A20D1
barcode	TGTCAACA	TGACAGGT	B3A17D2
barcode	TGTCAACA	AGGCTGTA	B3A01D1
barcode	TGTCAACA	TCGCTGAA	B1A17D2
barcode	TGTCAACA	CTAGGCTT	B3A21D2
barcode	TGTCAACA	TCGATCTC	B1A19D3

29

barcode	TGTCAACA	TGTCATGC	B3A11D2
barcode	TGTCAACA	AACGTGGA	B3A19D1
barcode	TGTCAACA	GACTCTTA	B3A06D3
barcode	TGTCAACA	CCTTAATG	B2A08D1
barcode	TGTCAACA	ACTGTGTA	B2A03D3
barcode	TGTCAACA	ATAGCTTG	B3A03D3
barcode	TGTCAACA	TTACTGCA	Mock
		Cercozoa	
Primer	GTTAAAARGCTCGTAGTYG	AAGARGAYATCCTTGGTG	
barcode	GTGAACTC	ACGATCAG	B4A06D1
barcode	GTGAACTC	GTGTCAAC	B4A20D2
barcode	GTGAACTC	CGTTCAAG	B2A20D1
barcode	GTGAACTC	TCTCTCAG	B3A04D2
barcode	GTGAACTC	TACTGTAG	B4A22D2
barcode	GTGAACTC	GACATATC	B4A13D1
barcode	GTGAACTC	ACAATGTG	B3A16D2
barcode	GTGAACTC	GTAACATG	B4A10D3
barcode	GTGAACTC	TTAGGAAC	B4A12D2
barcode	GTGAACTC	AATCAGGT	B4A11D2
barcode	GTGAACTC	TACGCTAT	B3A17D3
barcode	GTGAACTC	GTCAGTAT	B1A01D1
barcode	GTGAACTC	TATCAGTC	B3A08D1
barcode	GCGTAATC	ACGATCAG	B3A23D3
barcode	GCGTAATC	GTGTCAAC	B3A07D3
barcode	GCGTAATC	CGTTCAAG	B4A09D3
barcode	GCGTAATC	ATTCTCGG	B3A14D2
barcode	GCGTAATC	AAGCTACT	B4A16D2
barcode	GCGTAATC	AATTGGTC	B1A13D2
barcode	GCGTAATC	ACAATGTG	B3A04D3
barcode	GCGTAATC	GTAACATG	B1A02D3
barcode	GCGTAATC	CCATTATG	B3A19D2
barcode	GCGTAATC	AATCAGGT	B3A07D1
barcode	GCGTAATC	GCTTCAAT	B4A21D2
barcode	GCGTAATC	TATCAGTC	B3A04D1
barcode	ATGTGACC	ACGATCAG	B4A11D3
barcode	ATGTGACC	GTGTCAAC	B4A12D1
barcode	ATGTGACC	CGTTCAAG	B4A09D2
barcode	ATGTGACC	ATTCTCGG	B1A03D1
barcode	ATGTGACC	TCTCTCAG	B1A16D2
barcode	ATGTGACC	TACTGTAG	B2A18D2
barcode	ATGTGACC	GACATATC	B1A14D2
barcode	ATGTGACC	GTAACATG	B4A01D3
barcode	ATGTGACC	CCATTATG	B3A24D3
barcode	ATGTGACC	TTAGGAAC	B3A14D3
barcode	ATGTGACC	TACGCTAT	B4A12D3
barcode	ATGTGACC	GTCAGTAT	B3A09D1
barcode	ATACGCAC	ACGATCAG	B4A17D2
barcode	ATACGCAC	GTGTCAAC	B1A08D3

30

barcode	ATACGCAC	CGTTCAAG	B4A17D1
barcode	ATACGCAC	ATTCTCGG	B1A08D1
barcode	ATACGCAC	TCTCTCAG	B4A10D1
barcode	ATACGCAC	AAGCTACT	B4A06D3
barcode	ATACGCAC	AATTGGTC	B2A04D2
barcode	ATACGCAC	ACAATGTG	B4A09D1
barcode	ATACGCAC	CCATTATG	B4A07D3
barcode	ATACGCAC	TTAGGAAC	B4A20D3
barcode	ATACGCAC	AATCAGGT	B4A17D3
barcode	ATACGCAC	GCTTCAAT	B4A02D2
barcode	ATACGCAC	TATCAGTC	B2A06D3
barcode	ATCTGGAC	ACGATCAG	B4A02D1
barcode	ATCTGGAC	GTGTCAAC	B4A02D3
barcode	ATCTGGAC	CGTTCAAG	B4A10D2
barcode	ATCTGGAC	ATTCTCGG	B2A13D2
barcode	ATCTGGAC	TCTCTCAG	B3A03D1
barcode	ATCTGGAC	TACTGTAG	B3A13D3
barcode	ATCTGGAC	GACATATC	B1A20D3
barcode	ATCTGGAC	GTAACATG	B1A19D1
barcode	ATCTGGAC	TTAGGAAC	B2A06D2
barcode	ATCTGGAC	AATCAGGT	B1A11D2
barcode	ATCTGGAC	TACGCTAT	B1A07D1
barcode	ATCTGGAC	GTCAGTAT	B3A20D3
barcode	ATCTGGAC	TATCAGTC	B2A12D2
barcode	GTCAATCC	ACGATCAG	B1A20D2
barcode	GTCAATCC	GTGTCAAC	B4A13D3
barcode	GTCAATCC	CGTTCAAG	B2A18D1
barcode	GTCAATCC	ATTCTCGG	B2A17D1
barcode	GTCAATCC	TCTCTCAG	B1A03D2
barcode	GTCAATCC	AAGCTACT	B1A15D3
barcode	GTCAATCC	TACTGTAG	B1A05D2
barcode	GTCAATCC	AATTGGTC	B1A21D1
barcode	GTCAATCC	ACAATGTG	B1A04D1
barcode	GTCAATCC	CCATTATG	B1A02D1
barcode	GTCAATCC	AATCAGGT	B1A14D3
barcode	GTCAATCC	TACGCTAT	B3A12D1
barcode	GTCAATCC	GCTTCAAT	B1A11D3
barcode	GTCAATCC	TATCAGTC	B1A16D3
barcode	GACCATAC	ACGATCAG	B4A22D3
barcode	GACCATAC	GTGTCAAC	B4A14D3
barcode	GACCATAC	CGTTCAAG	B3A24D1
barcode	GACCATAC	ATTCTCGG	B1A06D2
barcode	GACCATAC	TCTCTCAG	B1A01D2
barcode	GACCATAC	AAGCTACT	B2A09D1
barcode	GACCATAC	TACTGTAG	B2A19D2
barcode	GACCATAC	AATTGGTC	B4A16D3
barcode	GACCATAC	GACATATC	B2A20D3
barcode	GACCATAC	GTAACATG	B1A17D1
barcode	GACCATAC	TTAGGAAC	B1A21D2

31

barcode	GACCATAC	TACGCTAT	B4A06D2
barcode	GACCATAC	GCTTCAAT	B1A15D2
barcode	GACCATAC	GTCAGTAT	B1A18D3
barcode	GAATACTC	ACGATCAG	B1A14D1
barcode	GAATACTC	GTGTCAAC	B2A13D3
barcode	GAATACTC	CGTTCAAG	B4A14D1
barcode	GAATACTC	ATTCTCGG	B1A18D1
barcode	GAATACTC	TCTCTCAG	B1A06D3
barcode	GAATACTC	AAGCTACT	B3A05D3
barcode	GAATACTC	TACTGTAG	B2A09D3
barcode	GAATACTC	AATTGGTC	B3A05D2
barcode	GAATACTC	CCATTATG	B4A18D2
barcode	GAATACTC	AATCAGGT	B3A07D2
barcode	GAATACTC	GCTTCAAT	B4A13D2
barcode	GAATACTC	GTCAGTAT	B4A15D2
barcode	GAATACTC	TATCAGTC	B1A12D3
barcode	GTTATAGC	ACGATCAG	B4A14D2
barcode	GTTATAGC	GTGTCAAC	B2A04D1
barcode	GTTATAGC	CGTTCAAG	B4A08D2
barcode	GTTATAGC	ATTCTCGG	B3A05D1
barcode	GTTATAGC	TCTCTCAG	B4A21D1
barcode	GTTATAGC	AAGCTACT	B1A13D3
barcode	GTTATAGC	TACTGTAG	B3A23D2
barcode	GTTATAGC	AATTGGTC	B3A02D1
barcode	GTTATAGC	GACATATC	B2A04D3
barcode	GTTATAGC	ACAATGTG	B3A06D2
barcode	GTTATAGC	TTAGGAAC	B1A04D3
barcode	GTTATAGC	TACGCTAT	B2A01D1
barcode	GTTATAGC	GTCAGTAT	B4A15D3
barcode	TAGTTACC	GTGTCAAC	B2A10D1
barcode	TAGTTACC	CGTTCAAG	B3A08D2
barcode	TAGTTACC	ATTCTCGG	B2A05D1
barcode	TAGTTACC	TCTCTCAG	B3A03D2
barcode	TAGTTACC	AAGCTACT	B2A17D3
barcode	TAGTTACC	TACTGTAG	B1A12D1
barcode	TAGTTACC	AATTGGTC	B2A02D3
barcode	TAGTTACC	GACATATC	B2A17D2
barcode	TAGTTACC	ACAATGTG	B1A08D2
barcode	TAGTTACC	GTAACATG	B1A05D3
barcode	TAGTTACC	CCATTATG	B4A07D2
barcode	TAGTTACC	AATCAGGT	B1A21D3
barcode	TAGTTACC	GCTTCAAT	B2A18D3
barcode	TAGTTACC	TATCAGTC	B3A22D1
barcode	AACTTAGC	ACGATCAG	B4A04D2
barcode	AACTTAGC	CGTTCAAG	B4A18D3
barcode	AACTTAGC	ATTCTCGG	B3A22D3
barcode	AACTTAGC	TCTCTCAG	B3A22D2
barcode	AACTTAGC	AAGCTACT	B4A04D3
barcode	AACTTAGC	TACTGTAG	B1A02D2

32

barcode	AACTTAGC	AATTGGTC	B3A21D1
barcode	AACTTAGC	GACATATC	B2A22D2
barcode	AACTTAGC	ACAATGTG	B2A20D2
barcode	AACTTAGC	GTAACATG	B3A21D3
barcode	AACTTAGC	CCATTATG	B4A01D2
barcode	AACTTAGC	TTAGGAAC	B4A22D1
barcode	AACTTAGC	TACGCTAT	B3A11D3
barcode	AACTTAGC	GTCAGTAT	B3A24D2
barcode	TATCTAGC	GTGTCAAC	B3A11D1
barcode	TATCTAGC	ATTCTCGG	B4A20D1
barcode	TATCTAGC	TCTCTCAG	B3A19D3
barcode	TATCTAGC	AAGCTACT	B4A07D1
barcode	TATCTAGC	TACTGTAG	B1A16D1
barcode	TATCTAGC	AATTGGTC	B2A14D1
barcode	TATCTAGC	GACATATC	B2A05D2
barcode	TATCTAGC	ACAATGTG	B2A16D1
barcode	TATCTAGC	GTAACATG	B1A06D1
barcode	TATCTAGC	CCATTATG	B1A05D1
barcode	TATCTAGC	TTAGGAAC	B3A06D1
barcode	TATCTAGC	AATCAGGT	B1A11D1
barcode	TATCTAGC	GCTTCAAT	B3A16D1
barcode	TATCTAGC	TATCAGTC	B4A08D3
barcode	TGATACAC	ACGATCAG	B4A16D1
barcode	TGATACAC	CGTTCAAG	B2A16D3
barcode	TGATACAC	TCTCTCAG	B4A04D1
barcode	TGATACAC	AAGCTACT	B3A14D1
barcode	TGATACAC	TACTGTAG	B3A09D2
barcode	TGATACAC	AATTGGTC	B2A08D2
barcode	TGATACAC	GACATATC	B2A08D3
barcode	TGATACAC	ACAATGTG	B1A13D1
barcode	TGATACAC	GTAACATG	B2A12D3
barcode	TGATACAC	CCATTATG	B2A13D1
barcode	TGATACAC	TTAGGAAC	B2A02D2
barcode	TGATACAC	AATCAGGT	B3A20D2
barcode	TGATACAC	TACGCTAT	B2A03D1
barcode	TGATACAC	GTCAGTAT	B3A13D2
barcode	GTAGATAC	GTGTCAAC	B2A21D3
barcode	GTAGATAC	ATTCTCGG	B2A09D2
barcode	GTAGATAC	AAGCTACT	B2A14D2
barcode	GTAGATAC	TACTGTAG	B1A12D2
barcode	GTAGATAC	AATTGGTC	B1A17D3
barcode	GTAGATAC	GACATATC	B2A21D2
barcode	GTAGATAC	ACAATGTG	B1A07D3
barcode	GTAGATAC	GTAACATG	B3A12D3
barcode	GTAGATAC	CCATTATG	B2A03D2
barcode	GTAGATAC	TTAGGAAC	B3A09D3
barcode	GTAGATAC	AATCAGGT	B2A14D3
barcode	GTAGATAC	TACGCTAT	B4A08D1
barcode	GTAGATAC	GCTTCAAT	B2A22D3

33

barcode	GTAGATAC	TATCAGTC	B3A16D3
barcode	AGTTCATC	ACGATCAG	B2A12D1
barcode	AGTTCATC	CGTTCAAG	B2A15D1
barcode	AGTTCATC	TCTCTCAG	B3A01D3
barcode	AGTTCATC	TACTGTAG	B3A13D1
barcode	AGTTCATC	AATTGGTC	B4A11D1
barcode	AGTTCATC	GACATATC	B3A12D2
barcode	AGTTCATC	ACAATGTG	B1A04D2
barcode	AGTTCATC	GTAACATG	B2A02D1
barcode	AGTTCATC	CCATTATG	B1A18D2
barcode	AGTTCATC	TTAGGAAC	B3A02D3
barcode	AGTTCATC	AATCAGGT	B2A05D3
barcode	AGTTCATC	TACGCTAT	B1A19D2
barcode	AGTTCATC	GCTTCAAT	B4A21D3
barcode	AGTTCATC	GTCAGTAT	B4A18D1
barcode	TTAGAGTC	GTGTCAAC	B4A15D1
barcode	TTAGAGTC	ATTCTCGG	B2A22D1
barcode	TTAGAGTC	AAGCTACT	B1A22D2
barcode	TTAGAGTC	AATTGGTC	B3A23D1
barcode	TTAGAGTC	GACATATC	B2A15D2
barcode	TTAGAGTC	ACAATGTG	B2A10D2
barcode	TTAGAGTC	GTAACATG	B1A22D1
barcode	TTAGAGTC	CCATTATG	B2A10D3
barcode	TTAGAGTC	TTAGGAAC	B2A21D1
barcode	TTAGAGTC	AATCAGGT	B2A15D3
barcode	TTAGAGTC	TACGCTAT	B2A16D2
barcode	TTAGAGTC	GCTTCAAT	B1A07D2
barcode	TTAGAGTC	GTCAGTAT	B2A19D3
barcode	TTAGAGTC	TATCAGTC	B2A19D1
barcode	TATATGCC	ACGATCAG	B3A01D2
barcode	TATATGCC	CGTTCAAG	B4A01D1
barcode	TATATGCC	TCTCTCAG	B2A06D1
barcode	TATATGCC	TACTGTAG	B3A08D3
barcode	TATATGCC	GACATATC	B2A01D2
barcode	TATATGCC	ACAATGTG	B1A01D3
barcode	TATATGCC	GTAACATG	B1A20D1
barcode	TATATGCC	CCATTATG	B1A15D1
barcode	TATATGCC	TTAGGAAC	B3A17D1
barcode	TATATGCC	AATCAGGT	B3A02D2
barcode	TATATGCC	TACGCTAT	B1A22D3
barcode	TATATGCC	GCTTCAAT	B2A01D3
barcode	TATATGCC	GTCAGTAT	B1A03D3
barcode	TATATGCC	TATCAGTC	B3A20D1
barcode	AATTGGTC	GTGTCAAC	B3A17D2
barcode	AATTGGTC	ATTCTCGG	B3A01D1
barcode	AATTGGTC	AAGCTACT	B1A17D2
barcode	AATTGGTC	AATTGGTC	B3A21D2
barcode	AATTGGTC	ACAATGTG	B1A19D3
barcode	AATTGGTC	GTAACATG	B3A11D2

34

barcode	AATTGGTC	CCATTATG	B3A19D1
barcode	AATTGGTC	AATCAGGT	B3A06D3
barcode	AATTGGTC	TACGCTAT	B2A08D1
barcode	AATTGGTC	GCTTCAAT	B2A03D3
barcode	AATTGGTC	GTCAGTAT	B3A03D3
barcode	AATTGGTC	TATCAGTC	Mock

Supplementary References

1. White TJ, Bruns T, Lee S, Taylor J. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: *PCR protocols: A Guide to Methods and Applications* (eds Innis MA, Gelfand DH, Sninsky JJ, White TJ). Academic Press (1990).
2. Ihrmark K, *et al*. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol. Ecol.* **82**, 666-677 (2012).
3. Kohout P, *et al*. Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: Is there a universal solution? *Soil Biol. Biochem.* **68**, 482-493 (2014).
4. Hennecke J, *et al*. Plant Species Richness and the Root Economics Space Drive Soil Fungal Communities. *Ecol. Lett.* **28**, e70032 (2025).
5. Schloss PD, *et al*. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537-7541 (2009).
6. Rognes T, Flouri T, Nichols B, Quince C, Mahe F. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**, e2584 (2016).
7. Nelson MC, Morrison HG, Benjamino J, Grim SL, Graf J. Analysis, optimization and verification of Illumina-generated 16S rRNA gene amplicon surveys. *PLoS One* **9**, e94249 (2014).
8. Bokulich NA, *et al*. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat. Methods* **10**, 57-59 (2013).

9. Abarenkov K, *et al*. The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. *Nucleic Acids Res.* **52**, D791-D797 (2023).
10. Camacho C, *et al*. BLAST+: architecture and applications. *BMC Bioinformatics* **10**, 421 (2009).
11. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**, 2194-2200 (2011).
12. Põlme S, *et al*. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Divers.* **105**, 1-16 (2021).
13. Fiore-Donno AM, Bonkowski M. Different community compositions between obligate and facultative oomycete plant parasites in a landscape-scale metabarcoding survey. *Biol. Fertility Soils* **57**, 245-256 (2021).
14. Solbach MD, *et al*. Legacies of consecutive summer droughts on soil-borne plant parasitic protists (Oomycota: Stramenopila and Phytomyxea: Rhizaria) and protistan consumers (Cercozoa: Rhizaria) along an experimental plant diversity gradient. *New Phytol.* **Early view** (2025).
15. Masigol H, *et al*. A glimpse into Oomycota diversity in freshwater lakes and adjacent forests using a metabarcoding approach. *Sci. Rep.* **15**, 19124 (2025).
16. Uzuhashi S, Kakishima M, Tojo M. Phylogeny of the genus *Pythium* and description of new genera. *Mycoscience* **51**, 337-365 (2010).
17. Fiore-Donno AM, Richter-Heitmann T, Bonkowski M. Contrasting Responses of Protistan Plant Parasites and Phagotrophs to Ecosystems, Land Management and Soil Properties. *Front. Microbiol.* **11**, 1823 (2020).
18. Guillou L, *et al*. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Res.* **41**, D597-D604 (2013).
19. Fiore-Donno AM, *et al*. New barcoded primers for efficient retrieval of cercozoan sequences in high-throughput environmental diversity surveys, with emphasis on worldwide biological soil crusts. *Mol. Ecol. Resour.* **18**, 229-239 (2018).
20. Dumack K, Fiore-Donno AM, Bass D, Bonkowski M. Making sense of environmental sequencing data: Ecologically important functional traits of the protistan groups Cercozoa and Endomyxa (Rhizaria). *Mol. Ecol. Resour.* **20**, 398-403 (2020).

21. Caporaso JG, *et al.* Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A* **108**, 4516-4522 (2011).
22. Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* **75**, 129-137 (2015).
23. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120 (2014).
24. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**, 2957-2963 (2011).
25. Caporaso JG, *et al.* QIIME allows analysis of highthroughput community sequencing data. *Nat. Methods* **7**, 335–336 (2010).
26. Pruesse E, *et al.* SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **35**, 7188-7196 (2007).
27. Quast C, *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590-596 (2013).
28. Glockner FO, *et al.* 25 years of serving the community with ribosomal RNA gene reference databases and tools. *J. Biotechnol.* **261**, 169-176 (2017).
29. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **73**, 5261-5267 (2007).

Chapter 3: JenaTron - An Experimental Approach to Study the Effects of Plant History and Soil History on Grassland Ecosystem Functioning

Publication:

Madaj A-M, Huang Y, Ebeling A, Ertel L, Gebler A, Gleixner G, Hines J, Roscher C, Weigelt A, Albracht C, Amyntas A, Bassi L, Bonato Asato AE, Bonkowski M, Bröcher M, Buscot F, De Giorgi F, Pinheiro Alves de Souza Y, Doan VC, Durka W, Heintz-Buschart A, Hennecke J, Lange M, Medina-van Berkum P, Meyer ST, Krawczyk S, Rai A, Reitz T, Ristok C, Scheu S, Schloter M, Schulz S, Solbach MD, Unsicker SB, Eisenhauer N. 2025. JenaTron - An Experimental Approach to Study the Effects of Plant History and Soil History on Grassland Ecosystem Functioning. *Journal of Visualized Experiments* **217**: e67496. https://doi.org/10.3791/67496

For high-resolution images and the video summary, readers are referred to the online version of the publication.

Declaration of personal contributions:

I contributed to the implementation of the experimental design and sampling design via regular meetings with A-M Madaj, who coordinated the experimental setup and sampling, and with all other PhD students of the Jena Experiment involved in this study. I contributed to the sampling and data acquisition of the plant height data presented in the publication, as well as subsequent biomass harvest, and the final, destructive sampling campaign used for future studies, including microbial analyses. I contributed to revising the article.

jove

JenaTron - An Experimental Approach to Study the Effects of Plant History and Soil History on Grassland Ecosystem Functioning

Anna-Maria Madaj[1,2], **Yuanyuan Huang**[1,2], **Anne Ebeling**[3], **Lisa Ertel**[1,2], **Alban Gebler**[1,2], **Gerd Gleixner**[1,4,5], **Jes Hines**[1,2], **Christiane Roscher**[1,6], **Alexandra Weigelt**[1,2], **Cynthia Albracht**[7,8,9], **Angelos Amyntas**[1,10], **Leonardo Bassi**[1,2], **Ana Elizabeth Bonato Asato**[1,2], **Michael Bonkowski**[11,12], **Maximilian Bröcher**[3], **François Buscot**[1,8], **Francesca De Giorgi**[1,6], **Yuri Pinheiro Alves de Souza**[13,14], **Van Cong Doan**[1,10], **Walter Durka**[1,15], **Anna Heintz-Buschart**[7], **Justus Hennecke**[1,2], **Markus Lange**[4], **Pamela Medina-van Berkum**[16], **Sebastian Tobias Meyer**[14], **Sören Krawczyk**[1,2], **Akanksha Rai**[4], **Thomas Reitz**[1,8], **Christian Ristok**[1,2], **Stefan Scheu**[17,18], **Michael Schloter**[13,14], **Stefanie Schulz**[13], **Marcel Dominik Solbach**[11], **Sybille Barbara Unsicker**[16,19], **Nico Eisenhauer**[1,2]

[1] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig [2] Institute of Biology, Leipzig University [3] Institute of Ecology and Evolution, Friedrich Schiller University Jena [4] Max Planck Institute for Biogeochemistry [5] Institute for Geosciences, Friedrich Schiller University Jena [6] Department Physiological Diversity, Helmholtz Centre for Environmental Research (UFZ) [7] Swammerdam Institute for Life Sciences, University of Amsterdam [8] Department Soil Ecology, Helmholtz Centre for Environmental Research (UFZ) [9] Institute Biosafety in Plant Biotechnology, Julius Kühn-Institute [10] Institute of Biodiversity, Friedrich Schiller University Jena [11] Institute of Zoology, University of Cologne [12] Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne [13] Research Unit Comparative Microbiome Analysis, Helmholtz Zentrum Munich [14] School of Life Science, Technical University Munich - TUM [15] Department of Community Ecology, Helmholtz Centre for Environmental Research (UFZ) [16] Department of Biochemistry, Max-Planck Institute for Chemical Ecology [17] JFB Institute of Zoology and Anthropology, University of Göttingen [18] Centre of Biodiversity and Sustainable Land Use, University of Göttingen [19] Botanical Institute, Plant-Environment-Interactions Group, Kiel University

Corresponding Author

Anna-Maria Madaj
anna-maria.madaj@idiv.de

Citation

Madaj, A.M., Huang, Y., Ebeling, A., Ertel, L., Gebler, A., Gleixner, G., Hines, J., Roscher, C., Weigelt, A., Albracht, C., Amyntas, A., Bassi, L., Bonato Asato, A.E., Bonkowski, M., Bröcher, M., Buscot, F., De Giorgi, F., Pinheiro Alves de Souza, Y., Doan, V.C., Durka, W., Heintz-Buschart, A., Hennecke, J., Lange, M., Medina-van Berkum, P., Meyer, S.T., Krawczyk, S., Rai, A., Reitz, T., Ristok, C., Scheu, S., Schloter, M., Schulz, S., Solbach, M.D., Unsicker, S.B., Eisenhauer, N. JenaTron - An Experimental Approach to Study the Effects of Plant History and Soil History on Grassland Ecosystem Functioning. *J. Vis. Exp.* (217), e67496, doi:10.3791/67496 (2025).

Abstract

The global loss of biodiversity has motivated many studies that experimentally vary plant species richness and examine the consequences for ecosystem functioning. Such experiments generally show a positive relationship between above- and below-ground biodiversity and the functioning of terrestrial ecosystems. Moreover, this relationship tends to strengthen over time, seen as enhanced functioning of diverse plant communities and reduced functioning of low-diversity plant communities. Differences in multitrophic community assembly and biotic interactions in high- versus low-diversity plant communities are hypothesized to affect plant performance by altering consumer community structure and function and driving plastic or micro-evolutionary responses of plant species in the plant communities. To resolve this complex interplay of community history, we separated these effects into plant and soil history. Plant history refers to all plant-level responses to past abiotic and biotic selection pressures experienced in their communities, while soil history relates to all abiotic and biotic soil properties developed as a legacy of plant-soil interactions under variable plant diversity. We set up a biodiversity experiment in an Ecotron, a terrestrial mesocosm facility that allows controlling environmental

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

jove

Date Published

March 21, 2025

DOI

10.3791/67496

URL

jove.com/video/67496

conditions above- and below-ground, to test whether the strengthening biodiversity-ecosystem functioning relationship is due to soil history, plant history, or a combination of both. We established a plant diversity gradient consisting of 1, 2, 3, and 6 grassland plant species and factorially nested with soil history and plant history treatments for each level of plant species richness. Representative results demonstrate the successful establishment of target treatments in the Ecotron experiment, observing the effects of plant and soil history on initial plant development and final plant growth. Additionally, we provide a case study for data analysis of individual response variables. We outline research objectives and methods to comprehensively assess the multifunctional responses to the experimental treatments necessary to ultimately address the overarching hypothesis.

Introduction

Over the past three decades, growing concerns about biodiversity loss have driven extensive research focused on experimentally manipulating plant species richness to investigate its impact on ecosystem functioning[1,2]. Experimental studies have demonstrated a positive relationship between plant diversity and ecosystem functioning[1], which tends to increase over time[3,4,5,6,7,8,9]. This is a significant finding, as it suggests that experimental results obtained from young, recently established communities likely underestimate the consequences of biodiversity loss while emphasizing the importance of conserving older, species-rich ecosystems[10].

Changes in biotic and abiotic processes over time, which can only be observed in long-term experiments, are one possible explanation for the amplifying effects of biodiversity on ecosystem functioning. High plant diversity has been associated with increases in soil carbon and nutrient concentrations[3,9,11,12,13], optimized resource complementarity[3,8,14], enhanced niche differentiation among plants[15], accumulation of mutualists at all trophic levels[10,16], and diminished threats from pathogens and herbivores via stronger top-down regulatory mechanisms[17,18,19]. Conversely, low plant diversity has been associated with selective pressure toward increased investment in plant defenses and less efficient resource use[10,20,21,22]. Taken together, differences in multitrophic community assembly and biotic interactions in high- versus low-diversity plant communities are hypothesized to influence plant performance by altering consumer community structure and function, as well as driving plastic or micro-evolutionary changes of plant species at the level of plant communities[23,24,25,26]. To resolve the complex interplay of community history, these effects were separated into two important components: plant history and soil history. Plant history refers to all plant responses to past abiotic and biotic selection pressures experienced in their respective communities, whereas soil history relates to all abiotic and biotic soil properties developed as a result of plant-soil interactions[23,24,27,28]. Both components

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

are increasingly recognized as key drivers in long-term biodiversity effects[29,30,31].

Experimental field studies provide valuable insights into the long-term effects of soil and plant history under realistic environmental conditions and on a large spatial scale[32,33]. However, field experiments are subject to various uncontrolled external factors, such as weather fluctuations, natural disturbances, and interactions with other organisms. In contrast, small-scale microcosm experiments, while limited in capturing the complexity and dynamics of natural ecosystems, offer precise control over abiotic factors like temperature, light, and water[34]. To combine the advantages of both approaches, novel experimental facilities called 'Ecotrons' emerged in the 1990s (e.g., Ecotron in Silkwood, UK[34]; Ecotron in Montpellier, France[35]; Ecotron in Hasselt, Belgium[36]). Roy et al. (2021)[37] define an Ecotron as an experimental facility consisting of replicated enclosures designed to accomodate ecosystem samples, allowing realistic simulation of above- and below-ground environmental conditions while facilitating the simultaneous and automated measurement of ecosystem processes. Thus, Ecotrons enable the integrated study of complex ecosystem processes, multitrophic interactions, and ecosystem functions. They can harbor a variety of above- and below-ground organisms from multiple trophic levels in a large series of independent mesocosm chambers, enabling precise controlling and monitoring of environmental conditions in both above- and below-ground systems[37,38].

In this study, we set up an experiment in the iDiv Ecotron[38,39], referred to as the "JenaTron Experiment" (**Figure 1** and **Figure 2**), to test whether biodiversity-ecosystem functioning (BEF) relationships in communities with different levels of plant species richness are modulated by soil history, shared plant history, or a combined effect of both. The iDiv Ecotron is specifically designed for multitrophic biodiversity experiments, focusing on the joint investigation of above- and below-ground interactions in terrestrial ecosystems[38,39,40]. It comprises 24 identical experimental units, termed 'EcoUnits' (**Figure 2F**,**G**), each capable of accommodating up to four individual ecosystems in isolated compartments (**Figure 2K**), allowing the construction of complex, near-natural ecosystems while minimizing the impact of environmental variability. One key advantage is the capacity to establish multiple replicates of intact soil monoliths (**Figure 2A-C**) under controlled environmental conditions, which enables the measurement of intricate ecosystem processes, including plant-plant and plant-soil feedback effects, that are often difficult to capture in small-scale microcosm experiments[38,39].

In Europe, semi-natural grasslands are among the most biodiverse and complex terrestrial ecosystems, providing vital ecosystem services. However, since the early 20th century, these extensively managed grasslands have faced increasing threats from land-use change, making them a key focus for biodiversity conservation and research. One of the world's longest-running biodiversity experiments on extensively managed grasslands is the Jena Experiment[32,33], located in Jena, Germany (50.951°N, 11.621°E, 139 m a.s.l.; website: https://the-jena-experiment.de). Established in 2002, the experiment investigates the relationships between grassland plant diversity and ecosystem functioning, particularly focusing on nutrient cycling and trophic interactions[32,33]. For the installation of the JenaTron Experiment within the iDiv Ecotron, we utilized the Trait-Based Experiment[41] (TBE), a component of the Jena Experiment initiated in 2010 and completed in 2021. In 2022, 23 plots of the TBE were selected to establish a plant diversity gradient

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

ranging from one to six species, spanning over 24 plant communities within the iDiv Ecotron. These communities comprised six grassland plant species, which overlapped with the Main Experiment of the Jena Experiment and represented three grass species (*Anthoxanthum odoratum* L., *Dactylis glomerata* L., *Holcus lanatus* L.) and three forb species (*Leucanthemum vulgare* agg., *Plantago lanceolata* L., and *Ranunculus acris* L.; see **Table 1** for details on plant species composition and replicates for each plant diversity level). These species, widespread and frequently coexisting in semi-natural European grasslands, additionally differ in their temporal resource acquisition traits, making them ideal for comprehensive studies of temporal resource use, such as plant phenology[41]. The 24 plant compositions were randomly assigned to all EcoUnits (**Figure 1C**), with each EcoUnit further subdivided into four compartments to accommodate the factorial combinations of soil history and plant history treatments.

Soil history (**Figure 1A**) was established by excavating a total of 96 soil monoliths from the Jena Experiment. The soil of the site is a nutrient-rich floodplain soil classified as Eutric Fluvisol, developed from loamy river sediments[32,33]. Detailed information regarding soil pH, changes in soil texture, and organic matter within the Jena Experiment soil profile can be found in Lange et al. (2023)[9]. Of the 96 monoliths, 48 were sourced by excavating two monoliths from each of 22 TBE plots, encompassing monocultures, two- and three-species mixtures. Additionally, four monoliths were taken from an eight-species plot, which will serve as a six-species mixture in the JenaTron Experiment. Each of these 48 soil monoliths (depth: 0.8 m; diameter: 0.5 m) harbored a plot-specific residence community since 2010, thus representing a plant community-specific soil history (+SH). The remaining 48 monoliths were excavated from four bare ground plots that were kept free of vegetation since the initiation of the Jena Experiment in 2002, thus lacking any plant community-specific soil history (-SH). From each of the four bare-ground plots, 12 monoliths were excavated. The two soil history treatments (+SH; -SH) were factorially combined with two plant history treatments by planting pre-grown plants that reflected the plant community-specific composition of the TBE at an equal density, with individual plants spaced 6 cm apart.

Plant history (**Figure 1B**) was established using two distinct seed sources for each of the selected plant species from the Jena Experiment species pool: The first source consisted of seeds collected from plants that had experienced a community-specific history since 2010, corresponding to the exact Trait-Based Experiment (TBE) plots from which the soil monoliths were excavated (+PH). These seeds were collected from May to September 2019 dependent on species flowering phenology, ensuring a minimum of five individuals per species and plot were sampled. After collection, seeds were cleaned and stored at -20°C until the initiation of the JenaTron Experiment. The second seed source comprised seeds obtained from a commercial seed supplier producing seeds of regional provenances. These seeds were used to establish the TBE plots in 2010, thus representing plants without a community-specific selection history within the Jena Experiment (-PH) and were stored at -20°C from 2010 until the start of the JenaTron Experiment. Both histories of the six perennial plant species were pre-cultivated from February to May 2022 in four chambers of the iDiv research greenhouse located in Leipzig, Germany (51.330°N, 12.393°E, 120 m a.s.l.).

The factorial combination of soil history (SH) and plant history (PH) at each level of plant species richness resulted in four treatments within each of the 24 EcoUnits: (1)

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

with soil history, with plant history (+SH/+PH), (2) with soil history, without plant history (+SH/-PH), (3) without soil history, with plant history (-SH/+PH), and (4) without soil history, without plant history (-SH/-PH; **Figure 1C**). The primary objective of the JenaTron Experiment is to investigate whether the strengthening relationship between biodiversity and ecosystem functioning is due to soil history, plant history, or a combination of both. We hypothesize that plant communities with shared plant and soil community-specific history will show the strongest BEF relationship, whereas an experimental exclusion of soil history, plant history, or both may have detrimental effects on the positive relationships of plant diversity and ecosystem functioning. Ultimately, if soil or plant history were to alter the relationship between plant species richness and ecosystem functioning, this would serve as an important consideration for the conservation and monitoring of locally preserved and novel restored communities.

Protocol

NOTE: Ensure that appropriate personal protective equipment (e.g., helmets, gloves, waistcoats, safety shoes, etc.) is provided at all protocol steps.

1. Soils: Preparation, excavation, installation

1. Soil sources, plot selection, and preparation (February 2022)
    1. Select two soil sources from the Jena Experiment field site:
        1. Select 23 TBE plots that harbored a plot-specific community for 12 years in order to cover a plant diversity gradient of 1, 2, 3, and 6 species. Designate these plots as JenaTron Experiment treatment "with plant community-specific soil history" (+SH).
        2. Select 4 bare ground plots with the same soil properties as the TBE plots (pH, sand content, etc.), but kept without vegetation cover since 2002. Designate these plots as JenaTron Experiment treatments "without a plant community-specific history" (-SH).
    2. Gently remove the upper 5 cm of soil from all selected plots using a mini-excavator to equalize conditions across soil history treatments.
2. Soil monolith excavation (March 2022)
    1. Set up the monolith extraction device at each plot, mount a cutting system on an empty steel cylinder, and insert it into the extraction device (**Figure 2A**).
    2. Switch on the device, and the cutting system will rotate around the outer cylinder wall. The system cuts a notch in the soil into which the extraction device simultaneously presses the steel cylinder. In parallel, use a mini-digger to excavate a pit at the side of the steel cylinder (**Figure 2B**).
    3. Once the steel cylinder is completely embedded in the soil, climb into the side pit and clip the monolith at the bottom edge of the steel cylinder by mounting a temporary bottom plate and use the monolith extraction device to lift it out of the excavation pit (**Figure 2C**).
    4. Dismantle the cutting system from the steel cylinder, mount a temporary top plate to the steel cylinder, lift the monolith using a suspension attached to the mini-digger, and turn it upside down.
3. Installation of the water suction system (March 2022)

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

1. Dismount the bottom plate, remove approximately 5 cm of soil layer with a trowel, and store it for later use.
2. Embed a ring of water suction probes (8 candles connected with Polyvinyl chloride [PVC] hoses) in quartz powder, moist it with demineralized water (VE), and refill the steel cylinder bottom with the previously removed soil.
3. Connect the PVC end piece of the water suction probe ring to the bottom plate and bolt it subsequently.
4. Turn the steel cylinder with the suspension on the mini-excavator upright again, label it individually, seal the openings in the cylinder walls with adhesive tape, and wrap the cylinder in plastic foil for protection during transportation.
5. Transport the soil monoliths with a truck to the iDiv Ecotron, located at the experimental field station of the Helmholtz Center for Environmental Research (UFZ) in Bad Lauchstädt, Germany (51.392°N, 11.876°E, 116 m a.s.l.).

4. Installation of soil sensors (April 2022)
    1. After unpacking, cut precisely fitting holes for the sensors horizontally in the soil monolith with a customized steel blade using the openings in the cylinder wall at three different depths (9.5 cm, 21.5 cm, and 43.5 cm).
    2. Place the soil sensors into the holes, use a wooden log to bring them in the right position, and bolt the openings with custom-made seal plugs.
5. Installation of the soil monoliths into the EcoUnits (April 2022)
    1. Carefully lift the technical and upper part of each EcoUnit from the lower part (**Figure 2G**). Transport the lower part to the steel cylinders harboring the intact soil monoliths equipped with soil sensors.
    2. Attach each steel cylinder to a suspension, lift it with a forklift, and mount a connector to the suction candle ring connected to the bottom plate.
    3. Lift four soil monoliths into the lower container of each of the 24 EcoUnits (**Figure 2F**), with two having the same plant community-specific soil history (+SH) and two without plant community-specific soil history (-SH). Lead the cables of the soil sensors through the openings of the ground container.
    4. Fix the steel cylinders to the lower part with screws and subsequently connect the water suction candle rings of each cylinder to the water suction system of the EcoUnit through the previously installed connector.
    5. Use the forklift to transport the container equipped with the soil monoliths into a hall (24 m x 24 m).
    6. Carefully place the technical and upper parts (with internal walls between subunits [**Figure 2K**]) of each EcoUnit back on top of the lower part.
    7. Arrange all 24 EcoUnits in three rows, each consisting of two groups of four EcoUnits, forming a total of six blocks. Ensure a center-to-center distance of 6 m between blocks within each row and 8.2 m between rows. In each block, randomly assign the four plant diversity levels (1, 2, 3, and 6) to the four EcoUnits, ensuring an even distribution of species across all EcoUnits wherever possible.

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

jove

2. Plants: Preparation and cultivation installation

1. Selection of species and seed material
    1. Select the following six plant species from the Jena Experiment species pool: *Anthoxanthum odoratum* L. (Poaceae), *Dactylis glomerata* L. (Poaceae), *Holcus lanatus* L. (Poaceae), *Leucanthemum vulgare* agg. *(Asteraceae), Plantago lanceolata* L. (Plantaginaceae), and *Ranunculus acris* L. (Ranunculaceae).
    2. For each species, select seed material originating from two different seed sources:
        1. Use seeds from plants that had been growing on the TBE plots of the Jena Experiment since 2010 (with plant history, +PH).
        2. Use the original seed material that was used to initiate the TBE in 2010 sourced from a regional seed producer (without plant history, -PH).
2. Substrate preparation (February 2022)
    1. Collect plot-specific soil from each of the selected plots at the Jena Experiment field site. Fill the soil into 20 L plastic boxes and store it at 4 °C until used for plant cultivation.
    2. Sieve each plot-specific soil through a 4 mm mesh. Mix the soil from the four bare ground plots before sieving.
    3. To remove unwanted biota, transfer quartz sand into stainless steel containers and heat in a drying oven for 4 h at 200 °C.
    4. Mix plot-specific soil and quartz sand by volume in a 3:1 ratio and fill the soil-sand mixture into multipot plates using a plastic shovel (total n = 152).
    5. Label each multipot plate individually and place them in a greenhouse with a day-night cycle of 16 h/18 °C and 8 h/12 °C, maintaining moderate humidity of 60%-70% relative humidity (RH).
    6. Water the plates on demand and keep them bare for two weeks to allow soil seed banks to germinate. Weed all plates twice to remove unwanted seedlings.
3. Sowing campaign (March-April 2022)
    1. Determine the sowing order based on species-specific germination time as follows:
        1. Sow *R. acris* 2 weeks prior to other herbs
        2. Sow *P. lanceolata* and *L. vulgare* 1 week ahead of grasses *A. odoratum*, *D. glomerata*, and *H. lanatus*.
    2. Sow all species, except *R. acris*, directly into the plot-specific soil-sand substrate (3:1) in multipot plates with two or three seeds per pot. After successful germination, thin out all species to single seedlings using spring steel tweezers.
    3. Pretreat *R. acris* seeds in Petri dishes on filter paper moistened with a gibberellic acid solution (1000 $mg.L^{-1}$) to break dormancy (**Figure 2D**). Incubate the seeds for 24 h.
    4. Transfer *R. acris* seeds to filter paper moistened with demineralized water (VE) until germination. Once seeds reach the radicle stage, prick seedlings into the plot-specific soil-sand substrate (3:1) in the prepared multipot plates using spring steel tweezers.

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

5. Water all plant individuals with deionized water on demand and keep them in the greenhouse until transport to the iDiv Ecotron (**Figure 2E-H**)

4. Planting campaign (May 2022)

    1. Design individual planting schemes for all soil monoliths with the following characteristics:

        1. Ensure a balanced number of individuals per species within the plant communities.

        2. Maintain a 6 cm distance between each plant individual and its neighbor.

        3. Randomize the planting pattern across EcoUnits, but keep it constant within subunits.

    2. Use a custom-made planting stencil to mark the exact position of each plant individual on the soil monoliths. Use different colored plastic toothpicks for marking, assigning a specific color to each species. After marking, remove the stencil (**Figure 2I**).

    3. Plant the pre-grown plants on all soil monoliths within one week using bulb planters with a 4 cm diameter (5280 total plant individuals; **Figure 2J**).

3. Course of the experiment

1. Three-week establishment phase (May 2022):

    1. Replace all individuals that did not survive the planting campaign within 2 weeks of initial planting (n = 124 [2.35%]).

    2. Turn on the lights and set the lighting regime to a 16/8 h day-night cycle. To replicate the regional day length in late spring and summer at the start of the experiment, simulate dusk by dimming the lights from 100% to 75% intensity over one hour, then to 0% over the next hour (100% intensity: ~370 µmol/(m²·s)). Simulate dawn by reversing this dimming pattern.

    3. Equalize the upper soil moisture to 20% (± 3%) by volume using a soil moisture measurement kit.

    4. Set the initial irrigation to 450 mL every 12 h for each subunit.

    5. Connect air and soil sensors to monitor temperature and humidity throughout the experiment.

2. Experimental phase (June-October 2022)

    1. Following the natural seasonality, shorten the day length by a full hour every 2 weeks from August to October to account for the regional day lengths from late summer to autumn.

    2. Increase the initial irrigation to 650 mL every 12 h in July and 750 mL every 12 h in August, adjusting to plant water demands.

    3. Constantly weed out unwanted seedlings at the cotyledon stage using tweezers to maintain plant density while minimizing disturbance to the soil structure.

    4. In accordance with the standard management for extensively managed meadows, perform two harvests of above-ground biomass, cutting the plants 3 cm above the soil surface with scissors.

Representative Results

To provide initial insights into the effectiveness of this approach in testing the independent and interactive effects of plant and soil history on biodiversity-ecosystem functioning relationships, we first evaluated photographs of two representative EcoUnits after the 3-week establishment

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

phase of the JenaTron Experiment, where treatment effects quickly became visible (**Figure 3**). Besides treatment-specific differences in plant community height and color (**Figure 3A**), we additionally recognized treatment-specific differences in the flowering time of *R. acris*. Specifically, plants grown from seeds with no plant history appeared to have an earlier flower onset than plants grown from seeds with the community-specific plant history of the Jena Experiment (**Figure 3B**).

To evaluate whether the newly established communities in the iDiv Ecotron accurately reflect the original field communities, a comparative analysis of species-specific vegetative plant height was performed as an indicator of final plant development between the JenaTron Experiment and TBE plots. In the JenaTron Experiment, plant height was determined as the mean value of the highest vegetative leaf of three individuals per plant species in each of the 24 communities and treatment combinations just prior to the first harvest campaign end of July 2022. In the TBE, vegetative plant height was determined as the mean value of the highest vegetative leaf of three individuals per plant species and plot in July 2012. Plant height measurements from the iDiv Ecotron exhibit a strong correlation with those from the Jena Experiment field plots (**Supplementary Figure 1**).

To further provide a case study for data analysis, the vegetative plant height in the iDiv Ecotron was investigated in more detail. We focused on species- and community-level analyses and tested whether plant history, soil history, and plant diversity influence vegetative plant growth. For this, we used linear mixed-effects models (LMM) and summarized results via ANOVA (type-I sum of squares; for detailed ANOVA results, see **Supplementary Table 1**). At plot level, response variables were mean plant vegetative height and the coefficient of variation of plant height (CV = sd/mean 100). Fixed effects included plant diversity (numerical), plant and soil history treatments, and their interactions. Random effects included EcoUnit nested within the Ecotron hall block. At the species level, we additionally included species and their interaction with plant diversity and history treatments in fixed terms. Data transformations (log for plant height and square root for height (CV) ensured model assumptions were met. Analyses were conducted using R (Version4.3.1)[42] and the nlme package for LMMs[43]. Results showed significant species differences in plant height, with grasses taller than forbs (**Figure 4, Supplementary Figure 2**). Across all species, soil history had a positive effect on plant height ($F_{(1,66)}$ = 11.11, P = 0.001), while plant history and diversity had no significant impact ($F_{(1,66)}$ = 0.22, P = 0.64 and $F_{(1,17)}$ = 1.52, P = 0.23, respectively; **Figure 4**). At the community level, plant diversity had no effect on height ($F_{(1,17)}$ = 0.01, P = 0.91; **Figure 5A**) but significantly increased plant height CV within communities ($F_{(1,17)}$ = 14.29, P = 0.002; **Figure 5B**). No significant effects of plant or soil history were found on plant height CV ($F_{(1,68)}$ = 0.55; P = 0.46; $F_{(1,68)}$ = 0.09, P = 0.76; **Figure 5**). We have uploaded the data and R script to the Jena Experiment database (https://doi.org/10.25829/QN9R-J839).

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

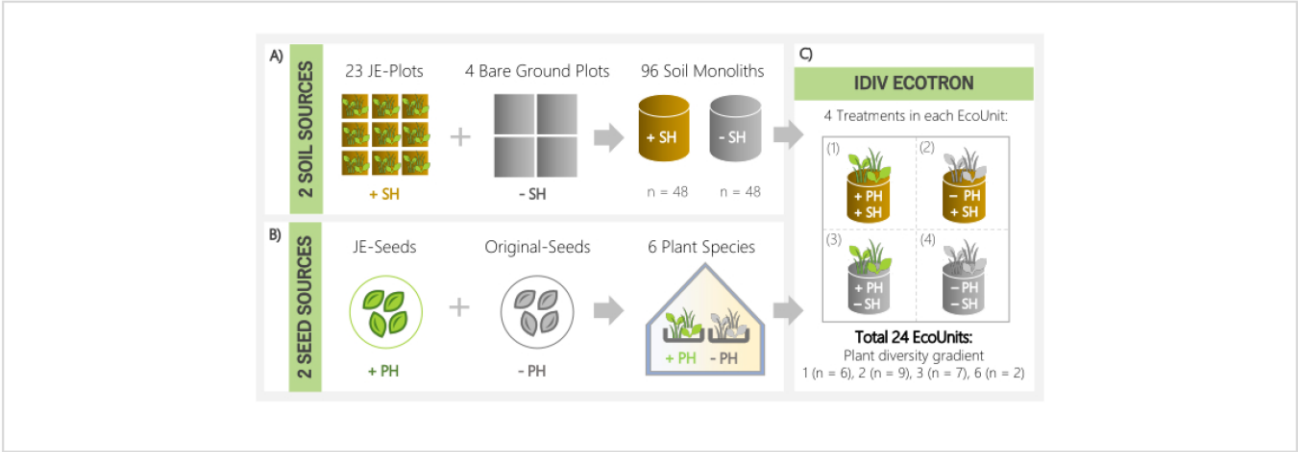


Figure 1: Setup of the JenaTron experiment. (**A**) Soil History (SH) was established by excavating 96 intact soil monoliths from the Jena Experiment (JE), of which half (n = 48) were obtained from 23 plots that harbored a plot-specific community for 12 years (+SH), while the other half (n = 48) was obtained from four bare ground plots held vegetation-free since the beginning of the Jena Experiment in 2002 (-SH). (**B**) Plant history (PH) was established using two seed sources for each of six plant species: seeds collected from plants grown since 2010 on the same plots, where the monoliths were excavated in the Jena Experiment (+ PH), and the original seeds as used to sow these communities in the field in 2010 (-PH). (**C**) Plant history and soil history were independently crossed for each composition at different plant diversity levels (1, 2, 3, and 6), resulting in four treatments in each EcoUnit of the iDiv Ecotron: (1) +SH/+PH, (2) +SH/-PH, (3) -SH/+PH, and (4) -SH/-PH.

Please click here to view a larger version of this figure.

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License



Figure 2: The iDiv Ecotron at the Research Station of the Helmholtz-Centre for Environmental Research (UFZ) in Bad Lauchstädt, Germany. (**A**) Soil monolith excavation equipment in the field, (**B**) Soil monolith excavation in the field, (**C**) Excavation pit in the field, (**D**) Preparation of *Ranunculus acris* seed material in petri-dishes on filter paper moistened with gibberellic acid solution in the greenhouse, (**E**) Pre-cultivation of plants with plot-specific soil substrate in multipot plates in the greenhouse, (**F**) EcoUnits in the iDiv Ecotron hall, (**G**) Schematic depiction of one experimental unit ("EcoUnit") with technical equipment (adapted from Eisenhauer et al. (2019)[23] and Schmidt et al. (2021)[38]), (**H**) Plates with all individuals to be planted in the JenaTron Experiment) after transport to the iDiv Ecotron, (**I**) Species-specific marking of the planting positions for all plant individuals on a soil monolith made with a planting stencil, (**J**) Planting of plant individuals on a soil monolith using bulb planters, (**K**) Four lysimeters in an EcoUnit with internal walls. Please click here to view a larger version of this figure.

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

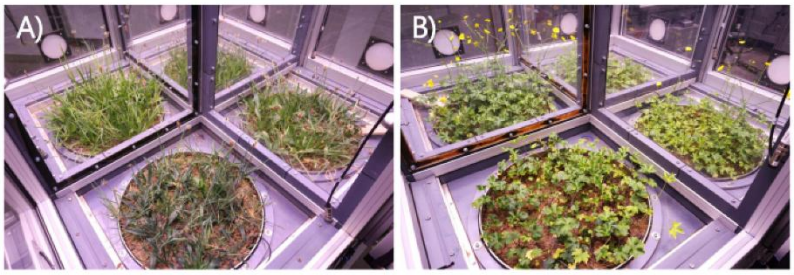


Figure 3: Two representatives of the 24 EcoUnits, each harboring one community in all treatment combinations of plant and soil history. (**A**) EcoUnit with a 3-species mixture (*A. odoratum*, *H. lanatus*, and *P. lanceolata*) with treatment specific differences in community vegetation height and color. (**B**) EcoUnit with a monoculture of *R. acris*, indicating plant history dependent differences in flowering time. Please click here to view a larger version of this figure.

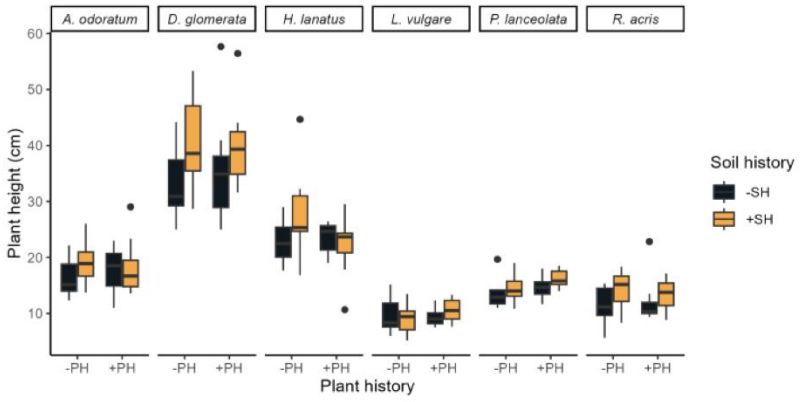


Figure 4: Effects of plant and soil history on vegetative plant height (cm) of the six grassland plant species before the first harvest in July 2022. Species are listed alphabetically within functional groups (grasses followed by forbs). Plant history treatments (+PH/-PH) are on the x-axis, and soil history (+SH/-SH) is color-coded (yellow/black) in the box plots. Please click here to view a larger version of this figure.

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

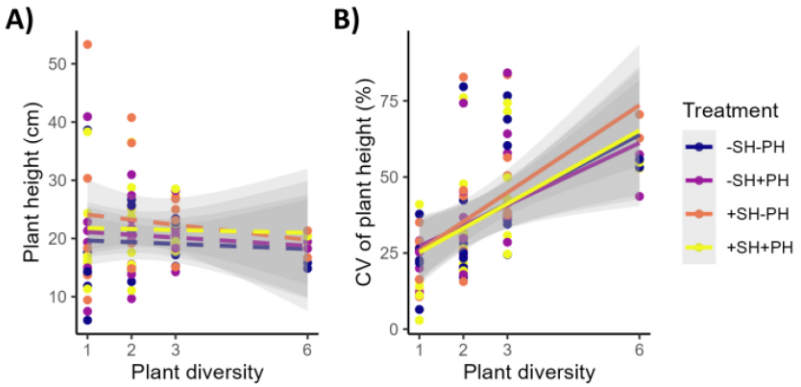


Figure 5: Effects of species richness on community mean and variation in vegetative plant height. (**A**) Community mean. (**B**) Variation in vegetative plant height. (+PH/-PH) for plant history, (+SH/-SH) for soil history. Plant height variation (CV) is shown per subunit. Dashed lines represent non-significant, solid lines represent significant effects on plant diversity. Please click here to view a larger version of this figure.

Table 1 Plant communities in the JenaTron Experiment. Monocultures of all selected plant species (n = 6); 2-species mixtures (n = 9); 3-species mixtures (n = 7) all with different compositions; and two identical replicates of the 6-species mixture (n = 2). Please click here to download this Table.

Supplementary Figure 1: Species' average plant height. Species' average plant height in the (**A**) JenaTron Experiment plots (July 2022) and in the field (**B**) TBE plots (July 2012). Only data from the treatment with plant history and soil history were included in panel **A**. There is a significant correlation between plant height in the JenaTron Experiment and the field plots (Pearson correlation $r_{(49)}$ = 0.45, p = 0.0008). Please click here to download this File.

Supplementary Figure 2: Effects of plant and soil history treatments on vegetative plant height (cm) of the six plant species at different levels of plant diversity (SR). Legend symbols +PH and -PH represent treatments with and without plant history, while +SH and -SH signify treatments with and without soil history, respectively. Please click here to download this File.

Supplementary Table 1: ANOVA results from linear mixed-effects models assessing the effects of plant diversity (species richness, SR), soil history (SH), and plant history (PH) on plant height at both species level and plot level. F-values and p-values are reported for each term. Note: numDF and denDF represent the numerator and the denominator degrees of freedom for each model term. P-values < 0.05 suggest a statistically significant effect on plant height. Please click here to download this File.

Supplementary Table 2: Above- and below-ground measurements, response variables, and methods. A spectrum of above- and below-ground measurements, response variables, and methods conducted in the JenaTron Experiment to study the independent and interactive effects

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

of plant history and soil history on BEF relationships for ecosystems as a whole. Please click here to download this File.

Discussion

With the JenaTron Experiment, we provide a complementary experimental approach to the Jena Experiment field site[32,33] to enhance our mechanistic understanding of BEF relationships. The Field Experiment allows us to study the long-term effects of soil history and plant history on ecosystem functioning under realistic environmental conditions, with large, undisturbed plots allowing repeated measurements over many years. The randomly selected combinations of plant species from a large species pool along a well-established plant diversity gradient from monocultures up to 60 species help to identify diversity effects by excluding species-specific effects as much as possible[32,33]. However, the Field Experiment, referring here explicitly to the 'ΔBEF Experiment' as part of the Jena Experiment (for further details, see Vogel et al. (2019)[29]), is limited in investigating soil history and plant history completely independent from each other and from potential influences of various external factors, such as weather fluctuations, natural disturbances, and interactions with other organisms[29]. At this point, the complementary approach of using Ecotron experiments comes into the focus of BEF research. Ecotron experiments represent an important link between small-scale, short-term microcosm experiments, which study only a few or even just a single species and thus reduce the high complexity of field experiments or even the real world[34,38]. Hence, the main aim of Ecotron experiments is not to imitate the full complexity of nature but rather to create simplified but biologically meaningful ecosystems. This allows to better understand many, but of course not all, biological processes and interactions that occur in the real world[34,37]. Moreover, the closed systems of an Ecotron allow for studying fluxes and processes, which often cannot be easily studied in the field[35].

Various modifications of the experimental technique have been utilized in previous studies, underscoring the significant adaptability of the iDiv Ecotron facility. For example, the "Insect Armageddon" experiment[44], conducted in 2018, involved a reconfiguration of the EcoUnits by removing the above-ground interior walls and below-ground dissection with steel cylinders. Instead, the below-ground containers were entirely filled with a soil-sand mixture, facilitating the establishment of grassland communities comprising 12 European plant species across a soil surface area of 1.5 m^2 per EcoUnit. This experimental design allowed for the introduction of two distinct abundance levels of above-ground invertebrates, enabling researchers to investigate the impacts of invertebrate biomass decline on ecosystem functions and services within Central European grassland ecosystems. Another illustrative example is the "EcoLux" experiment, conducted in 2020, which demonstrated a modification in the light regime to investigate the effects of light pollution at night on plant diversity and performance[45] as well as its influence on soil communities and functions[46]. Looking ahead, the potential for further modifications remains substantial. The upcoming "ResCUE" experiment, scheduled for 2026, aims to examine the relationships between plant diversity and ecosystem stability under climatic extremes[26]. This experiment will implement a standardized hot drought, an environmental condition difficult to replicate in natural settings, by simulating reduced precipitation alongside increased temperatures across a gradient of grassland plant diversity established in the iDiv Ecotron. The study will focus on assessing the resistance and recovery of multiple

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

ecosystem functions in response to the controlled climatic extreme event[26].

However, using Ecotron facilities to bridge field experiments and small-scale microcosm experiments also entails limitations, such as mostly a still relatively small spatial scale, a low number of replicates, lower biological complexity and the lack of representation of natural climatic variability[37]. Furthermore, the iDiv Ecotron facility cannot simulate all kinds of climatic scenarios, such as winters, limiting its ability to study individual plants in a realistic annual cycle across several generations. Hence, as mentioned before, it is very important to consider Ecotron studies as complementary research approaches that must ultimately be considered in combination with results from other experimental approaches[37]. Nevertheless, the iDiv Ecotron facility offers us the unique opportunity to study intact soil monoliths under controlled environmental conditions, to orthogonally cross plants with different plant community-specific histories with soils with different plant community-specific histories and to study their effects on ecosystem functioning along a plant diversity gradient, which is difficult to achieve under field conditions[23,35]. Additionally, the technique allows for studying plants as a whole, both above- and below-ground, under controlled environmental conditions, which is not easily achievable in the field. Lastly, in the JenaTron Experiment, the planted (rather than sown) plant communities were established to ensure equal total plant densities, with each species represented in equal proportions. In contrast, in sown communities, species often establish at different rates, and some target species may fail completely to establish or could remain at very low abundance.

To ensure the reproducibility of the presented protocol, several critical steps must be meticulously considered during execution: (1) Soil monolith excavation: One key advantage of the iDiv Ecotron facility is its capability to establish a substantial number of replicates of intact soil monoliths under controlled environmental conditions. To ensure the integrity of the soil monoliths, it is essential to maintain optimum field conditions for soil monolith extraction. Specifically, excessive soil moisture can lead to compaction of the soil column, while soils that are either too dry or frozen may result in the formation of cracks. In instances where the monolith is compromised due to these factors, it must be discarded, necessitating a repetition of the excavation process. (2) Species selection: The protocol described uses six grassland plant species of the TBE, which were selected based on overlap with the main experiment, a balanced representation of grasses and forbs, and the possibility of studying different resource use strategies (e.g., plant phenology)[41]. For a biodiversity experiment, six species are a low species number, and the selection of other species possessing other trait combinations could lead to different experimental results and must be carefully considered. (3) Plant density and equal proportions of all species: A notable advantage of the JenaTron Experiment setup is that plant communities are established with equal total plant densities and that each species is represented with the same number of individuals. Ensuring consistent plant density and equal planting proportions is crucial for experimental reproducibility, as it prevents confounding effects associated with a differential establishment of individual species. Hence, precise control of plant density is essential to preserve the experimental design and ensure reliable outcomes.

After initiating the JenaTron Experiment following the protocol with special emphasis of considering the mentioned

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

most critical steps, discernible treatment effects on plant community height, color, and flowering time swiftly emerged. Subsequent comparative analyses of species-specific vegetative plant heights between JenaTron and TBE clearly confirmed that communities established in the iDiv Ecotron indeed resemble the original communities in the field. To further provide a case study for data analysis, we analyzed the vegetative plant height in the JenaTron experiment in more detail. Analyses of species-specific vegetative plant heights in the JenaTron confirmed the successful establishment of all target species and revealed significant differences among species and functional groups. This result is expected, as grasses are known to grow taller than other functional groups in the Jena Experiment[47]. A significant positive effect of soil history was detected across species, indicating that prior soil conditions shape plant growth in the Ecotron, potentially driven by positive feedback effects from soils that previously harbored a plant community (independent of the diversity level) and negative feedback from soils held vegetation-free. The biotic and abiotic properties of soil histories appear to differ, giving plants in soils with plant community-specific history an advantage over plants in soils without this respective history, as plant height plays an important role in their ability to compete for light and is thus a vital trait for carbon gain[48]. In contrast, no significant effect of plant community-specific plant history on mean vegetative height was found, implying that soil properties may have a stronger influence than the seed sources under the Ecotron's environmental conditions. However, this hypothesis needs to be tested in a holistic way by investigating the multitude of ecosystem functions that were measured in the JenaTron Experiment.

Neither species- nor community-specific analyses provided evidence that species richness affects mean vegetative plant height, contrasting with previous findings from the Field Experiment, where plant height increased with higher species[49] likely due to stronger light competition[47,50]. Greater complementarity among species in more diverse communities may lead to higher plant densities[51], potentially additionally enhancing plant height growth[52]. The lack of plant diversity effects on plant height in the JenaTron Experiment could thus be due to the absence of biodiversity-dependent variation in plant density, indicating the potential importance of this mechanism for diversity-dependent individual plant performance in the real world[52]. Nevertheless, species richness had a significant effect on plant height CV, suggesting that plant diversity increases vegetation structural complexity, consistent with studies linking diversity to more complex above-ground canopies[49,50,52,53,54].

Taken together, results confirm the successful establishment of the JenaTron Experiment's target treatments, providing first insights into the effectiveness of our approach through the rapid manifestation of significant treatment effects (**Figure 3**, **Figure 4**, and **Figure 5**). Additionally, comparative analysis of species-specific vegetative plant height indicates that the newly established communities in the iDiv Ecotron indeed resemble the original field observations. The detailed analysis of vegetative plant height serves as a case study for data analysis and visualization of single response variables. However, this case study highlights the limitations of focusing on a single response variable in holistically addressing the overarching hypothesis, whether the strengthening relationship between biodiversity and ecosystem functioning over time is due to soil history, plant history, or a combination of both. This underscores the importance of our highly collaborative approach to investigating the independent and interactive effects of plant history and soil history

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

on BEF relationships[23]. Eleven sub-projects of the Jena Experimental Research Unit (FOR 5000) worked closely together in the JenaTron Experiment, measuring a wide range of response variables across various research areas, such as microbiology, chemical ecology, phenology, proximal sensing, genetics, epigenetics, soil ecology, and food web modeling. This integrative effort aims to (1) separate plant history effects from soil history effects on multiple trophic levels and ecosystem functions, (2) investigate plant species-specific and interaction-specific effects at all levels of plant species richness, and (3) explore the underlying mechanisms of BEF relationships[23] (**Supplementary Table 2**[49,50,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84]).

Disclosures

The authors have nothing to disclose.

Acknowledgments

Exceptional thanks go to L. Boockmann, T. Meier, N. Naderi, and L.-M. Walter for their support as research assistants during the entire duration of the JenaTron Experiment. Many thanks to all employees of the Umwelt-Geräte-Technik GmbH (UGT) for their professional and warm cooperation during the soil monolith excavation in the Jena Experiment. We would also like to thank C. Lauterwasser, H.-C. Schulz, and R. Widuwils for supporting UGT in soil monolith excavation. Many thanks go to the technical staff of the Jena Experiment S. Eismann, F. Freter, G. Kratzsch, U. Köber, K. Kunze, and H. Scheffler for their support in the field and their diligent help during the planting campaign at the iDiv Ecotron. We are grateful to I. Merbach and the technical staff of the Research Station of the Helmholtz Centre for Environmental Research (UFZ) in Bad Lauchstädt, especially to O. Bednorz and K. Kirsch, for their vital support. We want to thank A.Q. Barth and R. Altmann for their excellent collaboration in the iDiv greenhouse. Many thanks to S. Haenzel, A. Lochner, and A. Zeuner for their indispensable help with administration, organization, and laboratory work. We thank the Mercator Fellows J. Cavender-Bares, F. Isbell, L. Mommer, and K. Mueller, as well as the close collaborator B. Schmid and the Principal investigators U. Brose, H. Schielzeth, N. van Dam, W.W. Weisser, C. Wirth and J. Wolf for their important contribution to the conception, design and realization of the JenaTron Experiment. Finally, we thank the German Research Foundation (DFG) for financial support (FOR 5000) as well as the German Centre for Integrative Biodiversity Research (iDiv, funded by the German Research Foundation [DFG-FZT 118, 202548816]), the University of Leipzig, the Friedrich Schiller University Jena and the Max Planck Institute for Biogeochemistry Jena for financial and logistic support.

References

1. Cardinale, B. J. et al. Biodiversity loss and its impact on humanity. *Nature.* **486** (7401), 59-67 (2012).
2. Eisenhauer, N. et al. Chapter One - A multitrophic perspective on biodiversity-ecosystem functioning research. *Adv Ecol Res.* **61**, 1-54 (2019).
3. Reich, P. B. et al. Impacts of biodiversity loss escalate through time as redundancy fades. *Science.* **336** (6081), 589-592 (2012).
4. Meyer, S. T. et al. Effects of biodiversity strengthen over time as ecosystem functioning declines at low and increases at high biodiversity. *Ecosphere.* **7** (12), e01619 (2016).
5. Guerrero-Ramírez, N. R. et al. Diversity-dependent temporal divergence of ecosystem functioning in

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

experimental ecosystems. *Nat Ecol Evol.* **1** (11), 1639-1642 (2017).

6. Huang, Y. et al. Impacts of species richness on productivity in a large-scale subtropical forest experiment. *Science.* **362** (6410), 80-83 (2018).
7. Bongers, F. J. et al. Functional diversity effects on productivity increase with age in a forest biodiversity experiment. *Nat Ecol Evol.* **5** (12), 1594-1603 (2021).
8. Wagg, C. et al. Biodiversity-stability relationships strengthen over time in a long-term grassland experiment. *Nat Commun.* **13** (1), 7752 (2022).
9. Lange, M., Eisenhauer, N., Chen, H., Gleixner, G. Increased soil carbon storage through plant diversity strengthens with time and extends into the subsoil. *Glob Change Biol.* **29** (9), 2627-2639 (2023).
10. Eisenhauer, N., Reich, P.B., Scheu, S. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic Appl Ecol.* **13** (7), 571-578 (2012).
11. Fornara, D. A., Tilman, D. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *J Ecol.* **96** (2), 314-322 (2008).
12. Leimer, S. et al. Mechanisms behind plant diversity effects on inorganic and organic N leaching from temperate grassland. *Biogeochemistry.* **131** (3), 339-353 (2016).
13. Dietrich, P., Eisenhauer, N., Roscher, C. Linking plant diversity-productivity relationships to plant functional traits of dominant species and changes in soil properties in 15-year-old experimental grasslands. *Ecol Evol.* **13** (3), e9883 (2023).
14. Roscher, C. et al. A functional trait-based approach to understand community assembly and diversity-productivity relationships over 7 years in experimental grasslands. *Perspect Pl Ecol Evol Syst.* **15** (3), 139-149 (2013).
15. Zuppinger-Dingley, D., Schmid, B., Petermann, J. S., Yadav, V., De Deyn, G. B., Flynn, D. F. B. Selection for niche differentiation in plant communities increases biodiversity effects. *Nature.* **515** (7525), 108-111 (2014).
16. Latz, E. et al. Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. *J Ecol.* **100** (3), 597-604 (2012).
17. Barnes, A. D. et al. Biodiversity enhances the multitrophic control of arthropod herbivory. *Sci. Adv.* **6** (45), eabb6603 (2020).
18. Huang, Y. et al. Effects of enemy exclusion on biodiversity-productivity relationships in a subtropical forest experiment. *J Ecol.* **110** (9), 2167-2178 (2022).
19. Wan, N.-F. et al. Species richness promotes plant productivity by suppressing plant antagonists. *Research Square.* (2023).
20. Schnitzer, S. A. et al. Soil microbes drive the classic plant diversity-productivity pattern. *Ecology.* **92** (2), 296-303 (2011).
21. Kulmatiski, A., Beard, K.H., Heavilin, J. Plant-soil feedbacks provide an additional explanation for diversity-productivity relationships. *Proc Biol Sci.* **279** (1740), 3020-3026 (2012).
22. Mommer, L. et al. Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. *New Phytol.* **218** (2), 542-553 (2018).

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

23. Eisenhauer, N. et al. Biotic interactions, community assembly, and eco-evolutionary dynamics as drivers of long-term biodiversity-ecosystem functioning relationships. *Res Ideas Outcomes.* 5, e47042 (2019).
24. Thakur, M.P. et al. Plant-soil feedbacks and temporal dynamics of plant diversity-productivity relationships. *Trends Ecol Evol.* **36** (7), 651-661 (2021).
25. Eisenhauer, N. Aboveground-below-ground interactions as a source of complementarity effects in biodiversity experiments. *Plant Soil.* **351** (1), 1-22 (2012).
26. Eisenhauer, N. et al. The multiple-mechanisms hypothesis of biodiversity-stability relationships. *Basic Appl Ecol.* **79**, 153-166 (2024).
27. Bever, J. D. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytol.* **157** (3), 465-473 (2003).
28. Schmid, M. W., Hahl, T., van Moorsel, S. J., Wagg, C., De Deyn, G. B., Schmid, B. Feedbacks of plant identity and diversity on the diversity and community composition of rhizosphere microbiomes from a long-term biodiversity experiment. *Mol Ecol.* **28** (4), 863-878 (2019).
29. Vogel, A. et al. A new experimental approach to test why biodiversity effects strengthen as ecosystems age. *Adv Ecol Res.* **61**, 221-264 (2019).
30. Dietrich, P., Eisenhauer, N., Otto, P., Roscher, C. Plant history and soil history jointly influence the selection environment for plant species in a long-term grassland biodiversity experiment. *Ecol Evol.* **11** (12), 8156-8169 (2021).
31. van Moorsel, S.J. et al. Co-occurrence history increases ecosystem stability and resilience in experimental plant communities. *Ecology.* **102** (1), e03205 (2021).
32. Roscher, C. et al. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic Appl Ecol.* **5** (2), 107-121 (2004).
33. Weisser, W.W. et al. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic Appl Ecol.* **23**, 1-73 (2017).
34. Lawton, J.H. et al. The Ecotron: a controlled environmental facility for the investigation of population and ecosystem processes. *Philos Trans R Soc Lond B Biol Sci.* **341** (1296), 181-194 (1993).
35. Milcu, A. et al. Functional diversity of leaf nitrogen concentrations drives grassland carbon fluxes. *Ecol Lett.* **17** (4), 435-444 (2014).
36. Rineau, F. et al. Towards more predictive and interdisciplinary climate change ecosystem experiments. *Nat Clim Chang.* **9** (11), 809-816 (2019).
37. Roy, J. et al. Ecotrons: Powerful and versatile ecosystem analysers for ecology, agronomy and environmental science. *Glob Change Biol.* **27** (7), 1387-1407 (2021).
38. Schmidt, A. et al. The iDiv Ecotron-A flexible research platform for multitrophic biodiversity research. *Ecol Evol.* **11** (21), 15174-15190 (2021).
39. Eisenhauer, N., Türke, M. From climate chambers to biodiversity chambers. *Front Ecol Environ.* **16** (3), 136-137 (2018).
40. Eisenhauer, N. et al. Ecosystem consequences of invertebrate decline. *Curr Biol.* **33** (20), 4538-4547.e5 (2023).
41. Ebeling, A. et al. A trait-based experimental approach to understand the mechanisms underlying biodiversity-

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

ecosystem functioning relationships. *Basic Appl Ecol.* **15** (3), 229-240 (2014).

42. R Core Team. *R: A Language and Environment for Statistical Computing.* At <https://www.R-project.org/> (2023).
43. *Theory and Computational Methods for Nonlinear Mixed-Effects Models. Mixed-Effects Models in S and S-PLUS. Statistics and Computing.* Springer, New York, NY (2000).
44. Ulrich, J. et al. Invertebrate decline leads to shifts in plant species abundance and phenology. *Front Plant Sci.* **11**, 542125 (2020).
45. Bucher, S. F. et al. Artificial light at night decreases plant diversity and performance in experimental grassland communities. *Philos Trans R Soc Lond B Biol Sci.* **378** (1892), 20220358 (2023).
46. Cesarz, S., Eisenhauer, N., Bucher, S. F., Ciobanu, M., Hines, J. Artificial light at night (ALAN) causes shifts in soil communities and functions. *Philos Trans R Soc Lond B Biol Sci.* **378** (1892), 20220366 (2023).
47. Schmidtke, A., Rottstock, T., Gaedke, U., Fischer, M. Plant community diversity and composition affect individual plant performance. *Oecologia.* **164** (3), 665-677 (2010).
48. Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., Wright, I. J. Plant ecological strategies: Some leading dimensions of variation between species. *Annu Rev Ecol Syst.* **33** (1), 125-159 (2002).
49. Guimarães-Steinicke, C. et al. Biodiversity facets affect community surface temperature via 3D canopy structure in grassland communities. *J Ecol.* **109** (5), 1969-1985 (2021).
50. Roscher, C. et al. Interspecific trait differences rather than intraspecific trait variation increase the extent and filling of community trait space with increasing plant diversity in experimental grasslands. *Perspect Pl Ecol Evol Syst.* **33**, 42-50 (2018).
51. Marquard, E., Weigelt, A., Roscher, C., Gubsch, M., Lipowsky, A., Schmid, B. Positive biodiversity-productivity relationship due to increased plant density. *J Ecol.* **97** (4), 696-704 (2009).
52. Lorentzen, S., Roscher, C., Schumacher, J., Schulze, E. -D., Schmid, B. Species richness and identity affect the use of aboveground space in experimental grasslands. *Perspect Pl Ecol Evol Syst.* **10** (2), 73-87 (2008).
53. Williams, L. J., Paquette, A., Cavender-Bares, J., Messier, C., Reich, P. B. Spatial complementarity in tree crowns explains overyielding in species mixtures. *Nat Ecol Evol.* **1** (4), 1-7 (2017).
54. Roscher, C. et al. Using plant functional traits to explain diversity-productivity relationships. *PLoS One.* **7** (5), e36760 (2012).
55. Abbas, M. et al. Biodiversity effects on plant stoichiometry. *PLoS One.* **8** (3), e58179 (2013).
56. Pérez-Harguindeguy, N. et al. New handbook for standardised measurement of plant functional traits worldwide. *Aust J Bot.* **61** (3), 167-234 (2013).
57. Bassi, L. et al. Uncovering the secrets of monoculture yield decline: trade-offs between leaf and root chemical and physical defence traits in a grassland experiment. *Oikos.* **2023**, e10061, (2023).
58. Medina-van Berkum, P. et al. Plant geographic distribution influences chemical defences in native and

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

introduced Plantago lanceolata populations. *Funct Ecol.* **38** (4), 883-896 (2024).

59. Motohka, T., Nasahara, K.N., Oguma, H., Tsuchida, S. Applicability of green-red vegetation index for remote sensing of vegetation phenology. *Remote Sens.* **2** (10), 2369-2387 (2010).
60. Yin, G., Verger, A., Descals, A., Filella, I., Peñuelas, J. A Broadband green-red vegetation index for monitoring gross primary production phenology. *J Remote Sens.* **2022**, 9764982 (2022).
61. Chen, B., Jin, Y., Brown, P. An enhanced bloom index for quantifying floral phenology using multi-scale remote sensing observations. *ISPRS J Photogramm Remote Sens.* **156**, 108-120 (2019).
62. Dean, J. M., Mescher, M. C., De Moraes, C. M. Plant dependence on rhizobia for nitrogen influences induced plant defenses and herbivore performance. *Int J Mol Sci.* **15** (1), 1466-1480 (2014).
63. McCormick, A. C., Reinecke, A., Gershenzon, J., Unsicker, S. B. Feeding experience affects the behavioral response of polyphagous gypsy moth caterpillars to herbivore-induced poplar volatiles. *J Chem Ecol.* **42** (5), 382-393 (2016).
64. Waldbauer, G. P. The consumption and utilization of food by insects. *Adv In Insect Phys.* **5**, 229-288 (1968).
65. Bachmann, D., Roscher, C., Buchmann, N. How do leaf trait values change spatially and temporally with light availability in a grassland diversity experiment? *Oikos.* **127** (7), 935-948 (2018).
66. van Gurp, T. P., Wagemaker, N. C. A. M., Wouters, B., Vergeer, P., Ouborg, J. N. J., Verhoeven, K. J. F. epiGBS: reference-free reduced representation bisulfite sequencing. *Nat Methods.* **13** (4), 322-324 (2016).
67. Eichenberg, D., Ristok, C., Kröber, W., Bruelheide, H. Plant polyphenols - implications of different sampling, storage and sample processing in biodiversity-ecosystem functioning experiments. *J Chem Ecol.* **30** (7), 676-692 (2014).
68. Scheu, S. Automated measurement of the respiratory response of soil microcompartments: Active microbial biomass in earthworm faeces. *Soil Biol Biochem.* **24** (11), 1113-1118 (1992).
69. Freschet, G.T. et al. A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *New Phytol.* **232** (3), 973-1122 (2021).
70. Fiore-Donno, A. M., Richter-Heitmann, T., Bonkowski, M. Contrasting responses of Protistan plant parasites and phagotrophs to ecosystems, land management and soil properties. *Front Microbiol.* **11**, 1823 (2020).
71. Fiore-Donno, A. M., Bonkowski, M. Different community compositions between obligate and facultative oomycete plant parasites in a landscape-scale metabarcoding survey. *Biol Fertil Soils.* **57** (2), 245-256 (2021).
72. Albracht, C. et al. Common soil history is more important than plant history for arbuscular mycorrhizal community assembly in an experimental grassland diversity gradient. *Biol Fertil Soils.* **60** (4), 547-562 (2024).
73. Breitkreuz, C., Herzig, L., Buscot, F., Reitz, T., Tarkka, M. Interactions between soil properties, agricultural management and cultivar type drive structural and functional adaptations of the wheat rhizosphere

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

microbiome to drought. *Environ Microbiol.* **23** (10), 5866-5882 (2021).

74. Barnes, A. D. et al. Energy flux: The link between multitrophic biodiversity and ecosystem functioning. *Trends Ecol Evol.* **33** (3), 186-197 (2018).
75. Gauzens, B. et al.*fluxweb*: An R package to easily estimate energy fluxes in food webs. *Methods Ecol Evol.* **10** (2), 270-279 (2019).
76. Anderson, J. P. E., Domsch, K.H. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol Biochem.* **10** (3), 215-221 (1978).
77. Pausch, J. et al. Small but active - pool size does not matter for carbon incorporation in below-ground food webs. *Funct Ecol.* **30** (3), 479-489 (2016).
78. Macfadyen, A. Improved funnel-type extractors for soil arthropods. *J Anim Ecol.* **30** (1), 171 (1961).
79. Kempson, D., Lloyd, M., Ghelardi, R. A new extractor for woodland litter. *Pedobiologia.* **3**, 1-21 (1963).
80. Cesarz, S., Eva Schulz, A., Beugnon, R., Eisenhauer, N. Testing soil nematode extraction efficiency using different variations of the Baermann-funnel method. *Soil Org.* **91** (2), 61-72 (2019).
81. Ohno, T., Sleighter, R. L., Hatcher, P. G. Comparative study of organic matter chemical characterization using negative and positive mode electrospray ionization ultrahigh-resolution mass spectrometry. *Anal Bioanal Chem.* **408** (10), 2497-2504 (2016).
82. Swenson, T. L., Jenkins, S., Bowen, B. P., Northen, T. R. Untargeted soil metabolomics methods for analysis of extractable organic matter. *Soil Biol Biochem.* **80**, 189-198 (2015).
83. Steinbeiss, S., Temperton, V. M., Gleixner, G. Mechanisms of short-term soil carbon storage in experimental grasslands. *Soil Biol Biochem.* **40** (10), 2634-2642 (2008).
84. Roth, V. N. et al. Persistence of dissolved organic matter explained by molecular changes during its passage through soil. *Nat Geosci.* **12**, 755-761 (2019).

Copyright © 2025 JoVE Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

jove Journal of Visualized Experiments www.jove.com

Materials List for

JenaTron - An Experimental Approach to Study the Effects of Plant History and Soil History on Grassland Ecosystem Functioning

Anna-Maria Madaj[1,2], Yuanyuan Huang[1,2], Anne Ebeling[3], Lisa Ertel[1,2], Alban Gebler[1,2], Gerd Gleixner[1,4,5], Jes Hines[1,2], Christiane Roscher[1,6], Alexandra Weigelt[1,2], Cynthia Albracht[7,8,9], Angelos Amyntas[1,10], Leonardo Bassi[1,2], Ana Elizabeth Bonato Asato[1,2], Michael Bonkowski[11,12], Maximilian Bröcher[3], François Buscot[1,8], Francesca De Giorgi[1,6], Yuri Pinheiro Alves de Souza[13,14], Van Cong Doan[1,10], Walter Durka[1,15], Anna Heintz-Buschart[7], Justus Hennecke[1,2], Markus Lange[4], Pamela Medina-van Berkum[16], Sebastian Tobias Meyer[14], Sören Krawczyk[1,2], Akanksha Rai[4], Thomas Reitz[1,8], Christian Ristok[1,2], Stefan Scheu[17,18], Michael Schloter[13,14], Stefanie Schulz[13], Marcel Dominik Solbach[11], Sybille Barbara Unsicker[16,19], Nico Eisenhauer[1,2]

[1]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig

[2]Institute of Biology, Leipzig University

[3]Institute of Ecology and Evolution, Friedrich Schiller University Jena

[4]Max Planck Institute for Biogeochemistry

[5]Institute for Geosciences, Friedrich Schiller University Jena

[6]Department Physiological Diversity, Helmholtz Centre for Environmental Research (UFZ)

[7]Swammerdam Institute for Life Sciences, University of Amsterdam

[8]Department Soil Ecology, Helmholtz Centre for Environmental Research (UFZ)

[9]Institute Biosafety in Plant Biotechnology, Julius Kühn-Institute

[10]Institute of Biodiversity, Friedrich Schiller University Jena

[11]Institute of Zoology, University of Cologne

[12]Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne

[13]Research Unit Comparative Microbiome Analysis, Helmholtz Zentrum Munich

[14]School of Life Science, Technical University Munich - TUM

[15]Department of Community Ecology, Helmholtz Centre for Environmental Research (UFZ)

[16]Department of Biochemistry, Max-Planck Institute for Chemical Ecology

[17]JFB Institute of Zoology and Anthropology, University of Göttingen

[18]Centre of Biodiversity and Sustainable Land Use, University of Göttingen

[19]Botanical Institute, Plant-Environment-Interactions Group, Kiel University

Correspondence to: Anna-Maria Madaj at anna-maria.madaj@idiv.de

URL: https://www.jove.com/video/67496
DOI: doi:10.3791/67496

Materials

Name	Company	Catalog Number	Comments
Bulb planter (4 cm diameter)	Kent & Stowe	hardware store	https://www.bauhaus.info/gartenhandgeraete/kent-stowe-hand-blumenzwiebelpflanzer/p/27516825?cid=SSAGoo8995092923_90652787883&gad_source=1&gclid=CjwKCAiAqNSsBhAvEiwAn_tmxcMcdbxDo72K6gO6FX7hNePwWoc_8JIcgwA4qrYsbv-diJjHf_osDhoCXxEQAvD_BwE
Connector suction candle	Umwelt Geräte Technik (UGT)	1871000	https://ugt-online.de/en/solutions/ugt-lysimeter-sampling-technology-for-mineral-soils/
Cultivation tray (without drainage)	Bonsai-shopping	59000/5-m.D._1	https://www.bonsai-shopping.eu/Anzuchtschale-59000-5er.html
Demineralized water (VE)	Alfiltra	VEWA1000B	https://www.alfiltra.de/zubehoer/1000-liter-ve-wasser-im-ibc-behaelter
Duct tape	T-Rex	hardware store	https://www.bauhaus.info/panzertape/t-rex-

Copyright © 2025 Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

jove Journal of Visualized Experiments www.jove.com

			gewebeband/p/25421491?cid=SSAGoo8843016955_150014388930&gad_source=1&gclid=CjwKCAiAqNSsBhAvEiwAn_tmxX5KDl2CRPyAgi7nx2lLVTSy7dUfNY2DvvCi0ZvtnU8opjA-stwmvRoCflwQAvD_BwE
iDiv Ecotron	iDiv	n.a.	https://www.idiv.de/en/research/platforms-and-networks/idiv-ecotron.html
Plastic boxes (20 L)	Auer Packaging	EG 43/22 HG	https://www.auer-packaging.com/de/de/Eurobeh%C3%A4lter-geschlossen/EG-4322-HG.html?customer_type=private&gad_source=1&gclid=Cj0KCQjw4Oe4BhCcARIsADQ0csk38dwQfgiSU63eKE-xapuDT3f2f6EpzwKCJdjknbGPNbEwv4iOg0YaApUKEALw_wcB
Filter paper (150 mm diameter)	Labsolute	7697996	https://www.thgeyer.com/lab/shop/qualitativ-rundfilter-sorte-2005/labsolute-/7697990_de?variant=7697996
Forklift	hirsch	NEW-10084	https://www.hirsch.expert/elektro-gabelstapler-2-tonnen_1794_13098/?gad_source=1&gclid=Cj0KCQjw4Oe4BhCcARIsADQ0csmqfjWn46WITGVGnZDoDv4WkqUhWT0eYrsGnvIWcjemrOYCp3c3QC4aAn3WEALw_wcB
Gibberellic acid (90%)	Rotichrom	7697996	https://www.carlroth.com/de/de/weitere-vergleichssubstanzen/gibberellinsaeure/p/7464.2
Measuring jugs (3000 mL)	Hünersdorff	hardware store	https://www.hornbach.de/p/messkanne-3000-ml-huenersdorff/6481831/
Measuring jugs (5000 mL)	Hünersdorff	hardware store	https://www.hornbach.de/p/messkanne-5000-ml-huenersdorff/6481832/
Metal sieves (4 mm mesh size)	Blau-Metall	711082	https://analysensiebe.de/shop/produkt/analysensieb-400-maschenweite-2000/
Mini-digger	Zeppelin Cat	ZCS196577	https://www.zeppelin-cat.de/shop/produkte/minibagger-kaufen/cat-301-8-minibagger-inkl-loeffel-und-schnellwechsler/?srsltid=AfmBOoqOG9_VONO0EmPK0GAYEr1onMxM-v3YyM14hZFK0i9YHXJ_0ddggcs
Mortar tubs (90 L)	Gripline	hardware store	https://www.hornbach.de/p/moertelkuebel-pe-eckig-78-5-x-46-3-x-31-0-cm-90-l/274293/
Multipot plates (QuickPotPlates - QP 96T)	Herkuplast	741238	https://www.meyer-shop.com/product/view/37052?variantProductId=29253
Petri dishes (150 mm diameter)	Labsolute	7696412	https://www.thgeyer.com/lab/shop/petrischalen-ps-mit-nocken--aseptis/labsolute-/7696390_de?variant=7696412
Planting stencil (custom-made)	UFZ	n.a.	Planting stencil manufactured by the workshop of the Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany,

Copyright © 2025 Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

jove Journal of Visualized Experiments www.jove.com

			specifically for planting the iDiv Ecotron metal cylinders.
Plastic foil	Cargo Point	hardware store	https://www.hornbach.de/p/cargo-point-stretchfolie-0-50-x-300-m-20/7152950/
Plastic shovels	Hünersdorff	hardware store	https://www.hornbach.de/p/abfuellschaufel-huenersdorff-1390-ml/6481824/
Pressure sprayer (1500 mL)	Bürkle	9223162	https://shop.thgeyer-lab.com/erp/catalog/search/search.action?model.query=9223162
Quartz powder	Quarzsande	n.a.	https://quarzsand-shop.de/p/quarzmehl-25-kg-papier-sack-feinpulverig?srsltid=AfmBOoptmSFCMO-9KTIKoStSkd6aV2OobmBa2__UUcpYHXJisHGdq0JG
Quartz sand HQS (0.3 - 0.8 mm size)	Quanzsande	WMQ-10040	https://quarzsand-shop.de/p/quarzsand-hqs?variant=1185
Seal plugs	Umwelt Geräte Technik (UGT)	1871000	https://ugt-online.de/en/solutions/ugt-lysimeter-sampling-technology-for-mineral-soils/
Seed material	Rieger-Hofmann GmbH	n.a.	https://www.rieger-hofmann.de/startseite-rieger-hofmann.html
Soil moisture measuring kit (ML3 ThetaKit)	Delta-T Devices	ML3 ThetaKit	https://delta-t.co.uk/product/ml3/
Soil monolith extraction device	Umwelt Geräte Technik (UGT)	1871000	https://ugt-online.de/en/solutions/ugt-lysimeter-sampling-technology-for-mineral-soils/
Soil sensors (SMT-100)	Truebner GmbH	SMT-100	https://www.truebner.de/en/smt100.php#scroll-target
Spring steel tweezers (105 mm)	Roth	AL99.1	https://www.carlroth.com/de/de/spezialpinzetten-wafer-hakenpinzetten/federstahlpinzette/p/al99.1
Stainless steel blade (custom-made)	UFZ	n.a.	Stainless steel blade manufactured by the workshop of the Helmholtz Centre for Environmental Research (UFZ), Bad Lauchstädt, Germany, specifically for inserting soil sensors into the iDiv Ecotron metal cylinders.
Stainless steel cylinder	Umwelt Geräte Technik (UGT)	1871000	https://ugt-online.de/en/solutions/ugt-lysimeter-sampling-technology-for-mineral-soils/
Stick labels (12 cm x 2 cm)	Meyer	160140	https://www.meyer-shop.com/product/view/36273?variantProductId=31841
Suspension	Umwelt Geräte Technik (UGT)	1871000	https://ugt-online.de/en/solutions/ugt-lysimeter-sampling-technology-for-mineral-soils/
Temporary top/bottom plate	Umwelt Geräte Technik (UGT)	1871000	https://ugt-online.de/en/solutions/ugt-lysimeter-sampling-technology-for-mineral-soils/
Toothpicks	Büroshop24	269946	https://www.bueroshop24.de/500-papstar-party-picker-drop-kunststoff-269946?srpId=c8e0fcec04cb5e7e68a9bfe94096a8aa&storeType=B2C&lkz=1131602&obt=20&kwdid=5440413105-16705681638-135

Copyright © 2025 Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

			559674952-pla-32800987645 8&gad_source=1&gclid=CjwK CAiAqNSsBhAvEiwAn_tmxfR EZBIPohPHqFs2n6v_elmZTx L6QgkYdjBjMgZ2eYONmM05 Iwo-CRoCfWsQAvD_BwE
Trowel	Kaufmann	hardware store	https://www.hornbach.de/p/ berner-edelstahl-putzkelle- kaufmann-mit-softgriff/7940328/? marketNo=566&wt_mc=de.paid.sea google.alwayson_assortment.bod. pla.868482949.79838688391.&wt _cc1=868482949&wt_cc2=798386 88391&wt_cc3=377419910580&wt _cc4=&wt_cc6=7940328&wt_cc7= 566&gad_source=1&gclid=Cj0KCQ jw4Oe4BhCcARIsADQ0cslOTaWzp RLoaIVADaAOi5ZcHJEtFbKXvXJU ocTnzbhZTWgEUTlXr0waAp1q EALw_wcB
Truck	Europcar	n.a.	https://www.europcar.de/ de-de/reservation/vehicles? utm_source=google&utm_medium= cpc&utm_campaign&utm_term& gad_source=1&gclid=Cj0KCQjw4 Oe4BhCcARIsADQ0csn2Ph3mak wxbwrSHzKvJNJq9jaD-aiUvo8-4l RdCaUy8Gw_hgNW6KsaAiR2 EALw_wcB&gclsrc=aw.ds&vehicle Type=TR&pickupLocation=ZJSC04 &dropoffLocation=ZJSC04&pickupYe ar=2024&pickupMonth=12&pickup Day=11&dropoffYear=2024&dropoff Month=12&dropoffDay=18&pickup Hour=10&pickupMinute=00&dropoff Hour=18&dropoffMinute=00&driver Age=26&countryOfResidence=DE
Tweezers	Bochem Instrumente	9171013	https://www.thgeyer.com/ lab/shop/pinzette-105- mm-18/10-stahl/bochem- instrumente-1000/9171010_de? variant=9171013
Water suction probes	Umwelt Geräte Technik (UGT)	1211000	https://ugt-online.de/en/solutions/ ugt-suction-probe-with-ceramic- cup/
Wood for the sensor log	Konsta	hardware store	https://www.hornbach.de/p/konsta- konstruktionsvollholz-40x60x3000- mm-fichte-nsi-nicht-sichtbarer- einbau/8465807/

Copyright © 2025 Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Chapter 4: Fungivorous Protists in the Rhizosphere of *Arabidopsis thaliana* – Diversity, Functions, and Publicly Available Cultures for Experimental Exploration

Publication:

Estermann AH, Teixeira Pereira Bassiaridis J, Loos A, Solbach MD, Bonkowski M, Hess S, Dumack K. 2023. Fungivorous protists in the rhizosphere of *Arabidopsis thaliana* – Diversity, functions, and publicly available cultures for experimental exploration. *Soil Biology and Biochemistry* **187**: 109206. https://doi.org/10.1016/j.soilbio.2023.109206

For high-resolution images and additional video material, readers are referred to the online version of the publication.

Declaration of personal contributions:
I guided the Bachelor's Thesis of AH Estermann together with K Dumack, who also conceived the topic of the Bachelor's Thesis. I was involved in the conception and establishment of the methodology (setup of the feeding experiments, fluorescent staining, imaging via microscopy) of the Amoebozoa strains (*Cryptodifflugia oviformis*). I contributed to revising the article.

Soil Biology and Biochemistry 187 (2023) 109206

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Fungivorous protists in the rhizosphere of *Arabidopsis thaliana* – Diversity, functions, and publicly available cultures for experimental exploration

Antonie H. Estermann [a,1], Justin Teixeira Pereira Bassiaridis [b,1], Anne Loos [a], Marcel Dominik Solbach [a], Michael Bonkowski [a], Sebastian Hess [b,c,*,2], Kenneth Dumack [a,2,*]

[a] Terrestrial Ecology, Institute for Zoology, University of Cologne, Zülpicher Str. 47b, 50674, Cologne, Germany
[b] Evolutionary Ecology of Eukaryotic Microbes, Institute for Zoology, University of Cologne, Zülpicher Str. 47b, 50674, Cologne, Germany
[c] Department of Biology, Technical University of Darmstadt, Schnittspahnstr. 3, 64287, Darmstadt, Germany

ARTICLE INFO

Keywords:
Amoebae
Mycophagy
Plant microbiome
Protozoa
Pathogen suppression
Holobiont

ABSTRACT

In the context of the soil food web, the transfer of plant-fixed energy and carbon to higher trophic levels has traditionally been attributed to two main energy channels: the fungal energy channel and the bacterial energy channel. Historically, protists were overlooked in the fungal energy channel, which was believed to be controlled by fungivorous microarthropods and nematodes. In this study, we investigated fungivorous protists in the rhizosphere of *Arabidopsis thaliana*. Our findings revealed a notable abundance and diversity of protists that have developed specialized strategies to overcome the protective cell wall of fungi. Among the identified species were two Vampyrellida (Rhizaria) species, namely *Theratromyxa weberi* and *Platyreta germanica*, as well as one Arcellinida (Amoebozoa) species, called *Cryptodifflugia oviformis*. While *T. weberi* typically consumed entire fungal cells, the other two species perforated fungal cell walls and extracted the cellular contents. We elucidate the feeding strategies and dietary ranges of the amoebae, highlighting the non-uniform nature of fungivory in protists, as different taxa have evolved distinct approaches to access fungi as a food source. Moreover, we provide publicly available cultures of these protists to facilitate further experimental investigations within the research community.

1. Introduction

In the soil food web, energy and carbon fluxes are traditionally considered to start at the plants, the photosynthetic primary producers. Subsequently, this plant-fixed carbon was expected to be channeled to higher trophic levels by two main energy channels, the fungal and bacterial energy channels (Moore et al., 1988; Holtkamp et al., 2011; Bradford, 2016). The bacterial energy channel was believed to rely on bacterivorous protists and nematodes. The fungal energy channel was traditionally seen to be controlled by fungivorous microarthropods and nematodes (Hunt et al., 1987; de Ruiter et al., 1995). However, the plant holobiont, i.e. the sum of the plant and its associated microorganisms, was found to be much more complex. These plant-associated microorganisms compete and interact with each other, form complex food webs, and their community composition is shaped by both top-down and bottom-up effects (Hartmann et al., 2009; Krashevska et al., 2016). The plant-related functions of microorganisms include nutrient acquisition, disease suppression, the increase in stress tolerance, but also parasitism or herbivory (Berendsen et al., 2012; Richardson, 2017; Hassani et al., 2018; Xiong et al., 2020).

Protists associated with soils and plants were long considered one-dimensional, simple bacterivores, likely because the majority of phagotrophic protists can be isolated and maintained with bacteria as a food source (Bass et al., 2009b; Howe et al., 2009; Glücksman et al., 2010; Geisen et al., 2015a). With the rise of the molecular era, several plant-associated taxa that were previously believed to be fungi were revealed to be distantly related protists (e.g. oomycetes and plasmodiophorids). This shows that protists are functionally much more

* Corresponding authors. University of Cologne, Zülpicher Str. 47b, 50674, Cologne, Germany.
E-mail addresses: sebastian.hess@uni-koeln.de (S. Hess), kenneth.dumack@uni-koeln.de (K. Dumack).
[1] The authors contributed equally to this manuscript.
[2] The authors contributed equally to this manuscript.

https://doi.org/10.1016/j.soilbio.2023.109206
Received 27 June 2023; Received in revised form 8 September 2023; Accepted 11 October 2023
Available online 12 October 2023
0038-0717/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

versatile by being plant-parasitic (Schwelm et al., 2018; Dumack and Bonkowski, 2021). However, there are also numerous reports of soil protists that specifically interact with fungi and microscopic animals, and, in addition, some protists can process multiple food sources (bacteria, fungal cells, algae). Those taxa can be considered non-specialized, omnivorous phagotrophs, which are of high functional versatility (Dumack et al., 2019). Ekelund (1998) found that omnivorous protists feeding on fungal cells may reach similar biomass as bacterivorous protists. This suggests that protists feeding on fungi, similar to bacterivorous protists, can have significant impact on soil food webs and plant health by altering the abundance and community composition of other microorganisms by predation (Trap et al., 2016; Huang et al., 2021).

So far, taxonomic surveys report a relatively limited diversity of soil-dwelling protists that evolved special adaptions to feed on fungi, including their large hyphal structures (Petz et al., 1985; Ogden and Pitta, 1990; Pakzad, 2003). The most prominent examples belong to the ciliates (Grossglockneridae, Ciliophora), vampyrellid amoebae (Vampyrellida, Rhizaria), and testate lobose amoebae (Arcellinida, Amoebozoa). It takes specialist knowledge to isolate and culture such fungivorous protists. Since soils and plant surfaces are opaque, common light microscopy techniques are not applicable and extra efforts need to be made to study the interactions of soil protists with other organisms.

Recent environmental sequencing studies suggest that certain protists shape the fungal community and best explain the suppression of certain fungal pathogens (Huang et al., 2021; Ren et al., 2023). However, the experimental exploration of the mechanisms that underlie these findings was hampered by the lack of microscopical observations and publicly available cultures. In this study, we screened roots of *Arabidopsis thaliana* from natural populations for fungivorous protists. We isolated and characterized several strains of more or less specialized, fungivorous amoebae, and provide their cultures to the scientific community to facilitate the experimental exploration of plant-fungi-protist relationships.

2. Material and methods

2.1. Sampling and isolation

Six *Arabidopsis thaliana* were sampled around Cologne, Germany. The sampled plants stem from technosols of urban areas, including municipal parks and sidewalks. Roots were washed and cut into 1 cm long pieces. These root pieces were transferred into the wells of a 24-well plate with the culture medium Waris-H + Si (Mcfadden and Melkonian, 1986), and incubated for one day. The wells were screened with an inverted phase contrast microscope for fungivorous protists (Nikon Eclipse TS100; 100x - 400x). From the total of 144 wells, almost every well contained numerous *Cryptodifflugia* specimens and four wells with vampyrellid cells were found. Cultures were established with *Saccharomyces cerevisiae* as prey. Cell sorting as described in Solbach et al. (2021) or serial manual washings in sterile medium were used to establish cultures free of environmental bacteria. Microscopic documentation was done with a Nikon Eclipse 90i and Motic AE2000.

2.2. Molecular identification and phylogenetics

To identify the strains by molecular means, the SSU rRNA gene was amplified and sequenced. For the vampyrellid strains, digestive cysts were picked and transferred to 1 ml of fresh culture medium. To avoid any contamination by food organisms during PCR, the cysts were starved for several days until they had hatched. The resulting amoebae were then transferred with approx. 1 μl of medium to PCR tubes filled with 4 μl ddH_2O and stored at −20 °C. For *Cryptodifflugia*, cells in 5 μl of medium were transferred into PCR tubes. The PCR mixture was added, consisting of 6.4 μl ddH2O, 1.7 μl Thermo Scientific Dream Taq Green Buffer, 1.7 μl of 10 μM forward and reverse primers each, 0.34 μl 10 μM dNTPs and 0.17 μl DreamTaq polymerase (Thermo Fisher Scientific, Dreieich, Germany). The SSU rRNA genes were amplified in two successive steps. First, the whole SSU rRNA gene was amplified with the general eukaryotic primers EukA and EukB (Medlin et al., 1988). In the second step, semi-nested re-amplifications were performed. For Vampyrellida, we used Vampyrellida-specific primers in combination with eukaryotic primers, i.e. EukA +947_Vamp (5′-AAGAAGATATCCTTGGT-3'; Fiore-Donno et al., 2020) targeting the 5′-part of the SSU rDNA and 615_Phyt (5′-CTTTSAARGCTCGTAGTTG-3'; Fiore-Donno et al., 2020) + EukB for the 3′-part of the gene. One microlitre of the first PCR product was used as a template for reamplification. For *Cryptodifflugia*, the semi-nested re-amplifications were performed by combining EukA and EukB with Phryganellina-specific primers, i.e. 590F_Phry (5′-CGGTAATTCCAGCTCCAATAGT-3'; Dumack et al., 2020) and 1300R_Phry (5′-GCATGGCCGTTTTTAGTTGGTG-3'; Dumack et al., 2020) as described above. PCR conditions were as follows: initial denaturation at 95 °C for 5 min, 24 cycles (denaturation at 96 °C for 32 s, annealing at 50 °C for 36 s, elongation at 72 °C for 2 min), terminal extension at 72 °C for 7 min, and hold at 4 °C. The PCR products were purified by adding 0.15 μl of Exonuclease I, 0.9 μl FastAP and 1.95 μl water to 8 μl of the second PCR product and then heated for 30 min at 37 °C, and subsequently for 20 min at 85 °C. The Big dye Terminator Cycle sequencing Kit (Thermo Fisher Scientific, Dreieich, Germany) and an ABI PRISM automatic sequencer were used for the sequencing. All four previously mentioned primers were used for sequencing.

For the vampyrellid phylogeny, the SSU rRNA gene sequences were added to a sub-alignment (Leptophryidae with Vampyrellidae as outgroup) of that used in Hess and Suthaus (2022), and then aligned with MUSCLE in Seaview 5.0.4 (Gouy et al., 2010) and curated manually. We then inferred maximum likelihood (ML) and Bayesian (BI) phylogenies with 1541 aligned sites. Twenty maximum likelihood inferences were done with raxmlGUI 2.0.10 (Edler et al., 2021) with the GTR + I + Γ model, and the best tree was selected for display. The branch support was retrieved by bootstrapping with 1000 replicates. The Bayesian analysis was done with Beast 2.7.3. (Bouckaert et al., 2014) with the same model, 5,000,000 generations (trees sampled every 1000 generations), and a 25% burn-in (1,250,000 generations discarded). We confirmed the stationarity of the BI with Tracer 1.7.2. (Rambaut et al., 2018).

For the Arcellinida phylogeny placing *Cryptodifflugia*, the sequences were added to a sub-alignment of that used in Dumack et al. (2020), and then aligned with MUSCLE in Seaview 5.0.4 and curated manually. We then inferred maximum likelihood (ML) and Bayesian (BI) phylogenies with 1321 aligned sites, which were 40.88% invariant. We used the model GTR + I + Γ. For phylogenetic calculations, we used MrBayes v3.2.6 (Ronquist and Huelsenbeck, 2003) and RAxML (Stamatakis, 2014). The Bayesian analysis was set up with a sampling of every 100 and a diagnosis of every 500 trees and 25% of burn-in. It was conducted on the online platform CIPRES (Miller et al., 2010) and reached final split frequencies of <0.01 after 870,000 generations.

2.3. Food range experiment

Twelve different fungal species were used as potential food sources for the amoebae and cultivated in Petri dishes on potato glucose agar (Sigma-Aldrich) according to the manufacturer's instructions. The fungi were chosen to represent evolutionary diverse taxa, i.e. Ascomycota, Basidiomycota and Mucoromycota, and distinct morphologies, i.e. yeasts and taxa forming hyphae of different thickness. For the vampyrellid amoebae, a small quantity of each fungal culture was given into half-strength Waris-H + Si and transferred to wells of a twelve-well plate. The wells were observed after one, seven, and fourteen days for growth of the protists and signs of predation with the AE2000 (Motic) inverted microscope and the MikroLive 6,4 MP camera. For *Cryptodifflugia*, a small quantity of each fungal culture was transferred to separate Petri dishes containing 1% water agar. After a few days of incubation, about 300 μl of a fungi-free *Cryptodifflugia* culture in WG medium

(Bonkowski, 2019) was added to each fungal subculture. The preparation was then filmed with the Nikon Eclipse TE2000 inverted microscope and the Nikon digital sight DS-U3 camera (program: NIS-Elements V4.13.04, Tokyo, Japan) after one, seven, and fourteen days for amoebal growth and signs of predation. Time-lapse videos were prepared to confirm predation on each fungal culture.

2.4. Phalloidin staining and fluorescence microscopy

To obtain first insights into the mechanism used by *Cryptodifflugia oviformis* to feed on fungi, cells were fixed and stained with a fluorescent phalloidin-conjugate before and during the feeding process. For this, gelatin-coated cover glasses were placed inside 6-well plates and sterilized with ultraviolet light for 20 min. Subsequently, WG medium with hyphae of *Amniculicola* sp. was pipetted onto the cover glasses. Excess medium around the hyphae was removed. When hyphae adhered to the cover glasses, a culture of *Cryptodifflugia oviformis* was added and the setup was incubated for up to 24 h. After incubation, the cells were fixed with 4% formaldehyde (freshly made from paraformaldehyde dissolved in PBS, pH 7) for 10 min at room temperature. The coverslips were subsequently washed with PBS (Sigma Aldrich) for 5 min. After fixation, the cells were permeabilized for 10 min with 0.1% Triton X-100 in TBS. Subsequently, slides were washed with PBS for 5 min and stained with Phalloidin-iFluor 488 (ab176753, abcam, 0.001%). Incubation took place for 1 h in the dark (in a moist chamber, room temperature), followed by three washing steps (PBS, each 5 min). Afterward, mounting medium (85% glycerol, 15% PBS) was added and the cells were documented with a Nikon Eclipse 90i.

2.4.1. Scanning electron microscopy

To obtain insights into the mechanism used by *Platyreta germanica* to feed on fungi, the supernatant of an old culture of *Platyreta germanica* strain G2.3 was carefully removed and the emptied yeast cells resuspended by shaking. The suspension was placed on poly-L-lysine coated coverslips and let settle for about 1 h. The coverslips were then transferred to ethanol/water mixtures of ascending concentrations (30% - 50% - 70% - 90% - 100%), and finally immersed twice in hexamethyldisilazane (HMDS) for at least 5 min. After careful removal of HMDS the coverslips were air-dried in a fume hood, sputter-coated with gold and imaged with a ZEISS Neon 40 scanning electron microscope using the secondary electron detector (ZEISS, Oberkochen, Germany).

3. Results

3.1. Genetic identity of the isolated strains

Thirteen strains of naked and testate amoebae were isolated from the roots of *Arabidopsis thaliana* and cultivated with *Saccharomyces cerevisiae* as food source. Based on their SSU rRNA gene sequences, the isolates could be assigned to two distinct and distantly related orders of amoeboid life forms, the Vampyrellida (Rhizaria) and Arcellinida (Amoebozoa) (Fig. 1A). Phylogenetic inferences revealed that the four

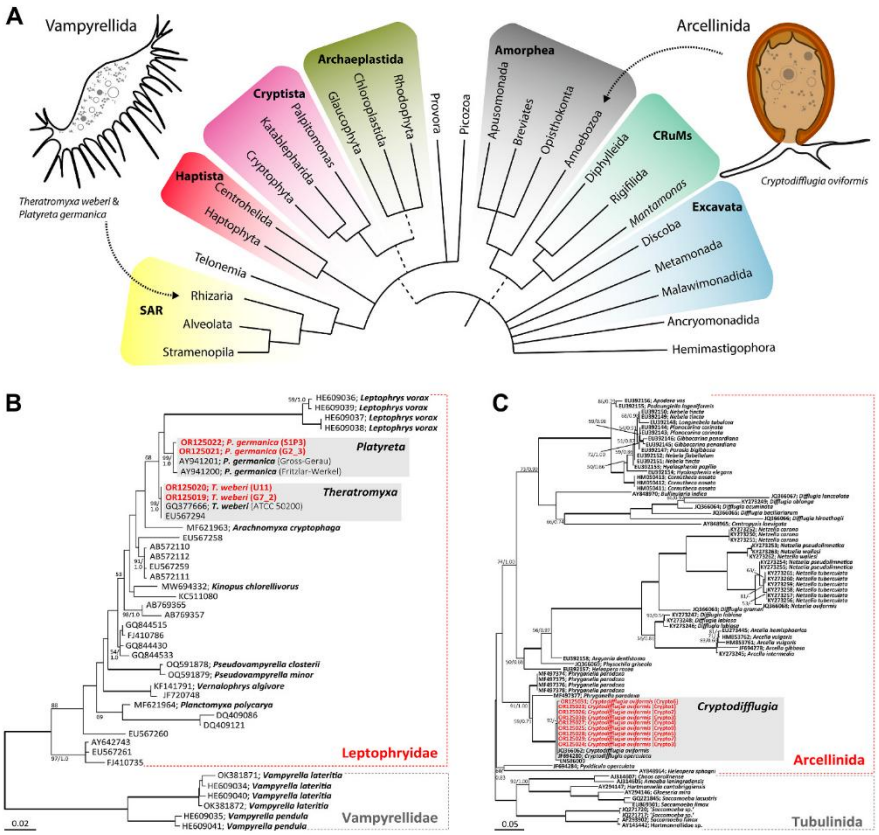


Fig. 1. Phylogenetic position of the studied amoebae. (A) Overview of the eukaryotic diversity based on Burki et al. (2020) with highlighted supergroups. Representative drawings indicate the overall morphologies (naked filose vs. testate lobose) and the phylogenetic affinity of the studied amoebae. Note that both taxa are morphologically and genetically vastly different. (B) ML phylogeny of the Leptophryidae (Vampyrellida, Rhizaria) with Vampyrellidae as outgroup. New strains are shown in red. (C) ML phylogeny of the Arcellinida (Amoebozoa) with the Tubulinida as outgroup. New strains are shown in bold. Branches of both phylogenies show bootstrap support and posterior probabilities (ML/BI) unless fully supported (bold) or with support below 50/0.9 (omitted). Branches show bootstrap support and posterior probabilities (ML/BI) unless fully supported (bold) or with support below 50/0.9 (omitted). Scale bars: 0.02 expected substitutions per site.

vampyrellid isolates branched in the family Leptophryidae with maximum support (Fig. 1B). Two strains (G7.2, U11) were closely related to *Theratromyxa weberi* (strain ATCC 50200), the other two (G2.3, S1P3) to *Platyreta germanica* (strains "Gross-Gerau" and "Fritzlar-Werkel"). As the genetic distances between the new strains and the published reference sequences were <0.5%, we assign the new strains to these two species (Table 1). The nine arcellinid strains branched within the family Cryptodifflugiidae with maximum support (Fig. 1C). They are all closely related to each other and to *Cryptodifflugia oviformis* (JQ366062) and comply with the morphological circumscription of this species (details below). The cultures of *Theratromyxa weberi* and *Platyreta germanica* could be grown without environmental bacteria, while bacteria-free cultures of *Cryptodifflugia oviformis* showed atypical behavior, i.e. daughter cells lacked shells and ceased to grow after approximately five divisions. Hence, the here presented cultures of *Cryptodifflugia oviformis* contained environmental bacteria throughout subsequent analyses.

3.2. Morphology and feeding processes of vampyrellid strains

Both vampyrellid species *Theratromyxa weberi* and *Platyreta germanica* had colorless, highly branched cells, best observed with phase contrast optics (Fig. 2A and B). The amoeboid cells (=trophozoites) exhibited thin filopodia without granules and moved by slow enlargement and advancement of pseudopodial structures. They showed a high propensity to form network-like structures of unlimited size in our cultures (Fig. 2C; Supplementary Video 1). Although both species could be readily grown with yeast as food, they differed markedly in their feeding strategies. *Theratromyxa weberi* incorporated entire yeast cells and cleared the bottom of the culture flask (Fig. 2A), while *Platyreta germanica* perforated the yeast cells and phagocytosed the cytoplasm (protoplast feeding). As typical for all known vampyrellids (Hess and Suthaus, 2022), the trophozoites of the studied strains entered an immobile, but highly active digestive cyst stage after feeding (Fig. 2D and E). The different feeding strategies observed in *Theratromyxa* and *Platyreta* were also evident from the undigested food remnants left behind in the empty cyst walls. *Theratromyxa* cysts contained an agglomerate of partially digested yeast cells (Fig. 2F), while *Platyreta* cysts contained an amorphous, dark brown food remnant (Fig. 2G). Such amorphous food remnants can also be found in other protoplast-feeding vampyrellids (Hess et al., 2012), and help identify the mode of feeding before the process itself was observed. As a result of *Platyreta's* feeding strategy, empty yeast cells accumulate as the cultures develop (Fig. 2B). Scanning electron microscopy revealed that these empty yeast cells exhibit large perforations caused by *Platyreta* (Fig. 2H).

3.3. Morphology and feeding processes of Cryptodifflugia strains

Cryptodifflugia oviformis cells exhibited an oval, smooth and transparent shell with a mean length of 19 ± 3 μm and a width of 15 ± 3 μm (n = 25; measured on strain Crypto3). The opening of the shell was terminal and circular with a diameter of 4 ± 0.6 μm (n = 17). These characters and measurements match the current circumscription of the species *C. oviformis* very well (Bobrov and Mazei, 2017). The cells bore several lobose and conical pseudopodia, which reached a length of up to 45 μm. *Cryptodifflugia oviformis* used these pseudopodia for locomotion and to initiate contact with fungal cells. Small fungal structures such as yeast cells and small spores were pulled through the shell's opening and phagocytosed entirely. We observed that the cells of *Saccharomyces cerevisiae* were frequently deformed during this process, indicating considerable forces applied to the prey cells (Fig. 3E; Supplementary Video 3). If cells were too large to be pulled through the shell's opening, the fungal cell wall ruptured and the amoebae incorporated both cytoplasm and cell wall material. Relatively thick hyphae were perforated by this process, resulting in a hole with rough edges, while thinner hyphae were broken in two and dragged into the shell (Fig. 3A–D; Supplementary Video 2). To study the distribution of F-actin during food uptake, we stained fixed *Cryptodifflugia* cells with a fluorescent phalloidin conjugate. The cell body of the amoebae contained conical bundles of F-actin that started from the point of contact with the fungal cells and extended far into the cell body (Fig. 3F). Often these bundles reached the epipodia, i.e. the contact zones of the amoeba's cell body with the inner wall of the shell (Fig. 3F). In motile, foraging amoebae, we did not observe a distinct phalloidin staining in the cell body, but a strong signal in the pseudopodia used for locomotion (Fig. 3G).

3.4. Consumption of fungi by the isolated soil amoebae

Our food range experiments indicated that a variety of fungi can be consumed by the fungivorous protists *Platyreta germanica, Theratromyxa weberi,* and *Cryptodifflugia oviformis* (Table 2). The range of fungal cultures that was preyed upon by each protistan species differed, as did the mechanisms that were used to access the fungal prey. *Platyreta germanica* perforated the cell walls of its prey and extracted the fungal cell's protoplast. We did not record a single instance of *Platyreta germanica* phagocytosing entire prey cells. In contrast, the closely related *Theratromyxa weberi* most often phagocytosed entire prey organisms (spores, yeasts, and small hyphae). However, occasional perforation of spores of *Umbelopsis isabellina* and *Mortierella* sp. and the yeasts *Saccharomyces cerevisiae* and *Cryptococcus aerius* were observed as well. *Cryptodifflugia oviformis* consumed small prey cells entirely, but perforated or ruptured hyphae. The hyphae of *Mortierella* sp. and *Rhizopus* sp. showed circular perforations with irregular margins, while we observed all other

Table 1
Investigated strains of fungivorous protists and corresponding data.

Species	Strain	Sequence length	Sampling sites	Coordinates	Sequence accession	Culture accession
Platyreta germanica	G2.3	1747	Germany Jüchen	51.070000, 6.514000	OR125021	CCAP 2559/1
	S1P3	1631	Germany Brühl	50.827000, 6.909000	OR125022	
Theratromyxa weberi	G7.2	1755	Germany Jüchen	51.070000, 6.514000	OR125019	CCAP 2573/1
	U11	1768	Germany Cologne	50.925750, 6.936083	OR125020	
Cryptodifflugia oviformis	Crypto1	1898	Germany Brühl	50.827000, 6.909000	OR125023	
	Crypto2	1897	Germany Brühl	50.827000, 6.909000	OR125026	
	Crypto3	1898	Germany Brühl	50.827000, 6.909000	OR125024	CCAP 1514/3
	Crypto4	1937	Germany Brühl	50.827000, 6.909000	OR125027	
	Crypto5	1897	Germany Brühl	50.827000, 6.909000	OR125028	
	Crypto6	1956	Germany Brühl	50.827000, 6.909000	OR125031	
	Crypto7	1951	Germany Brühl	50.827000, 6.909000	OR125029	
	Crypto8	1947	Germany Brühl	50.827000, 6.909000	OR125030	
	Crypto9	1949	Germany Brühl	50.827000, 6.909000	OR125025	

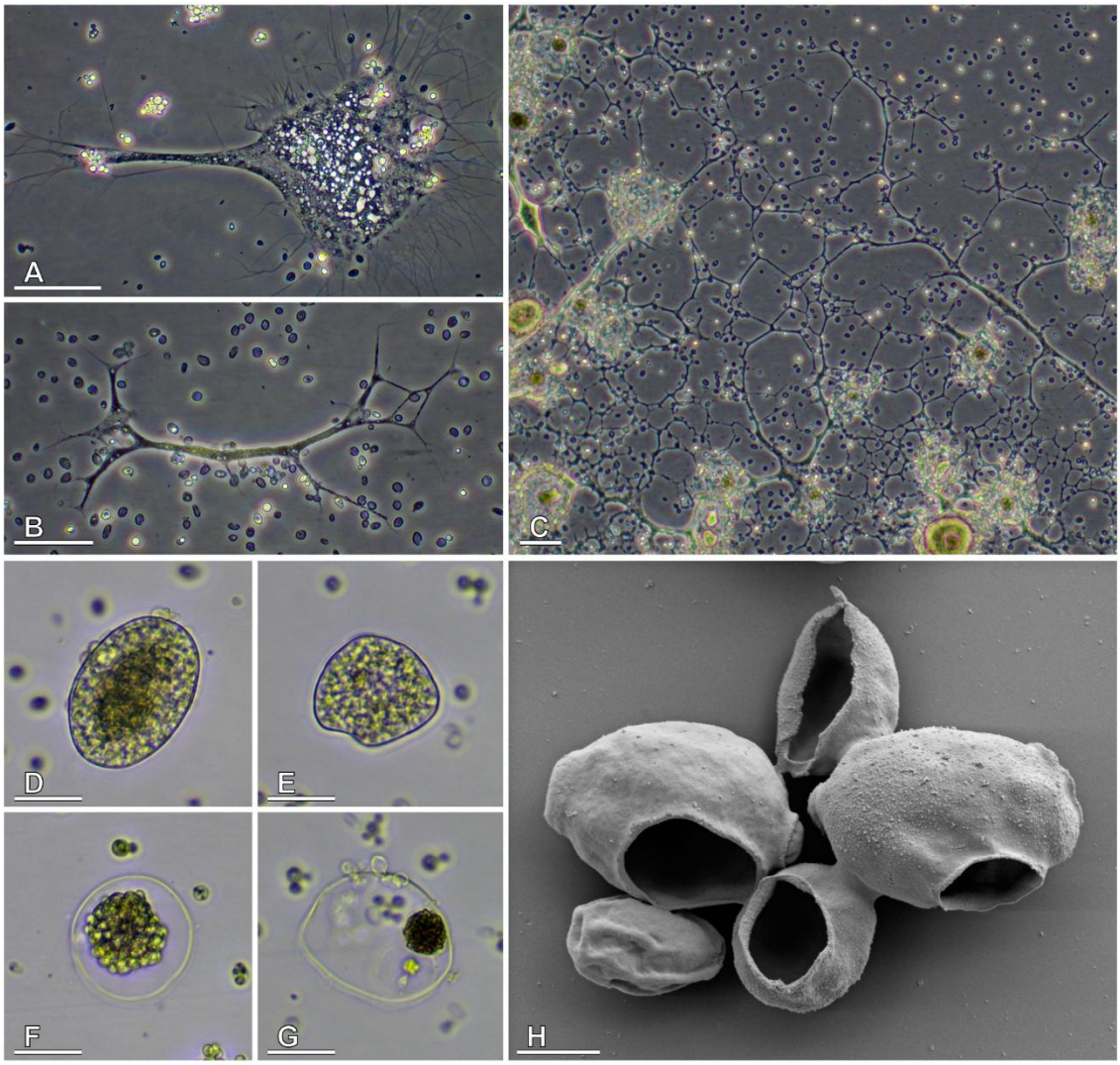


Fig. 2. Morphology of soil-dwelling vampyrellids (strains G2.3 and G7.2) and aspects of their feeding behavior. (A) Trophozoite of *Theratromyxa weberi* strain G7.2, phase contrast. (B, C) Trophozoites of *Platyreta germanica* strain G2.3, phase contrast. Note that the cells can fuse and form large networks (D, E; see also Supplementary Video 1). Full digestive cysts of *T. weberi* (D) and *P. germanica* (E), brightfield. (F, G) Empty digestive cysts left behind by *T. weberi* (F) and *P. germanica* (G) after digestion of the food, brightfield. Note the difference in the food remnants between the engulfing (F) and extracting (G) vampyrellid. (H) Scanning electron micrograph of yeast cells (*Saccharomyces cerevisiae*) perforated and emptied by *P. germanica*. Scale bars indicate 50 μm (A–C), 20 μm (D–G) and 2 μm (H).

filamentous fungi to be cut in two during predation. *Cryptodifflugia oviformis* also consumed environmental bacteria, which, however, were not determined.

4. Discussion

4.1. Mechanisms of fungivory in amoebae

Fungivory by soil amoebae has been documented for more than 50 years. However, the first reports were still a mystery: Old (1967) observed peculiar, round perforations in the walls of fungal spores which had been incubated in natural soils, but the true nature of the "perforating agent" remained unknown for several years (Old and Wong, 1976; Clough and Patrick, 1976). Finally, Old (1977) and shortly afterward Anderson and Patrick (1980) provided evidence that the perforations were caused by giant terrestrial amoebae. The latter authors also documented several distinct amoebae, which caused perforations of different sizes (from 1 to 5 μm). In our opinion, the applied genus names (e.g. *Vampyrella*, *Arachnula*) are, however, incorrect. A similar fungivorous amoeba was later cultivated and studied by Pakzad (2003), and finally described as a new taxon, *Platyreta germanica* Bass et al. (2009). It was also shown that this amoeba belongs to the rhizarian protists (and not the Amoebozoa) – relatively closely related to the genus *Vampyrella* (Bass et al., 2009a; Hess et al., 2012). During the past 10 years, the vampyrellid amoebae have been extensively explored by cultivation and molecular methods (Hess et al., 2012; Berney et al., 2013; Gong et al., 2015; Hess, 2017a, 2017b; More et al., 2018, 2021; Zhang et al., 2022), but these studies mainly focused on aquatic representatives. For the terrestrial vampyrellids preying on fungi and animals, we still lack the link between in-depth ecological observations and the genetic identity of particular species. Furthermore, there were no cultures available for experimentation, as all strains studied before were lost. In this study, we isolated *Platyreta germanica* from the rhizosphere of *A. thaliana* and established a bacteria-free culture for our feeding experiments. In addition, we demonstrate that two other terrestrial amoebae, *Theratromyxa weberi* and *Cryptodifflugia oviformis* readily feed on diverse fungi – including hyphal structures. This came as a surprise, as *T. weberi* was originally described as a carnivorous amoeba feeding on

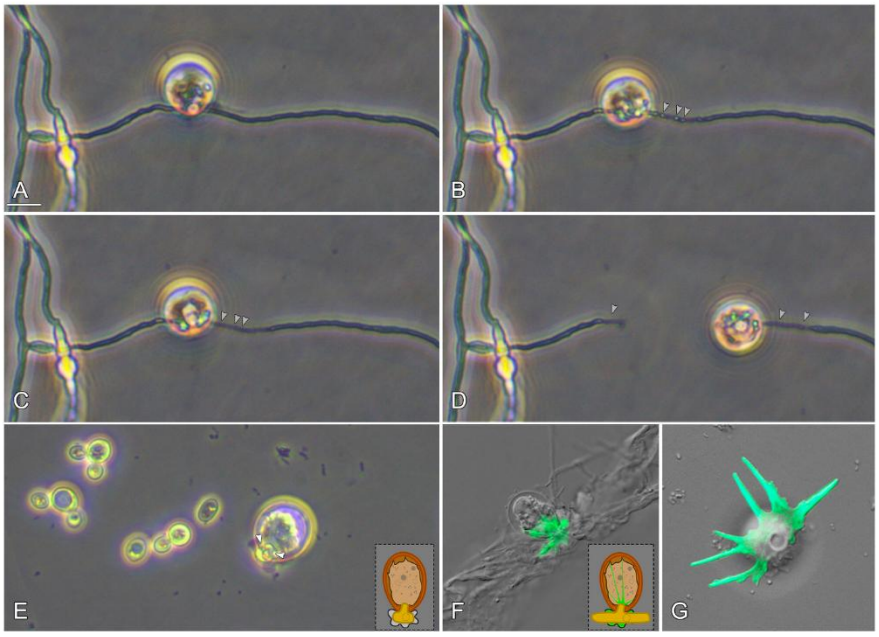


Fig. 3. The figure shows the fungivorous protist *Cryptodifflugia oviformis*. Figures A–D show a time series of *C. oviformis* feeding on hyphae of *Fusarium sporotrichoides* (see also Supplementary Video 2). The protist attaches to the hypha and starts to manipulate, pull and break the fungal cells (A). The hypha's cell wall is broken (B), note that the fungal cell's contents are fragmented, but still inside of the hypha (see grey arrows). *C. oviformis* empties the fungal hypha (C) and migrates along the hypha while phagocytosis further parts of it (D). Note the grey arrows that indicate empty fragments of the hypha. Figure E shows *C. oviformis* feeding on a yeast cell (*Saccharomyces cerevisiae*). Note that the ovoid and spherical yeast cell is forcefully deformed while being pulled into the shell of the protist (see also drawing and Supplementary Video 3). Figures F–G show F-actin-stained cells. During the rupture of fungal hyphae (here *Amniculicola* sp.), F-actin bundles stretching through the cell body of the protist could be seen (F). During locomotion the cell body was almost entirely devoid of stained F-actin, while pseudopodia were highly stained. Scale bar indicates 10 μm.

Table 2
Results of the food range experiments. "+" indicates observed feeding events, "–" indicates the absence of observed feeding events. No feeding rates are indicated.

Fungi				Vampyrellida (Rhizaria)		Arcellinida (Amoebozoa)
			morphotype	*Platyreta germanica*	*Theratromyxa weberi*	*Cryptodifflugia oviformis*
Ascomycota	Hypocerales	*Fusarium* sp.	filamentous	+	–	+
		Fusarium solani	filamentous	+	–	+
		Fusarium sporotrichioides	filamentous	+	–	+
		Gliomastix murorum	filamentous	–	–	+
		Purpureocillium lilacinum	filamentous	–	–	+
	Eurotiales	*Paecilomyces marquandii*	filamentous	+	+	–
	Saccharomycetales	*Saccharomyces cerevisiae*	unicellular	+	+	+
Mucoromycota	Mortierellales	Mortierella sp.	filamentous	+	+	+
	Mucorales	*Rhizopus* sp.	filamentous	–	–	+
		Umbelopsis isabellina	filamentous	+	+	–
Basidiomycota	Filobasidiales	*Cryptococcus aerius*	unicellular	+	+	+
		Cryptococcus laurentii	unicellular	+	+	+

nematodes (Weber et al., 1952; Sayre, 1973). It was also shown that *Cryptodifflugia* and some relatives, e.g. *Nebela* and *Difflugia,* are able to consume nematodes (Yeates and Foissner, 1995; Gilbert et al., 2000; Geisen et al., 2015b). Hence, we presume that several soil amoebae have a fairly wide prey range.

Furthermore, our results exemplify that fungivory is not a uniform protistan trait. All three studied soil amoebae, *Platyreta germanica*, *Theratromyxa weberi* and *Cryptodifflugia oviformis*, were able to manipulate, ingest and/or perforate fungal cells. However, how exactly these amoebae fed on the same fungal prey differed strikingly – even among the two very closely related vampyrellid species. While *Platyreta* extracts fungal cells after local perforation of the wall, *Theratromyxa* usually ingested entire cells, which might have an impact on the possible prey range (size-limited ingestion). In fact, *Theratromyxa* showed a somewhat narrower prey range and was not able to feed on the filamentous *Fusarium* species. The different prey ranges of *Platyreta germanica* and *Cryptodifflugia oviformis* cannot be explained by prey size, as both organisms can access the cytoplasm of spores and hyphae by the destruction of the fungal wall. How exactly these amoebae open fungal cells remains unclear. Based on the regular morphology and smooth margins of the perforations produced by vampyrellid amoebae such as *Platyreta*, the action of enzymes was hypothesized (Old, 1978) but not tested. In fact, the annular perforations of *Platyreta* closely resemble those found in other rhizarian protoplast feeders (*Orciraptor agilis*) which evidently use lytic carbohydrate-active enzymes to open prey cell walls (Moye et al., 2022). There are a number of reports on testate amoebae feeding on fungi, but most often they exclusively consumed spores and yeasts, which can be ingested entirely (Ogden and Pitta, 1990; Jassey et al., 2012; Dumack et al., 2016). Besides *Cryptodifflugia*, there are a few reports of other arcellinid amoebae preying on and/or perforating large organisms. These observations were mainly made on limnic species (Hoogenraad and Groot, 1941; Gilbert et al., 2000;

Siemensma and Opitz, 2014; Geisen et al., 2015b; Dumack et al., 2018a). For example, *Difflugia rubescens* and *Pseudonebela africana* were found to perforate cell walls of green algae, leaving roughly-edged holes in the remaining cell wall (Hoogenraad and Groot, 1941; Siemensma and Opitz, 2014). Dumack et al., 2018a speculated that this process is mechanical, potentially being facilitated by the cytoskeleton of these amoebae. They observed *Phryganella paradoxa*, a specialized predator of diatoms and close relative of *Cryptodifflugia oviformis* (see Fig. 1C), to bend and break the siliceous thecae of their prey. The aperture of the amoeba's shell seemed to function as a pivot point in this process and thus its diameter might be the limiting factor in terms of prey size. Here, we found F-actin bundles in attacking cells of *Cryptodifflugia oviformis*, which supports the idea that the cytoskeleton in testate amoebae exerts mechanical force during the feeding process. In contrast to the diatom-feeding *Phryganella paradoxa*, the prey range of *Cryptodifflugia oviformis* seems not to be limited by prey size, which we explain by the flexibility of fungal cells (e.g. note the deformation of yeasts cells when passing the aperture). Hence, the observed differences in prey range between *Cryptodifflugia oviformis* and the vampyrellid amoebae may also be related to the evolutionary and functionally different mechanisms of fungivory in these two distantly related groups of amoebae. We conclude that fungivory evolved repeatedly in protist lineages, and that fungivorous soil amoebae evolved functionally distinct strategies that result in different prey ranges. Fungivory in protists is thus not a uniform trait, and depending on whether the feeding pressure is directed at yeasts, fungal spores, filamentous fungi or all of them, fungivorous protists might have different functions in the soil food web. In previous attempts to assign traits to protists, fungivory was considered (Dumack et al., 2019). However, here we show that more fine-grained categories for the type of fungivory need to be created to reflect the actual feeding mechanisms. For example, one should consider the prey morphology (yeasts, spores, and hyphae) and the mechanism (enzymatic lysis, mechanic rupturing, and the engulfment of whole prey organisms). We argue that it is important to acknowledge these mechanistic differences, in particular, if protists are selected as biocontrol agents. Plant-pathogenic *Fusarium* species with hyphae and large, multicellular spores, for example, are unlikely to be affected by fungivores that exclusively feed on yeasts. Equally, these mechanistic differences between fungivores make it impossible to assess fungivory in natural soils by chitinolytic activity or by the expression of chitinases. Chitinases are unlikely to be involved in fungi-rupturing amoebae and instead play various roles in processes unrelated to fungivory (plant immunity, spore germination, decay of exoskeletons).

4.2. Fungivory and its consequences for the soil food web

Arabidopsis thaliana is widely used as a model plant, but associated eukaryotic microorganisms remain largely unexplored (Urbina et al., 2018). Although *A. thaliana* does not engage in mycorrhizal symbiosis, the growth and health of *A. thaliana* depend on root-associated fungi (Micallef et al., 2009; Urbina et al., 2018). Junker et al. (2012) isolated fungi from *Arabidopsis thaliana*. Among others, they found *Fusarium* sp. and *Rhizopus* sp., species that – as we demonstrated in our experiments – were consumed by *Platyreta germanica* (*Fusarium* spp.) and *Cryptodifflugia oviformis* (*Fusarium* spp. and *Rhizopus* sp.). Yeasts are neglected in rhizosphere studies, but evidence by stable isotope probing suggests that yeasts could be important competitors of rhizobacteria for root exudates and there are numerous protist species feeding on yeasts (Dumack et al., 2018b; Hünninghaus et al., 2019). Geisen et al. (2015) showed that vampyrellid amoebae represent a small fraction of the protist community, but they are consistently present in various terrestrial habitats (see also Fiore-Donno et al., 2019, 2020). *Cryptodifflugia oviformis* is long known to be a ubiquitous and abundant protist (Bobrov and Mazei, 2017), which we found along the roots of *Arabidopsis thaliana* in high numbers. The amount of energy that is channeled by fungivorous protists may thus be considerable (Hedley et al., 1950; Kramer et al., 2016). Here, we provide publicly available cultures of three potentially important soil amoebae, which may facilitate future experimental research to understand the functions, relevance and potential use of fungivorous protists.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data has been published and the cultures are publicly available.

Acknowledgments

This work was funded by the German Research Foundation in the framework of the priority programme SPP 1991 (Taxon-Omics: New approaches for discovering and naming biodiversity), grant 447190101 to S.H. and 447013012 to M.B. and K.D. Furthermore, we acknowledge funding by the German Research Foundation through the Emmy Noether Programme, grant 417585753, to S.H.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109206.

References

Anderson, T.R., Patrick, Z.A., 1980. Soil Vampyrellid amoebae that cause small perforations in conidia of Cochliobolus sativus. Soil Biology and Biochemistry 12, 159–167. https://doi.org/10.1016/0038-0717(80)90053-X.

Bass, D., Chao, E.E.-Y., Nikolaev, S., Yabuki, A., Ishida, K.-I., Berney, C., Pakzad, U., Wylezich, C., Cavalier-Smith, T., 2009a. Phylogeny of novel naked filose and reticulose cercozoa: granofilosea cl. n. and proteomyxidea revised. Protist 160, 75–109. https://doi.org/10.1016/j.protis.2008.07.002.

Bass, D., Howe, A.T., Mylnikov, A.P., Vickerman, K., Chao, E.E., Edwards Smallbone, J., Snell, J., Cabral, C., Cavalier-Smith, T., 2009b. Phylogeny and classification of cercomonadida (Protozoa, cercozoa): cercomonas, eocercomonas, paracercomonas, and cavernomonas gen. Nov. Protist 160, 483–521. https://doi.org/10.1016/j.protis.2009.01.004.

Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. Trends in Plant Science 17, 478–486. https://doi.org/10.1016/j.tplants.2012.04.001.

Berney, C., Romac, S., Mahé, F., Santini, S., Siano, R., Bass, D., 2013. Vampires in the oceans: predatory cercozoan amoebae in marine habitats. The ISME Journal 7, 2387–2399. https://doi.org/10.1038/ismej.2013.116.

Bobrov, A., Mazei, Y., 2017. A Review of Testate Amoeba Genus Cryptodifflugiidae with a ... 1890. https://doi.org/10.11646/zootaxa.4282.2.4.

Bonkowski, M., 2019. Microcosm approaches to investigate multitrophic interactions between microbial communities in the rhizosphere of plants. In: Reinhardt, D., Sharma, A.K. (Eds.), Methods in Rhizosphere Biology Research. Springer Singapore, Singapore, pp. 255–270.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. Beast 2: a software platform for bayesian evolutionary analysis. PLoS Computational Biology 10, e1003537. https://doi.org/10.1371/journal.pcbi.1003537.

Bradford, M.A., 2016. Re-visioning soil food webs. Soil Biology and Biochemistry 102, 1–3. https://doi.org/10.1016/j.soilbio.2016.08.010.

Burki, F., Roger, A.J., Brown, M.W., Simpson, A.G.B., 2020. The new tree of eukaryotes. Trends in Ecology & Evolution 1, 43–55. https://doi.org/10.1016/j.tree.2019.08.008.

Clough, K.S., Patrick, Z.A., 1976. Characteristics of the perforating agent of chlamydospores of Thielavlopsis basicola. Berk. & Br.) ferraris. Soil Biology and Biochemistry. https://doi.org/10.1016/0038-0717(76)90088-2.

de Ruiter, P.C., Neutel, A., Moore, J.C., 1995. Energetics, Patterns of Interaction Strengths, and Stability in Real Ecosystems, vol. 269.

Dumack, K., Bonkowski, M., 2021. Protists in the plant microbiome: an untapped field of research. In: The Plant Microbiome: Methods and Protocols, Methods in Molecular Biology, pp. 162–173.

Dumack, K., Fiore-Donno, A.M., Bass, D., Bonkowski, M., 2019. Making sense of environmental sequencing data: ecologically important functional traits of the protistan groups Cercozoa and Endomyxa (Rhizaria). Molecular Ecology Resources 20, 398–403. https://doi.org/10.1111/1755-0998.13112.

Dumack, K., Görzen, D., González-Miguéns, R., Siemensma, F., Lahr, D.J.G., Lara, E., Bonkowski, M., 2020. Molecular investigation of Phryganella acropodia Hertwig et Lesser, 1874 (Arcellinida, Amoebozoa). European Journal of Protistology 75, 125707. https://doi.org/10.1016/j.ejop.2020.125707.

Dumack, K., Kahlich, C., Lahr, D.J.G., Bonkowski, M., 2018a. Reinvestigation of Phryganella paradoxa (Arcellinida, Amoebozoa) penard 1902. The Journal of Eukaryotic Microbiology 66, 232–243. https://doi.org/10.1111/jeu.12665.

Dumack, K., Müller, M., Bonkowski, M., 2016. Description of lecythium terrestris sp. nov. (Chlamydophryidae, cercozoa), a soil dwelling protist feeding on fungi and algae. Protist 572, 51–58. https://doi.org/10.1016/j.protis.2016.01.001.

Dumack, K., Pundt, J., Bonkowski, M., 2018b. Food choice experiments indicate selective fungivorous predation in fisculla terrestris (thecofilosea, cercozoa). The Journal of Eukaryotic Microbiology 66, 525–527. https://doi.org/10.1111/jeu.12680.

Edler, D., Klein, J., Antonelli, A., Silvestro, D., 2021. raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. Methods in Ecology and Evolution 12, 373–377.

Ekelund, F., 1998. Enumeration and abundance of mycophagous protozoa in soil, with special emphasis on heterotrophic flagellates. Soil Biology and Biochemistry 30, 1343–1347. https://doi.org/10.1016/S0038-0717(97)00266-6.

Fiore-Donno, A.M., Richter-Heitmann, T., Bonkowski, M., 2020. Contrasting responses of protistan plant parasites and phagotrophs to ecosystems, land management and soil properties. Frontiers in Microbiology 11, 1–13. https://doi.org/10.3389/fmicb.2020.01823.

Fiore-Donno, A.M., Richter-Heitmann, T., Degrune, F., Dumack, K., Regan, K.M., Marhan, S., Boeddinghaus, R.S., Rillig, M.C., Friedrich, M.W., Kandeler, E., Bonkowski, M., 2019. Functional traits and spatio-temporal structure of a major group of soil protists (Rhizaria: cercozoa) in a temperate grassland. Frontiers in Microbiology 10, 1332. https://doi.org/10.3389/fmicb.2019.01332.

Geisen, S., Koller, R., Hünninghaus, M., Dumack, K., Urich, T., Bonkowski, M., 2015a. The soil food web revisited: diverse and widespread mycophagous soil protists. Soil Biology and Biochemistry 94, 10–18. https://doi.org/10.1016/j.soilbio.2015.11.010.

Geisen, S., Rosengarten, J., Koller, R., Mulder, C., Urich, T., Bonkowski, M., 2015b. Pack hunting by a common soil amoeba on. nematodes 17, 4538–4546. https://doi.org/10.1111/1462-2920.12949.

Gilbert, D., Amblard, C., Bourdier, G., André-Jean, F., Mitchell, E.A.D., 2000. Le régime alimentaire des Thécamoebiens (Protista, Sarcodina). l'Annee Biologique 39, 57–68. https://doi.org/10.1016/S0003-5017(00)80001-X.

Glücksman, E., Bell, T., Griffiths, R.I., Bass, D., 2010. Closely related protist strains have different grazing impacts on natural bacterial communities. Environmental Microbiology 12, 3105–3113. https://doi.org/10.1111/j.1462-2920.2010.02283.x.

Gong, Y., Patterson, D.J., Li, Y., Hu, Z., Sommerfeld, M., Chen, Y., Hu, Q., 2015. Identification and characterization of vernalophrys algivore n. g. n. sp. (Rhizaria: cercozoa: Vampyrellida), a new algal predator isolated from outdoor mass culture of scenedesmus dimorphus. Applied and Environmental Microbiology 81. https://doi.org/10.1128/AEM.00160-15. AEM.00160-15.

Gouy, M., Guindon, S., Gascuel, O., 2010. Sea view version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution 27, 221–224. https://doi.org/10.1093/molbev/msp259.

Hartmann, A., Schmid, M., van Tuinen, D., Berg, G., 2009. Plant-driven selection of microbes. Plant and Soil 321, 235–257. https://doi.org/10.1007/s11104-008-9814-y.

Hassani, M.A., Durán, P., Hacquard, S., 2018. Microbial interactions within the plant holobiont. Microbiome 6, 58. https://doi.org/10.1186/s40168-018-0445-0.

Hedley, R.H., Ogden, C.G., Mordan, N.J., 1950. Biology and fine structure of Cryptodifflugia oviformis (Rhizopodea: Protozoa). Bulletin of the British Museum (Natural History) 26, 269–343.

Hess, S., 2017a. Description of hyalodiscus flabellus sp . nov . (Vampyrellida , Rhizaria) and identification of its bacterial endosymbiont , " candidatus megaira polyxenophila " (rickettsiales , alphaproteobacteria). Annals of Anatomy 168, 109–133. https://doi.org/10.1016/j.protis.2016.11.003.

Hess, S., 2017b. Hunting for Agile Prey : Trophic Specialisation in Leptophryid Amoebae (Vampyrellida , Rhizaria) Revealed by Two Novel Predators of Planktonic Algae, pp. 1–12. https://doi.org/10.1093/femsec/fix104.

Hess, S., Sausen, N., Melkonian, M., 2012. Shedding light on vampires: the phylogeny of vampyrellid amoebae revisited. PLoS One 7, e31165. https://doi.org/10.1371/journal.pone.0031165.

Hess, S., Suthaus, A., 2022. The vampyrellid amoebae (Vampyrellida, Rhizaria). Protist 173, 125854. https://doi.org/10.1016/j.protis.2021.125854.

Holtkamp, R., Wal, A. Van Der, Kardol, P., Putten, W.H. Van Der, Ruiter, P.C. De, Dekker, S.C., 2011. Soil Biology & Biochemistry Modelling C and N mineralisation in soil food webs during secondary succession on ex-arable land. Soil Biology and Biochemistry 43, 251–260. https://doi.org/10.1016/j.soilbio.2010.10.004.

Hoogenraad, H.R., Groot, A.A.D., 1941. Observations on a special manner of feeding of a species of Difflugia (D. rubescens Penard). Proceedings Nederlandse Akademie van Wetenschappen 44, 217–228.

Howe, A.T., Bass, D., Vickerman, K., Chao, E.E., Cavalier-Smith, T., 2009. Phylogeny, taxonomy, and astounding genetic diversity of glissomonadida ord. nov., the dominant gliding zooflagellates in soil (Protozoa: cercozoa). Protist 160, 159–189. https://doi.org/10.1016/j.protis.2008.11.007.

Huang, X., Wang, J., Dumack, K., Liu, W., Zhang, Q., He, Y., Di, H., Bonkowski, M., Xu, J., Li, Y., 2021. Protists modulate fungal community assembly in paddy soils across climatic zones at the continental scale. Soil Biology and Biochemistry 160, 108358. https://doi.org/10.1016/j.soilbio.2021.108358.

Hünninghaus, M., Dibbern, D., Kramer, S., Koller, R., Pausch, J., Schloter-Hai, B., Urich, T., Kandeler, E., Bonkowski, M., Lueders, T., 2019. Disentangling carbon flow across microbial kingdoms in the rhizosphere of maize. Soil Biology and Biochemistry 134, 122–130. https://doi.org/10.1016/j.soilbio.2019.03.007.

Hunt, H.W., Coleman, D.C., Ingham, E.R., Ingham, R.E., Elliott, E.T., Moore, J.C., Rose, S. L., Reid, C.P.P., Morley, C.R.I., 1987. The Detrital Food Web in a Shortgrass Prairie, pp. 57–68. https://doi.org/10.1007/bf00260580.

Jassey, V.E.J., Shimano, S., Dupuy, C., Toussaint, M.-L., Gilbert, D., 2012. Characterizing the feeding habits of the testate amoebae hyalosphenia papilio and nebela tincta along a narrow "fen-bog" gradient using digestive vacuole content and 13C and 15N isotopic analyses. Protist 163, 451–464. https://doi.org/10.1016/j.protis.2011.07.006.

Junker, C., Draeger, S., Schulz, B., 2012. A fine line - endophytes or pathogens in Arabidopsis thaliana. Fungal Ecology 5, 657–662. https://doi.org/10.1016/j.funeco.2012.05.002.

Kramer, S., Dibbern, D., Moll, J., Huenninghaus, M., Koller, R., Krueger, D., Marhan, S., Urich, T., Wubet, T., Bonkowski, M., Buscot, F., Lueders, T., Kandeler, E., 2016. Resource partitioning between bacteria, fungi, and protists in the detritusphere of an agricultural soil. Frontiers in Microbiology 7, 1–12. https://doi.org/10.3389/fmicb.2016.01524.

Krashevska, V., Klarner, B., Widyastuti, R., Maraun, M., Scheu, S., 2016. Changes in structure and functioning of protist (testate amoebae) communities due to conversion of lowland rainforest into rubber and oil palm plantations. PLoS One 11, e0160179. https://doi.org/10.1371/journal.pone.0160179.

Mcfadden, G., Melkonian, M., 1986. Use of Hepes buffer for micro algal culture media and fixation for electron microscopy. Phycologia 25, 551–557.

Medlin, L., Elwood, H.J., Stickel, S., Sogin, M.L., 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71, 491–499. https://doi.org/10.1016/0378-1119(88)90066-2.

Micallef, S.A., Shiaris, M.P., Colón-Carmona, A., 2009. Influence of Arabidopsis thaliana accessions on rhizobacterial communities and natural variation in root exudates. Journal of Experimental Botany 60, 1729–1742. https://doi.org/10.1093/jxb/erp053.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop. GCE 2010. https://doi.org/10.1109/GCE.2010.5676129.

Moore, J.C., Walter, D.E., William, H., 1988. Arthropod regulation of micro- and mesobiota in below-ground detrital food webs. Annual Review of Entomology 33, 419–439. https://doi.org/10.1146/annurev.en.33.010188.002223.

More, K., Simpson, A.G.B., Hess, S., 2021. Description of the marine predator *Sericomyxa perlucida* gen. et sp. nov., a cultivated representative of the deepest branching lineage of vampyrellid amoebae (Vampyrellida, Rhizaria). The Journal of Eukaryotic Microbiology 68. https://doi.org/10.1111/jeu.12864.

More, K., Simpson, A.G.B., Hess, S., 2018. Two New Marine Species of Placopus (Vampyrellida , Rhizaria) that Perforate the Theca of Tetraselmis, pp. 1–14. https://doi.org/10.1111/jeu.12698.

Moye, J., Schenk, T., Hess, S., 2022. Experimental evidence for enzymatic cell wall dissolution in a microbial protoplast feeder (Orciraptor agilis, Viridiraptoridae). BMC Biology. https://doi.org/10.1186/s12915-022-01478-x.

Ogden, C.G., Pitta, P., 1990. Biology and ultrastructure of the mycophagus, soil testate amoeba, Phryganella acropodia (Rhizopoda, Protozoa). Biology and Fertility of Soils 9, 101–109.

Old, K.M., 1978. Fine structure of perforation of Cochliobolus sativus conidia by giant amoebae. Soil Biology and Biochemistry 10, 509–516.

Old, K.M., 1977. Giant soil amoebae cause perforation of conidia of Cochliobolus satious. Transactions of the British Mycological Society 68, 277–281.

Old, K.M., 1967. Effects of natural soil on survival of Cochliobolus sativus. Transactions of the British Mycological Society 50, 615. https://doi.org/10.1016/S0007-1536(67)80093-7. IN2.

Old, K.M., Wong, J.N.F., 1976. Perforation and lysis of fungal spores in natural soils. Soil Biology and Biochemistry. https://doi.org/10.1016/0038-0717(76)90058-4.

Pakzad, U., 2003. Untersuchungen einer mykophagen vampiramöbe.

Petz, W., Foissner, W., Adam, H., 1985. ULTURE, FOOD SELECTION AND GROWTH RATE IN THE MYCOPHAGOUS CILIATE GROSSGLOCKNERIA ACUTA FOISSNER, 1980: FIRST EVIDENCE OF AUTOCHTHONOUS SOIL CILIATES, vol. 17, pp. 871–875.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in bayesian phylogenetics using tracer 1.7. Systematic Biology 67, 901–904. https://doi.org/10.1093/sysbio/syy032.

Ren, P., Sun, A., Jiao, X., Shen, J.-P., Yu, D.-T., Li, F., Wu, B., He, J.-Z., Hu, H.-W., 2023. Predatory protists play predominant roles in suppressing soil-borne fungal pathogens under organic fertilization regimes. Science of the Total Environment 863, 160986. https://doi.org/10.1016/j.scitotenv.2022.160986.

Richardson, L.A., 2017. Evolving as a holobiont. PLoS Biology 15, 4–7. https://doi.org/10.1371/journal.pbio.2002168.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. https://doi.org/10.1093/bioinformatics/btg180.

Sayre, R.M., 1973. Theratromyxa weberi, an amoeba predatory on plant-parasitic nematodes. Journal of Nematology 5, 258–264.

Schwelm, A., Badstöber, J., Bulman, S., Desoignies, N., Etemadi, M., Falloon, R.E., Gachon, C.M.M., Legreve, A., Lukes, J., Merz, U., Nenarokova, A., Strittmatter, M., Sullivan, B.K., Neuhauser, S., 2018. Not in your usual Top 10 : protists that infect plants and algae. Molecular Plant Pathology 19, 1029–1044. https://doi.org/10.1111/mpp.12580.

Siemensma, F., Opitz, A.M., 2014. Beobachtungen an Pseudonebela africana, einer seltenen, doch weltweit verbreiteten Schalenamöbe. Mikrokosmos 103, 248–255.

Solbach, M.D., Bonkowski, M., Dumack, K., 2021. Novel endosymbionts in rhizarian amoebae imply universal infection of unrelated free-living amoebae by legionellales.

Frontiers in Cellular and Infection Microbiology 11, 642216. https://doi.org/10.3389/fcimb.2021.642216.

Stamatakis, A., 2014. RAxML Version 8 : a Tool for Phylogenetic Analysis and Post-analysis of Large Phylogenies, vol. 30, pp. 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Trap, J., Bonkowski, M., Plassard, C., Villenave, C., Blanchart, E., 2016. Ecological importance of soil bacterivores for ecosystem functions. Plant and Soil 398, 1–24. https://doi.org/10.1007/s11104-015-2671-6.

Urbina, H., Breed, M.F., Zhao, W., Lakshmi Gurrala, K., Andersson, S.G.E., Ågren, J., Baldauf, S., Rosling, A., 2018. Specificity in Arabidopsis thaliana recruitment of root fungal communities from soil and rhizosphere. Fungal Biology 122, 231–240. https://doi.org/10.1016/j.funbio.2017.12.013.

Weber, A., Zwillenberg, L., van der Laan, P., 1952. A predacious amoeboid organism destroying larvae of the potato root eelworm and other nematodes. Nature 169, 834–835.

Xiong, W., Song, Y., Yang, K., Gu, Y., Wei, Z., Kowalchuk, G.A., Xu, Y., Jousset, A., Shen, Q., Geisen, S., 2020. Rhizosphere protists are key determinants of plant health. Microbiome 8, 27. https://doi.org/10.1186/s40168-020-00799-9.

Yeates, G.W., Foissner, W., 1995. Testate amoebae as predators of nematodes. Biology and Fertility of Soils 20, 1–7. https://doi.org/10.1007/BF00307834.

Zhang, H., Patterson, D.J., He, Y., Wang, H., Yuan, D., Hu, Q., Gong, Y., 2022. *Kinopus chlorellivorus* gen. Nov., sp. nov. (Vampyrellida, Rhizaria), a new algivorous protist predator isolated from large-scale outdoor cultures of *Chlorella sorokiniana*. Applied and Environmental Microbiology 88, e01215-e01222. https://doi.org/10.1128/aem.01215-22.

Addendum: Further publications

The following publications were produced during the time of this doctoral work but are unrelated to the Jena Experiment and are therefore not presented in full in this dissertation. They address related questions on plant–microbe interactions or on the ecology of the focal microbial groups (Oomycota and Cercozoa) across a range of ecosystems, thereby providing complementary context to the grassland-based results presented in the main chapters. For the articles and any included supplementary material, readers are referred to the respective online versions of the publications.

Addendum 1:

Masigol H*, Solbach MD*, Pourmoghaddam MJ, Ahadi R, Mostowfizadeh-Ghalamfarsa R, Taheri SR, Tobias-Hünefeldt SP, Bonkowski M, Grossart H-P. 2025. A glimpse into *Oomycota* diversity in freshwater lakes and adjacent forests using a metabarcoding approach. *Scientific Reports* **15**: 19124. https://doi.org/10.1038/s41598-025-01727-3

*Masigol H and **Solbach MD** contributed equally to this work.

This study broadens the ecological scope of the thesis by extending the investigation of Oomycota beyond the semi-natural grassland systems of the Jena Experiment to freshwater lakes and their adjacent forests. By applying the same DNA-based metabarcoding framework as used in Chapters 1 and 2, this work enables a direct methodological comparison across contrasting ecosystems and highlights shared as well as ecosystem-specific patterns of Oomycota diversity and community composition. The inclusion of freshwater and forest habitats provides complementary insights into the environmental breadth, habitat specificity, and potential dispersal pathways of Oomycota, thereby placing the grassland-focused findings of the Jena Experiment into a broader ecological context.

Declaration of personal contributions:
The study was conceived by H Masigol, H-P Grossart, and R Mostowfizadeh-Ghalamfarsa, and H Masigol, SP Tobias-Hünefeldt, and H-P Grossart designed the sampling. I performed the laboratory work for the metabarcoding together with H Masigol. I performed the bioinformatic data processing and statistical analyses, and contributed to illustrating the figures. I wrote the majority of the original draft together with H Masigol (shared first-authorship).

Addendum 2:

Solbach MD, Bonkowski M, Dumack K. 2021. Novel endosymbionts in rhizarian amoebae imply universal infection of unrelated free-living amoebae by Legionellales. *Frontiers in Cellular and Infection Microbiology* **11**: 642216. https://doi.org/10.3389/fcimb.2021.642216

This work illustrates that cercozoan protists, beyond their roles as consumers or plant pathogens, can also serve as hosts for intracellular bacterial symbionts. By documenting novel endosymbionts in Thecofilosea strains, many of which were isolated from soil or plant material, this study highlights an additional layer of interactions between bacteria and protists, offering broader insights into the ecological complexity of protist communities in plant-associated environments.

Declaration of personal contributions:
This work is based on my Master's Thesis (submitted in 2019 at the University of Cologne), guided and conceived by K Dumack, and formally supervised by M Bonkowski. I isolated the majority of novel Thecofilosea strains, performed the sequencing of Thecofilosea and their endosymbiotic bacteria, designed the FISH (fluorescence *in situ* hybridization) probes, and performed the FISH staining, including imaging via light microscopy, all with guidance from K Dumack. Phylogenetic analyses were performed together with K Dumack. I wrote the original draft of the study (Master's Thesis), which was then revised by K Dumack and M Bonkowski. I handled the submission and the review process of the article.

Addendum 3:

Solbach MD, Bonkowski M, Dumack K. 2025. *Katarium polorum* n. sp., n. g., a novel thecofilosean amoeba (Cercozoa, Rhizaria) from the polar oceans. *Journal of Eukaryotic Microbiology* **72**(2): e13071. https://doi.org/10.1111/jeu.13071

This study provides an example of a full taxonomic description of a novel cercozoan genus and species within the class Thecofilosea. Although *Katarium polorum* originates from polar marine waters rather than a plant-associated environment, the methodological framework used for its isolation, cultivation, sequencing, phylogenetic placement, and morphological diagnosis is broadly applicable to protists in general. The study exemplifies how future work could use novel sequences detected by metabarcoding in plant-associated ecosystems from the Jena Experiment to locate

and isolate previously undescribed protists, which could then be formally described, as exemplified in this study.

Declaration of personal contributions:
I isolated, cultivated, and sequenced the first of the two strains of the novel organism *Katarium polorum* (strain CCAP 1938/1). Phylogenetic analysis was performed together with K Dumack. I performed the imaging via light microscopy, illustrated the organism, formally described the organism, and wrote the initial manuscript. K Dumack and M Bonkowski revised the manuscript. I handled the submission and the review process of the article.

General Discussion

Synthesis of Main Findings

This thesis investigated the role of microbes with a focus on protist plant pathogens as components of soil legacy effects along the plant diversity gradient of the long-term Jena Experiment. Chapter 1 demonstrated that repeated summer droughts as well as plant diversity induce long-lasting soil legacy effects on protistan communities, with droughts enriching plant species-specific parasites (H2). These results suggest that drought weakens plant defense and thereby promotes persistent pathogen legacies, and also creates persistent shifts in cercozoan consumer communities (Solbach *et al.*, 2025a). Chapter 2 showed that plant-induced soil microbial legacies strengthen with time, driven by both plant species identity and plant species richness (H3). After 19 years of shared history, soil microbial community shifts were substantially stronger than after only five years, and they were largely unaffected by the replacement of the plant individuals. These legacies were characterized not only by enrichment but also by widespread reduction of microbial taxa, proposing microbial suppression as an overlooked mechanism underlying long-term plant–soil feedbacks. Chapter 3 introduced the JenaTron Experiment to disentangle plant and soil history effects along a reduced plant diversity gradient in a fully crossed indoor experiment (Madaj *et al.*, 2025). Finally, Chapter 4 demonstrated that protistan consumers actively feed on fungi in the rhizosphere using distinct feeding strategies (Estermann *et al.*, 2023), thereby potentially shaping the microbiome and legacy effects.

Directionality and Causality in BEF–Microbiome Research

Linking the observed patterns in microbial communities to BEF relationships is not trivial, largely because plant–soil feedbacks are inherently reciprocal: Plants shape soil microbial communities through root exudation, litter inputs, changes in resource availability, etc., while soil microbes in turn influence plant performance (Kulmatiski *et al.*, 2008; van der Putten *et al.*, 2013), making it difficult to disentangle cause and effect using observational data alone. In the Jena Experiment, plant species richness is the primary manipulated factor (Roscher *et al.*, 2004), and the present analyses therefore predominantly capture directional effects of plant communities on microbial assemblages. However, because sampling was conducted after several years of

continuous plant–soil interaction, microbial feedbacks will also have contributed to shaping plant community composition and productivity, which then further modified soil microbial communities.

Experimental designs that directly manipulate soil communities are required to more explicitly assess microbial effects on plant performance (Berlinches de Gea *et al.*, 2023; Guo *et al.*, 2024). Such approaches are partially implemented in the ΔBEF (Vogel *et al.*, 2019) and JenaTron (Chapter 3 (Madaj *et al.*, 2025)) experiments, where soils differing in conditioning history were exchanged, including all biotic and abiotic soil legacies. While this holistic manipulation reflects ecological realism, it precludes isolating the effects of individual microbial taxa or functional groups.

Soil legacy effects likely reflect the cumulative outcome of multiple interacting biotic and abiotic processes rather than isolated microbial mechanisms. Plant communities alter soil nutrient availability and organic matter content (Dybzinski *et al.*, 2008; Lange *et al.*, 2015; Furey & Tilman, 2021), soil structure (Leff *et al.*, 2018), moisture (Caldwell *et al.*, 1998), temperature (Huang *et al.*, 2023), pH (Hinsinger *et al.*, 2003), etc. All these processes occur simultaneously with biotic changes in communities of nematodes (Dietrich *et al.*, 2021), earthworms (Eisenhauer *et al.*, 2009), arthropods (Ebeling *et al.*, 2018), microbes (Eisenhauer *et al.*, 2010; Sasse *et al.*, 2018; Albracht *et al.*, 2023; Albracht *et al.*, 2024; de Souza *et al.*, 2024; Hennecke *et al.*, 2025), etc., all potentially contributing to BEF relationships (Eisenhauer *et al.*, 2024; Li *et al.*, 2024), making it extremely difficult to disentangle individual drivers.

PSF studies are often quantified exclusively from the plant perspective, such that measured effects on plant performance reflect only the net outcome of multiple, potentially opposing soil-mediated processes (van der Putten *et al.*, 2013). Positive feedbacks driven by the accumulation of mutualists, such as arbuscular mycorrhizal fungi or plant growth–promoting bacteria, may therefore occur simultaneously with negative feedbacks caused by the build-up of soil-borne antagonists, such as parasitic protists, fungi, nematodes, or insects, leading to partial or complete cancellation of their effects and resulting in apparently neutral PSFs. Consequently, neutral feedbacks do not necessarily imply the absence of soil conditioning or legacy formation, but could potentially indicate that positive and negative microbial influences balance out each other at the level of plant performance. Similarly, the observation of changes in

microbial communities and the formation of microbial legacies does not necessarily imply the presence of positive or negative PSFs.

Interpretation is further complicated by the limited taxonomic and functional resolution of many detected Cercozoa and Oomycota, because a substantial fraction of sequences obtained from metabarcoding often show low sequence similarity to described species in reference databases (Jauss *et al.*, 2021; Walden *et al.*, 2021). As most functional knowledge on plant-associated parasites is derived from crop systems (Neuhauser *et al.*, 2014; Kamoun *et al.*, 2015; Schwelm *et al.*, 2018), predicting host specificity and pathogenicity for grassland-associated taxa remains highly uncertain. Addressing these limitations will require more targeted approaches, including plant species-specific sampling of rhizosphere and root-associated microbiomes. Future work could use novel sequences detected by metabarcoding to locate, isolate, cultivate, and describe previously undescribed protists (as exemplified in Addendum 3 (Solbach *et al.*, 2025b)). Isolated parasites could then be used to investigate host ranges and pathogenic potential by infecting putative host plants, and consumers could be used to assess their effects on plant growth and disease suppression in laboratory experiments.

Experimental Trade-offs: From the Field to the JenaTron

An important caveat of the ΔBEF experiment (Vogel *et al.*, 2019) is that the soil used for the novel interactions (−SH −PH) treatment originated from an adjacent, intensively managed agricultural field that had been regularly fertilized. Consequently, differences between short (−SH; 5 years) and long (+SH; 19 years) soil history treatments confound plant-driven microbial legacies caused by the plants from the Jena Experiment with legacies from previous agricultural history and nutrient status. Rather than constituting a methodological flaw or shortcoming, this design mirrors the starting conditions of the original main plots of the Jena Experiment, which were likewise established on former agricultural land (Roscher *et al.*, 2004), and thus captures an ecologically realistic trajectory of an ecosystem transitioning from fertilized cropland toward a (semi-natural) grassland (hay meadow). Nevertheless, differences in soil texture between the experimental field and the adjacent agricultural field, particularly in sand and clay content, may have contributed to treatment effects unrelated to soil community history. In addition, while soils and plant communities in the coupled history

(+SH +PH) treatment remained undisturbed, the removal of the plant sod and soil mixing in the treatment with only soil history (+SH −PH) as well as novel interactions (−SH −PH) likely disrupted the soil structure including physical features such as root channels and earthworm burrows, and partially disturbed biotic legacies by redistributing microbial resting stages and plant-derived compounds to lower soil depths.

In the main field experiment (Roscher *et al.*, 2004), the plant communities were initially established with predefined species compositions and sowing densities to ensure equal starting abundances among species. Over time, however, relative abundances naturally diverged as a consequence of competitive interactions, environmental filtering, and stochastic processes, leading to shifts in dominance and local extinctions (Weisser *et al.*, 2017).

The abovementioned limitations were explicitly addressed in the JenaTron Experiment (Chapter 3 (Madaj *et al.*, 2025)), which used soils originating exclusively from the Jena Experiment field site for both soil history treatments and implements the previously missing -SH +PH combination by using seeds obtained from plants with growth history from the Trait-Based Experiment (Ebeling *et al.*, 2014), thereby enabling a fully crossed design that better disentangles soil and plant history effects. By standardizing plant density during treatment establishment, the JenaTron Experiment further minimized the confounding effects of plant abundance and relative dominance.

This refined experimental control comes at the cost of reduced plant diversity, with only six species representing two functional groups and a smaller total number of plant communities (24 EcoUnits in the JenaTron vs. 80 plots in the main Jena Experiment). However, the destructive final harvest allowed for targeted sampling of roots and rhizosphere soil, providing a level of resolution that was not achievable in the bulk-soil sampling of the ΔBEF experiment.

Plant Species Richness and the Dilution Effect

The expectation that increasing plant diversity dilutes host-specific pathogens (H1) was only partially supported by the data. This hypothesis assumes high host specificity and an increasing number of incompetent hosts with higher species diversity (Ostfeld & Keesing, 2000; Mitchell *et al.*, 2002). This assumption appears to be only partially

met for the parasites investigated in the present thesis, providing a plausible explanation for the absence of consistent alpha-diversity responses to plant species richness. Therefore, differences among microbial groups likely reflect contrasting lifestyle strategies. While plant diversity clearly structured pathogen community composition, consistent reductions in dominance or increases in evenness of pathogenic taxa were not observed across all parasite groups.

Among the investigated taxa, only Phytomyxea showed a reproducible increase in evenness with increasing plant species richness (Chapters 1 and 2). However, the host ranges and pathogenic potential of most phytomyxeans remain poorly resolved (Neuhauser *et al.*, 2014), limiting firm conclusions about their degree of host specificity. Phytomyxea are obligate biotrophs (Neuhauser *et al.*, 2014; Bulman & Neuhauser, 2017; Hittorf *et al.*, 2020), whereas the majority of oomycetes detected in this thesis belonged to the hemibiotrophic genera *Pythium sensu stricto* and *Globisporangium* (order Pythiales), which can alternate between saprotrophic growth on dead plant material and opportunistic infection of living hosts (Uzuhashi *et al.*, 2010; Beakes & Thines, 2016; Thines & Choi, 2016). Although the class Peronosporomycetes in Oomycota is generally well studied due to its role as plant pathogens, the near absence of obligate biotrophs such as *Albugo* or *Peronospora* is notable. Comparable metabarcoding studies using the same primer sets as used in this thesis (Fiore-Donno & Bonkowski, 2021) across grasslands, forests, and freshwater systems recovered substantial proportions of Saprolegniales (class Saprolegniomycetes) and Peronosporales in addition to Pythiales (Fiore-Donno & Bonkowski, 2021; Addendum 1 (Masigol *et al.*, 2025); Wakelin *et al.*, 2025), indicating that the observed pattern reflects a true ecological signal rather than a methodological artifact. Although *Pythium* and *Globisporangium* are often described as opportunistic pathogens with broad host ranges (Thines & Kamoun, 2010; Uzuhashi *et al.*, 2010), differences in host selectivity (van der Plaats-Niterink, 1981; Mitchell & Deacon, 1986; Augspurger & Wilkinson, 2007) and pathogenicity (Boie *et al.*, 2024) have also been documented for several species. In line with this, plant functional group identity influences oomycete communities (Chapter 1 (Solbach *et al.*, 2025a)), suggesting a higher degree of host preferences than commonly assumed.

Additionally, plant species are known to structure the entire soil microbiome, including bacteria, fungi, and heterotrophic protists, with recent studies providing increasing

evidence that non-pathogenic microbes also form plant species- or plant functional group-specific consortia (Berg *et al.*, 2014; Fitzpatrick *et al.*, 2018; Sasse *et al.*, 2018; Rüger *et al.*, 2021; Dumack *et al.*, 2022; Albracht *et al.*, 2023; d'Entremont & Kivlin, 2023; Hennecke *et al.*, 2023; Solbach *et al.*, 2025a; Wang *et al.*, 2025). This raises the question of why dilution effects should be limited to parasites/pathogens, rather than representing a general property of plant-associated microbial communities. Consistent with this view, we found increases in evenness also in non-plant-pathogenic fungi and bacteria (Chapter 2).

Taken together, while this thesis provides evidence consistent with dilution effects for certain microbial taxa, it also highlights important constraints of applying community-wide alpha-diversity metrics to test this concept. Microbial communities consist of mixtures of taxa with heterogeneous lifestyles and host preferences, and accordingly, the concept of dilution effects has been increasingly questioned (Randolph & Dobson, 2012; Salkeld *et al.*, 2013). Consequently, alpha diversity alone appears to be a weak predictor of dilution effects. Future studies should focus on resolving host ranges and life-history strategies of individual microbial taxa and explicitly test how these taxa respond to the plant diversity gradient.

Protist Plant Pathogens as Drivers of Eco-Evolutionary Adaptations

Soil-borne protistan plant pathogens may contribute to eco-evolutionary dynamics in plant communities by imposing persistent and species-specific selection pressure on the plants (H4). In long-established plant communities, the gradual accumulation of soil legacies of host-specific pathogens is expected to increase selection pressure towards enhanced plant defense traits against these pathogens. In contrast, recently established communities of naïve plants in conditioned soil (soil history; +SH −PH) may lack such eco-evolutionary adaptations. In line with this expectation, we observed an increased effect of plant species richness and an increased number of differentially abundant operational taxonomic units affiliated with the oomycete genus *Pythium* in old soils with new plants (Chapter 2, Figs. 3-5), which may indicate enhanced susceptibility of naïve plants to opportunistic infection from accumulated oospores under soil legacy conditions. Notably, comparable patterns were not detected for *Globisporangium* oomycetes, Phytomyxea, or plant-pathogenic fungi, suggesting that potential eco-evolutionary responses may be taxon-specific. However, since we did

not investigate plant defense traits, these observations should be interpreted as indicative of potential eco-evolutionary feedbacks rather than evidence, suggesting a promising direction for future research.

Protistan Consumers as Underappreciated Modulators of Soil Legacies?

Protistan consumers remain an overlooked component in studies on BEF relationships, despite their central role in the release of mineral nutrients through predation on bacteria, fungi, and other microeukaryotes (Coleman, 1994; Bonkowski *et al.*, 2000; Bonkowski, 2004; Fenchel, 2008; Geisen *et al.*, 2018). Although cercozoan consumers responded to the plant species richness gradient in both the Drought (Chapter 1 (Solbach *et al.*, 2025a)) and ΔBEF (Chapter 2) experiments (H5), these effects were generally less consistent than those observed for parasitic protists. Notably, the effect of plant diversity on cercozoan alpha diversity differed between experiments, with increasing inverse Simpson index observed along the plant richness gradient in the Drought Experiment (see Fig. 2 in Chapter 1 (Solbach *et al.*, 2025a)) but no significant relationship in the ΔBEF Experiment (see Fig. 6 in Chapter 2). Furthermore, cercozoan patterns in alpha diversity from the Drought Experiment (Solbach *et al.*, 2025a) matched those of bacteria (de Souza *et al.*, 2024), suggesting a tight association between bacterivorous Cercozoa and their potential prey. However, in the ΔBEF Experiment (Chapter 2), cercozoan and bacterial patterns did not match. As these experiments were sampled in different years and seasons (Drought Experiment: August 2017; ΔBEF Experiment: May/June 2021), these contrasting patterns likely reflect annual or seasonal fluctuations (Herold *et al.*, 2020; Walden *et al.*, 2021), such as differences in temperature (Tsyganov *et al.*, 2011), precipitation (Zhao *et al.*, 2023), or plant phenology (Bonato Asato *et al.*, 2023), likely related to root exudation (Gao *et al.*, 2024; Wannenmacher *et al.*, 2025). It is noteworthy that some cercozoan consumers can host bacterial endosymbionts (Addendum 2 (Solbach *et al.*, 2021)), suggesting that predation is not the only pathway by which the patterns of cercozoa and bacteria are linked.

While protistan consumers also have the capacity to form resting stages (Ekelund, 1996; Foissner, 2011; Schaap & Schilde, 2018), they appear to be more strongly influenced by short-term fluctuations in the active fraction of organisms (H5). In

contrast, oomycete and phytomyxean communities exhibited more consistent responses across experiments, supporting the idea that long-term accumulation of resting stages is the strongest driver of their patterns (H3).

In Chapters 1 and 2, protistan consumers were represented only by Cercozoa, whereas soil protistan consumers comprise a much broader phylogenetic spectrum, especially Amoebozoa and Ciliophora (Geisen *et al.*, 2018; Oliverio *et al.*, 2020; Fiore-Donno *et al.*, 2024). Cercozoan consumers therefore represent only a subset of the total predatory microeukaryote diversity in soils, and more comprehensive surveys of soil microeukaryotes would be needed to fully capture consumer-driven processes in the Jena Experiment. Approaches targeting the metabolically active fraction of the whole microbial community, such as RNA-based metabarcoding or metatranscriptomics, may be particularly informative in this context.

Cercozoan consumers such as members of the Thecofilosea have been shown to consume unicellular fungi and fungal spores (Dumack *et al.*, 2016; Dumack *et al.*, 2019). However, one of the most abundant consumer groups detected in our cercozoan datasets was the order Vampyrellida. Vampyrellida are predators and grazers of a wide range of microeukaryotes, including other protists and even small metazoans such as nematodes (Weber *et al.*, 1952; Hess *et al.*, 2012; Hess & Suthaus, 2022). Feeding on fungal and oomycete hyphae and spores has been reported for Vampyrellida as well as Amoebozoa (Old & Darbyshire, 1978; Chakraborty & Old, 1982; Chakraborty *et al.*, 1983; Ogden & Pitta, 1990), a pattern consistent with the observations from feeding experiments on fungi shown in Chapter 4 (Estermann *et al.*, 2023). Protistan consumers may act as top-down regulators of microbial community composition (Nguyen *et al.*, 2023; Xue *et al.*, 2023; Bodur *et al.*, 2024; Degrune *et al.*, 2024; Guo *et al.*, 2024), thereby potentially shaping, buffering, and eroding soil legacies over time.

Outlook

This thesis demonstrates that protists, including plant parasites and consumers, are integral but still underappreciated components of plant–soil feedbacks and biodiversity research. Disentangling the effects of plant species identity and plant species richness remains challenging, yet resolving species- and functional group-specific plant–microbe interactions is essential for predicting microbiome assembly in plant mixtures.

Given the accumulating evidence that individual plant species and functional groups harbor distinct microbial consortia, approaches focusing solely on the plant diversity gradient are oversimplifying the underlying mechanisms. Future research should therefore prioritize the targeted characterization of microbiomes associated with individual plant species and functional groups by combining taxon-specific sequencing with root- and rhizosphere-focused sampling and isolation experiments to identify key taxa and their ecological roles, and subsequently assess how these taxa respond to increasing plant diversity. Integrating protists into the complex BEF frameworks will be essential for developing a more complete understanding of how biodiversity shapes ecosystem functioning over long timescales.

General References

References for the General Introduction and General Discussion.

Adams MJ, Swaby AG, Jones P. 1988. Confirmation of the transmission of barley yellow mosaic virus (BaYMV) by the fungus *Polymyxa graminis*. *Annals of Applied Biology* **112**: 133-141.

Adl MS, Gupta VVSR. 2006. Protists in soil ecology and forest nutrient cycling. *Canadian Journal of Forest Research* **36**(7): 1805-1817.

Adl SM, Bass D, Lane CE, Lukes J, Schoch CL, Smirnov A, Agatha S, Berney C, Brown MW, Burki F, et al. 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. *Journal of Eukaryotic Microbiology* **66**(1): 4-119.

Adl SM, Simpson AG, Lane CE, Lukes J, Bass D, Bowser SS, Brown MW, Burki F, Dunthorn M, Hampl V, et al. 2012. The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology* **59**(5): 429-493.

Aist JR, Williams PH. 1971. The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. *Canadian Journal of Botany* **49**(11): 2023-2034.

Albracht C, Eisenhauer N, Vogel A, Wagg C, Buscot F, Heintz-Buschart A. 2023. Effects of recurrent summer droughts on arbuscular mycorrhizal and total fungal communities in experimental grasslands differing in plant diversity and community composition. *Frontiers in Soil Science* **3**: 1129845.

Albracht C, Solbach MD, Hennecke J, Bassi L, van der Ploeg GR, Eisenhauer N, Weigelt A, Buscot F, Heintz-Buschart A. 2024. Common soil history is more important than plant history for arbuscular mycorrhizal community assembly in an experimental grassland diversity gradient. *Biology and Fertility of Soils* **60**: 547–562.

Augspurger CK, Wilkinson HT. 2007. Host Specificity of Pathogenic *Pythium* Species: Implications for Tree Species Diversity. *Biotropica* **39**(6): 702-708.

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F. 1983. The Ecological Role of Water-Column Microbes in the Sea. *Marine Ecology Progress Series* **10**: 257-263.

Bachy C, Hehenberger E, Ling Y-C, Needham DM, Strauss J, Wilken S, Worden AZ 2022. Marine Protists: A Hitchhiker's Guide to their Role in the Marine Microbiome. In: Stal LJ, Cretoiu MS eds. *The Marine Microbiome, Second Edition*, 159-241.

Bakker MG, Manter DK, Sheflin AM, Weir TL, Vivanco JM. 2012. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant and Soil* **360**(1-2): 1-13.

Balvanera P, Pfisterer AB, Buchmann N, He JS, Nakashizuka T, Raffaelli D, Schmid B. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters* **9**(10): 1146-1156.

Bardgett RD, van der Putten WH. 2014. Belowground biodiversity and ecosystem functioning. *Nature* **515**(7528): 505-511.

Beakes GW, Glockling SL, Sekimoto S. 2012. The evolutionary phylogeny of the oomycete "fungi". *Protoplasma* **249**(1): 3-19.

Beakes GW, Thines M 2016. Hyphochytriomycota and Oomycota. In: Archibald JM, Simpson AGB, Slamovits CH eds. *Handbook of the Protists*. Cham: Springer, 435-505.

Bengtsson J, Bullock JM, Egoh B, Everson C, Everson T, O'Connor T, O'Farrell PJ, Smith HG, Lindborg R. 2019. Grasslands—more important for ecosystem services than you might think. *Ecosphere* **10**(2): e02582.

Bennett JA, Klironomos J. 2019. Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. *New Phytologist* **222**(1): 91-96.

Bennett JA, Maherali H, Reinhart KO, Lekberg Y, Hart MM, Klironomos J. 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* **355**: 181-184.

Berendsen RL, Pieterse CM, Bakker PA. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science* **17**(8): 478-486.

Berg G, Grube M, Schloter M, Smalla K. 2014. Unraveling the plant microbiome: looking back and future perspectives. *Frontiers in Microbiology* **5**: 148.

Bergmann J, Verbruggen E, Heinze J, Xiang D, Chen B, Joshi J, Rillig MC. 2016. The interplay between soil structure, roots, and microbiota as a determinant of plant–soil feedback. *Ecol Evol* **6**(21): 7633-7644.

Berlinches de Gea A, Li G, Chen JO, Wu W, Kohra A, Aslan SK, Geisen S. 2023. Increasing soil protist diversity alters tomato plant biomass in a stress-dependent manner. *Soil Biology and Biochemistry* **186**: 109179.

Bever JD. 1994. Feedback between plants and their soil communities in an old field community. *Ecology* **75**(7): 1965-1977.

Bever JD. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist* **157**(3): 465-473.

Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M. 2010. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution* **25**(8): 468-478.

Bever JD, Westover KM, Antonovics J. 1997. Incorporating the Soil Community into Plant Population Dynamics: The Utility of the Feedback Approach. *Journal of Ecology* **85**(5): 561-573.

Bircher U, Hohl HR. 1997. Environmental signalling during induction of appressorium formation in *Phytophthora*. *Mycological Research* **101**(4): 395-402.

Blagodatskaya E, Kuzyakov Y. 2013. Active microorganisms in soil: Critical review of estimation criteria and approaches. *Soil Biology and Biochemistry* **67**: 192-211.

Bodur SO, Suzuki K, Harada N, Asiloglu R. 2024. Top-down predators shape soil bacterial community composition while bottom-up nutrients drive bacterial abundance. *Science of the Total Environment* **957**: 177634.

Bodył A. 2005. Do Plastid-Related Characters Support the Chromalveolate Hypothesis? *Journal of Phycology* **41**(3): 712-719.

Boie W, Schemmel M, Ye W, Hasler M, Goll M, Verreet JA, Cai D. 2024. An assessment of the species diversity and disease potential of *Pythium* communities in Europe. *Nature Communications* **15**(1): 8369.

Bonato Asato AE, Wirth C, Eisenhauer N, Hines J. 2023. On the phenology of soil organisms: Current knowledge and future steps. *Ecology and Evolution* **13**(4): e10022.

Bonkowski M. 2004. Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist* **162**(3): 617-631.

Bonkowski M, Cheng W, Griffiths BS, Alphei J, Scheu S. 2000. Microbial-faunal interactions in the rhizosphere and effects on plant growth. *European Journal of Soil Biology* **36**(3-4): 135−147.

Bonkowski M, Dumack K, Fiore-Donno AM 2019. The Protists in Soil—A Token of Untold Eukaryotic Diversity. In: van Elsas JD, Trevors JT, Soares Rosado A,

Nannipieri P eds. *Modern Soil Microbiology, Third Edition*. Boca Raton: CRC Press.

Boscaro V, Husnik F, Vannini C, Keeling PJ. 2019. Symbionts of the ciliate Euplotes: diversity, patterns and potential as models for bacteria-eukaryote endosymbioses. *Proceedings of the Royal Society B: Biological Sciences* **286**(1907): 20190693.

Brisson J, Rodriguez M, Martin CA, Proulx R. 2020. Plant diversity effect on water quality in wetlands: a meta-analysis based on experimental systems. *Ecological Applications* **30**(4): e02074.

Bruelheide H, Böhnke M, Both S, Fang T, Assmann T, Baruffol M, Bauhus J, Buscot F, Chen X-Y, Ding B-Y, et al. 2011. Community assembly during secondary forest succession in a Chinese subtropical forest. *Ecological Monographs* **81**(1): 25–41.

Buaya A, Tsai I, Thines M. 2023. *Pontisma blauvikense* sp. nov. the first member of the early-diverging oomycete genus *Pontisma* parasitizing brown algae. *Journal of Eukaryotic Microbiology* **70**(2): e12957.

Buaya AT, Scholz B, Thines M. 2021. A New Marine Species of *Miracula* (*Oomycota*) Parasitic to *Minidiscus* sp. in Iceland. *Mycobiology* **49**(4): 355-362.

Buaya AT, Thines M. 2020. *Diatomophthoraceae* - a new family of olpidiopsis-like diatom parasitoids largely unrelated to *Ectrogella*. *Fungal Systems and Evolution* **5**: 113-118.

Bulman S, Neuhauser S 2017. Phytomyxea. In: Archibald JM, Simpson AGB, Slamovits CH eds. *Handbook of the Protists*. Cham: Springer, 783-803.

Burki F, Roger AJ, Brown MW, Simpson AGB. 2020. The New Tree of Eukaryotes. *Trends in Ecology & Evolution* **35**(1): 43-55.

Burki F, Sandin MM, Jamy M. 2021. Diversity and ecology of protists revealed by metabarcoding. *Current Biology* **31**(19): R1267-R1280.

Buzhdygan OY, Meyer ST, Weisser WW, Eisenhauer N, Ebeling A, Borrett SR, Buchmann N, Cortois R, De Deyn GB, de Kroon H, et al. 2020. Biodiversity increases multitrophic energy use efficiency, flow and storage in grasslands. *Nature Ecology and Evolution* **4**(3): 393-405.

Caldwell M, Dawson T, Richards J. 1998. Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia* **113**: 151–161.

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, et al. 2012. Biodiversity loss and its impact on humanity. *Nature* **486**(7401): 59-67.

Cardinale BJ, Wright JP, Cadotte MW, Carroll IT, Hector A, Srivastava DS, Loreau M, Weis JJ. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences of the United States of America* **104**(46): 18123-18128.

Carter R, Mendis KN. 2002. Evolutionary and historical aspects of the burden of malaria. *Clinical Microbiology Reviews* **15**(4): 564-594.

Casas SM, Reece KS, Li Y, Moss JA, Villalba A, La Peyre JF. 2008. Continuous culture of Perkinsus mediterraneus, a parasite of the European flat oyster Ostrea edulis, and characterization of its morphology, propagation, and extracellular proteins in vitro. *Journal of Eukaryotic Microbiology* **55**(1): 34-43.

Cavalier-Smith T. 2002. The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. *International Journal of Systematic and Evolutionary Microbiology* **52**: 297–354.

Cavalier-Smith T. 2003. Protist phylogeny and the high-level classification of Protozoa. *European Journal of Protistology* **39**(4): 338-348.

Cavalier-Smith T. 2018. Kingdom Chromista and its eight phyla: a new synthesis emphasising periplastid protein targeting, cytoskeletal and periplastid evolution, and ancient divergences. *Protoplasma* **255**(1): 297-357.

Cavalier-Smith T, Chao EE-Y. 2003. Phylogeny and Classification of Phylum Cercozoa (Protozoa). *Protist* **154**: 341-358.

Cavalier-Smith T, Chao EE, Lewis R. 2018. Multigene phylogeny and cell evolution of chromist infrakingdom Rhizaria: contrasting cell organisation of sister phyla Cercozoa and Retaria. *Protoplasma* **255**(5): 1517-1574.

Chakraborty S, Old KM. 1982. Mycophagous soil amoeba: Interactions with three plant pathogenic fungi. *Soil Biology and Biochemistry* **14**: 247-255.

Chakraborty S, Old KM, Warcup JH. 1983. Amoebae from a take-all suppressive soil which feed on *Gaeumannomyces graminis tritici* and other soil fungi. *Soil Biology and Biochemistry* **15**(1): 17-24.

Chapin FS, Matson PA, Vitousek PM. 2002. *Principles of Terrestrial Ecosystem Ecology*. NY: Springer New York.

Clarholm M. 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. *Soil Biology and Biochemistry* **17**(2): 181-187.

Coleman DC. 1994. The microbial loop concept as used in terrestrial soil ecology studies. *Microbial Ecology* **28**: 245–250.

Corlett RT. 2016. Plant diversity in a changing world: Status, trends, and conservation needs. *Plant Diversity* **38**(1): 10-16.

Cowie RH, Bouchet P, Fontaine B. 2022. The Sixth Mass Extinction: fact, fiction or speculation? *Biological Reviews of the Cambridge Philosophical Society* **97**(2): 640-663.

Crews TE, Carton W, Olsson L. 2018. Is the future of agriculture perennial? Imperatives and opportunities to reinvent agriculture by shifting from annual monocultures to perennial polycultures. *Global Sustainability* **1**: e11.

Crockford PW, Bar On YM, Ward LM, Milo R, Halevy I. 2023. The geologic history of primary productivity. *Current Biology* **33**(21): 4741-4750.

d'Entremont TW, Kivlin SN. 2023. Specificity in plant-mycorrhizal fungal relationships: prevalence, parameterization, and prospects. *Frontiers in Plant Science* **14**: 1260286.

de Souza YPA, Siani R, Albracht C, Huang Y, Eisenhauer N, Vogel A, Wagg C, Schloter M, Schulz S. 2024. The effect of successive summer drought periods on bacterial diversity along a plant species richness gradient. *FEMS Microbiology Ecology* **100**(8): fiae096.

Degrune F, Dumack K, Ryo M, Garland G, Romdhane S, Saghai A, Banerjee S, Edlinger A, Herzog C, Pescador DS, et al. 2024. The impact of fungi on soil protist communities in European cereal croplands. *Environmental Microbiology* **26**(7): e16673.

Dennis PG, Miller AJ, Hirsch PR. 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiology Ecology* **72**(3): 313-327.

Derevnina L, Petre B, Kellner R, Dagdas YF, Sarowar MN, Giannakopoulou A, De la Concepcion JC, Chaparro-Garcia A, Pennington HG, van West P, et al. 2016. Emerging oomycete threats to plants and animals. *Philosophical Transactions of the Royal Society B: Biological Sciences* **371**(1709): 20150459.

Dietrich P, Cesarz S, Liu T, Roscher C, Eisenhauer N. 2021. Effects of plant species diversity on nematode community composition and diversity in a long-term biodiversity experiment. *Oecologia* **197**(2): 297-311.

Dixon GR. 2009. *Plasmodiophora brassicae* in its Environment. *Journal of Plant Growth Regulation* **28**(3): 212-228.

Ducklow H, Steinberg D, Buesseler K. 2001. Upper Ocean Carbon Export and the Biological Pump. *Oceanography* **14**(4): 50-58.

Dumack K, Feng K, Flues S, Sapp M, Schreiter S, Grosch R, Rose LE, Deng Y, Smalla K, Bonkowski M. 2022. What Drives the Assembly of Plant-associated Protist Microbiomes? Investigating the Effects of Crop Species, Soil Type and Bacterial Microbiomes. *Protist* **173**(6): 125913.

Dumack K, Müller MEH, Bonkowski M. 2016. Description of *Lecythium terrestris* sp. nov. (Chlamydophryidae, Cercozoa), a Soil Dwelling Protist Feeding on Fungi and Algae. *Protist* **167**(2): 93-105.

Dumack K, Pundt J, Bonkowski M. 2019. Food Choice Experiments Indicate Selective Fungivorous Predation in *Fisculla terrestris* (Thecofilosea, Cercozoa). *Journal of Eukaryotic Microbiology* **66**(3): 525-527.

Dybzinski R, Fargione JE, Zak DR, Fornara D, Tilman D. 2008. Soil fertility increases with plant species diversity in a long-term biodiversity experiment. *Oecologia* **158**(1): 85-93.

Ebeling A, Hines J, Hertzog LR, Lange M, Meyer ST, Simons NK, Weisser WW. 2018. Plant diversity effects on arthropods and arthropod-dependent ecosystem functions in a biodiversity experiment. *Basic and Applied Ecology* **26**: 50-63.

Ebeling A, Klein AM, Schumacher J, Weisser WW, Tscharntke T. 2008. How does plant richness affect pollinator richness and temporal stability of flower visits? *Oikos* **117**(12): 1808-1815.

Ebeling A, Pompe S, Baade J, Eisenhauer N, Hillebrand H, Proulx R, Roscher C, Schmid B, Wirth C, Weisser WW. 2014. A trait-based experimental approach to understand the mechanisms underlying biodiversity–ecosystem functioning relationships. *Basic and Applied Ecology* **15**(3): 229-240.

Eggertson QA, Rintoul TL, Levesque CA. 2023. Resolving the *Globisporangium ultimum* (*Pythium ultimum*) species complex. *Mycologia* **115**(6): 768-786.

Ehrenfeld JG, Ravit B, Elgersma K. 2005. Feedback in the Plant-Soil System. *Annual Review of Environment and Resources* **30**(1): 75-115.

Eisenhauer N, Beßler H, Engels C, Gleixner G, Habekost M, Milcu A, Partsch S, Sabais ACW, Scherber C, Steinbeiss S, et al. 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* **91**(2): 485–496.

Eisenhauer N, Bonkowski M, Brose U, Buscot F, Durka W, Ebeling A, Fischer M, Gleixner G, Heintz-Buschart A, Hines J, et al. 2019. Biotic interactions, community assembly, and eco-evolutionary dynamics as drivers of long-term biodiversity–ecosystem functioning relationships. *Research Ideas and Outcomes* **5**: e47042.

Eisenhauer N, Milcu A, Nitschke N, Sabais AC, Scherber C, Scheu S. 2009. Earthworm and belowground competition effects on plant productivity in a plant diversity gradient. *Oecologia* **161**(2): 291-301.

Eisenhauer N, Mueller K, Ebeling A, Gleixner G, Huang Y, Madaj A-M, Roscher C, Weigelt A, Bahn M, Bonkowski M, et al. 2024. The multiple-mechanisms hypothesis of biodiversity–stability relationships. *Basic and Applied Ecology* **79**: 153-166.

Eisenhauer N, Reich PB, Scheu S. 2012. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic and Applied Ecology* **13**(7): 571-578.

Ekelund F. 1996. Growth kinetics of five common heterotrophic soil flagellates. *European Journal of Soil Biology.* **32**: 15-24.

Estermann AH, Teixeira Pereira Bassiaridis J, Loos A, Solbach MD, Bonkowski M, Hess S, Dumack K. 2023. Fungivorous protists in the rhizosphere of *Arabidopsis thaliana* – Diversity, functions, and publicly available cultures for experimental exploration. *Soil Biology and Biochemistry* **187**: 109206.

Falkowski P. 2012. Ocean Science: The power of plankton. *Nature* **483**: S17–S20.

Falloon RE, Merz U, Butler RC, Curtin D, Lister RA, Thomas SM. 2015. Root infection of potato by *Spongospora subterranea*: knowledge review and evidence for decreased plant productivity. *Plant Pathology* **65**(3): 422-434.

Fawke S, Doumane M, Schornack S. 2015. Oomycete interactions with plants: infection strategies and resistance principles. *Microbiology and Molecular Biology Reviews* **79**(3): 263-280.

Fenchel T. 2008. The microbial loop – 25 years later. *Journal of Experimental Marine Biology and Ecology* **366**(1-2): 99-103.

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* **281**(5374): 237-240.

Fiore-Donno AM, Bonkowski M. 2021. Different community compositions between obligate and facultative oomycete plant parasites in a landscape-scale metabarcoding survey. *Biology and Fertility of Soils* **57**(2): 245-256.

Fiore-Donno AM, Freudenthal J, Dahl MB, Rixen C, Urich T, Bonkowski M. 2024. Biotic interactions explain seasonal dynamics of the alpine soil microbiome. *ISME Communications* **4**(1): ycae028.

Fiore-Donno AM, Richter-Heitmann T, Degrune F, Dumack K, Regan KM, Marhan S, Boeddinghaus RS, Rillig MC, Friedrich MW, Kandeler E, et al. 2019. Functional Traits and Spatio-Temporal Structure of a Major Group of Soil Protists (Rhizaria: Cercozoa) in a Temperate Grassland. *Frontiers in Microbiology* **10**: 1332.

Fiore-Donno AM, Rixen C, Rippin M, Glaser K, Samolov E, Karsten U, Becker B, Bonkowski M. 2018. New barcoded primers for efficient retrieval of cercozoan sequences in high-throughput environmental diversity surveys, with emphasis on worldwide biological soil crusts. *Molecular Ecology Resources* **18**(2): 229-239.

Fitzpatrick CR, Copeland J, Wang PW, Guttman DS, Kotanen PM, Johnson MTJ. 2018. Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences of the United States of America* **115**(6): E1157-E1165.

Fogg GE. 1983. The Ecological Significance of Extracellular Products of Phytoplankton Photosynthesis. *Botanica Marina* **26**: 3-14.

Foissner W 2011. Dispersal of protists: the role of cysts and human introductions. *Biogeography of Microscopic Organisms*, 61-87.

Frouz J. 2024. Plant-soil feedback across spatiotemporal scales from immediate effects to legacy. *Soil Biology and Biochemistry* **189**: 109289.

Fry W. 2008. *Phytophthora infestans*: the plant (and R gene) destroyer. *Molecular Plant Pathology* **9**(3): 385-402.

Furey GN, Tilman D. 2021. Plant biodiversity and the regeneration of soil fertility. *Proceedings of the National Academy of Sciences of the United States of America* **118**(49).

Gao Y, Wang H, Yang F, Dai X, Meng S, Hu M, Kou L, Fu X. 2024. Relationships between root exudation and root morphological and architectural traits vary with growing season. *Tree Physiology* **44**(1).

Garcia Arredondo M, Kew W, Chu R, Jones ME, Boiteau RM, Cardon ZG, Keiluweit M. 2024. Differential Exudation Creates Biogeochemically Distinct Microenvironments during Rhizosphere Evolution. *Environmental Science & Technology* **58**(42): 18713-18722.

Gaulin E, Jacquet C, Bottin A, Dumas B. 2007. Root rot disease of legumes caused by *Aphanomyces euteiches*. *Molecular Plant Pathology* **8**(5): 539-548.

Geisen S, Mitchell EAD, Adl S, Bonkowski M, Dunthorn M, Ekelund F, Fernandez LD, Jousset A, Krashevska V, Singer D, et al. 2018. Soil protists: a fertile frontier in soil biology research. *FEMS Microbiology Reviews* **42**(3): 293-323.

Gleason FH, Lilje O. 2009. Structure and function of fungal zoospores: ecological implications. *Fungal Ecology* **2**(2): 53-59.

Gortz HD. 2001. Intracellular bacteria in ciliates. *International Microbiology* **4**(3): 143-150.

Gough CM. 2012. Terrestrial primary production: Fuel for life. *Nature Education Knowledge* **3**(10): 28.

Guerrero-Ramirez NR, Craven D, Reich PB, Ewel JJ, Isbell F, Koricheva J, Parrotta JA, Auge H, Erickson HE, Forrester DI, et al. 2017. Diversity-dependent temporal divergence of ecosystem functioning in experimental ecosystems. *Nature Ecology and Evolution* **1**(11): 1639-1642.

Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, Boutte C, Burgaud G, de Vargas C, Decelle J, et al. 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Research* **41**(Database issue): D597-D604.

Guo S, Geisen S, Mo Y, Yan X, Huang R, Liu H, Gao Z, Tao C, Deng X, Xiong W, et al. 2024. Predatory protists impact plant performance by promoting plant growth-promoting rhizobacterial consortia. *ISME Journal* **18**(1): wrae180.

Hannula SE, Heinen R, Huberty M, Steinauer K, De Long JR, Jongen R, Bezemer TM. 2021. Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. *Nature Communications* **12**(1): 5686.

Hansen EM. 2015. Phytophthora Species Emerging as Pathogens of Forest Trees. *Current Forestry Reports* **1**(1): 16-24.

Hardham AR. 2007. Cell biology of plant-oomycete interactions. *Cellular Microbiology* **9**(1): 31-39.

Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, et al. 1999. Plant diversity and productivity experiments in european grasslands. *Science* **286**(5442): 1123-1127.

Helbert W, Sugiyama J, Ishihara M, Yamanaka S. 1997. Characterization of native crystalline cellulose in the cell walls of Oomycota. *Journal of Biotechnology* **57**(1-3): 29-37.

Hennecke J, Bassi L, Albracht C, Amyntas A, Bergmann J, Eisenhauer N, Fox A, Heimbold L, Heintz-Buschart A, Kuyper TW, et al. 2025. Plant Species Richness and the Root Economics Space Drive Soil Fungal Communities. *Ecology Letters* **28**(1): e70032.

Hennecke J, Bassi L, Mommer L, Albracht C, Bergmann J, Eisenhauer N, Guerra CA, Heintz-Buschart A, Kuyper TW, Lange M, et al. 2023. Responses of rhizosphere fungi to the root economics space in grassland monocultures of different age. *New Phytologist* **240**(5): 2035-2049.

Herold M, Martinez Arbas S, Narayanasamy S, Sheik AR, Kleine-Borgmann LAK, Lebrun LA, Kunath BJ, Roume H, Bessarab I, Williams RBH, et al. 2020.

Integration of time-series meta-omics data reveals how microbial ecosystems respond to disturbance. *Nature Communications* **11**(1): 5281.

Hess S, Sausen N, Melkonian M. 2012. Shedding light on vampires: the phylogeny of vampyrellid amoebae revisited. *PLoS One* **7**(2): e31165.

Hess S, Suthaus A. 2022. The Vampyrellid Amoebae (Vampyrellida, Rhizaria). *Protist* **173**(1): 125854.

Hess S, Suthaus A, Melkonian M. 2016. "*Candidatus* Finniella" (*Rickettsiales*, *Alphaproteobacteria*), Novel Endosymbionts of Viridiraptorid Amoeboflagellates (Cercozoa, Rhizaria). *Applied and Environmental Microbiology* **82**(2): 659-670.

Hinsinger P, Plassard C, Tang C, Jaillard B. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. *Plant and Soil* **248**: 43–59.

Hittorf M, Letsch-Praxmarer S, Windegger A, Bass D, Kirchmair M, Neuhauser S. 2020. Revised Taxonomy and Expanded Biodiversity of the Phytomyxea (Rhizaria, Endomyxa). *Journal of Eukaryotic Microbiology* **67**(6): 648-659.

Hu L, Robert CAM, Cadot S, Zhang X, Ye M, Li B, Manzo D, Chervet N, Steinger T, van der Heijden MGA, et al. 2018. Root exudate metabolites drive plant–soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* **9**(1): 2738.

Huang Y, Stein G, Kolle O, Kübler K, Schulze E-D, Dong H, Eichenberg D, Gleixner G, Hildebrandt A, Lange M, et al. 2023. Enhanced stability of grassland soil temperature by plant diversity. *Nature Geoscience* **17**(1): 44-50.

Hundt R. 1961. Die Auswirkungen der Saaletalsperren auf die Grünlandvegetation des mittleren Saaletales. *Mitteilungen des Instituts für Wasserwirtschaft* **14**: 21–60.

Huston M. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* **110**: 449–460.

Irwin NAT, Tikhonenkov DV, Hehenberger E, Mylnikov AP, Burki F, Keeling PJ. 2019. Phylogenomics supports the monophyly of the Cercozoa. *Molecular Phylogenetics and Evolution* **130**: 416-423.

Jacoby RP, Chen L, Schwier M, Koprivova A, Kopriva S. 2020. Recent advances in the role of plant metabolites in shaping the root microbiome [version 1; peer review: 3 approved]. *F1000Research* **9(F1000 Faculty Rev)**: 151.

Jandt U, Bruelheide H, Jansen F, Bonn A, Grescho V, Klenke RA, Sabatini FM, Bernhardt-Romermann M, Bluml V, Dengler J, et al. 2022. More losses than gains during one century of plant biodiversity change in Germany. *Nature* **611**(7936): 512-518.

Jauss R-T, Walden S, Fiore-Donno AM, Schaffer S, Wolf R, Feng K, Bonkowski M, Schlegel M. 2021. A Parasite's Paradise: Biotrophic Species Prevail Oomycete Community Composition in Tree Canopies. *Frontiers in Forests and Global Change* **4**: 668895.

Judelson HS, Blanco FA. 2005. The spores of Phytophthora: weapons of the plant destroyer. *Nature Reviews: Microbiology* **3**(1): 47-58.

Kageyama K, Asano T. 2009. Life Cycle of *Plasmodiophora brassicae*. *Journal of Plant Growth Regulation* **28**(3): 203-211.

Kamoun S, Furzer O, Jones JD, Judelson HS, Ali GS, Dalio RJ, Roy SG, Schena L, Zambounis A, Panabieres F, et al. 2015. The Top 10 oomycete pathogens in molecular plant pathology. *Molecular Plant Pathology* **16**(4): 413-434.

Kannan R, James DA. 2009. Effects of climate change on global biodiversity: a review of key literature. *Tropical Ecology* **50**(1): 31-39.

Kanyuka K, Ward E, Adams MJ. 2003. *Polymyxa graminis* and the cereal viruses it transmits: a research challenge. *Molecular Plant Pathology* **4**(5): 393-406.

Karakashian SJ, Karakashian MW. 1965. Evolution and Symbiosis in the Genus *Chlorella* and Related Algae. *Evolution* **19**(3): 368-377.

Kaur S, Bedi M, Singh S, Kour N, Bhatti SS, Bhatia A, Kumar M, Kumar R 2024. Chapter Eight - Monoculture of crops: A challenge in attaining food security. In: Sharma A, Kumar M, Sharma P eds. *Advances in Food Security and Sustainability*: Elsevier, 197-213.

Keeling PJ. 2010. The endosymbiotic origin, diversification and fate of plastids. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**(1541): 729-748.

Keeling PJ. 2013. The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. *Annual Review of Plant Biology* **64**: 583-607.

Keesing F, Ostfeld RS. 2021. Dilution effects in disease ecology. *Ecology Letters* **24**(11): 2490-2505.

Keskin B, Fuchs WH. 1969. Der Infektionsvorgang bei *Polymyxa betae*. *Archiv für Mikrobiologie* **68**: 218-226.

Klironomos J. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* **417**: 67-70.

Kulmatiski A, Beard KH, Heavilin J. 2012. Plant–soil feedbacks provide an additional explanation for diversity–productivity relationships. *Proceedings of the Royal Society B: Biological Sciences* **279**(1740): 3020-3026.

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM. 2008. Plant-soil feedbacks: a meta-analytical review. *Ecology Letters* **11**(9): 980-992.

Lange M, Eisenhauer N, Sierra CA, Bessler H, Engels C, Griffiths RI, Mellado-Vazquez PG, Malik AA, Roy J, Scheu S, et al. 2015. Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications* **6**: 6707.

Latijnhouwers M, de Wit PJ, Govers F. 2003. Oomycetes and fungi: similar weaponry to attack plants. *Trends in Microbiology* **11**(10): 462-469.

Latz E, Eisenhauer N, Rall BC, Allan E, Roscher C, Scheu S, Jousset A. 2012. Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. *Journal of Ecology* **100**(3): 597-604.

Latz E, Eisenhauer N, Rall BC, Scheu S, Jousset A. 2016. Unravelling Linkages between Plant Community Composition and the Pathogen-Suppressive Potential of Soils. *Scientific Reports* **6**: 23584.

Leff JW, Bardgett RD, Wilkinson A, Jackson BG, Pritchard WJ, De Long JR, Oakley S, Mason KE, Ostle NJ, Johnson D, et al. 2018. Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *ISME Journal* **12**(7): 1794-1805.

Lentendu G, Wubet T, Chatzinotas A, Wilhelm C, Buscot F, Schlegel M. 2014. Effects of long-term differential fertilization on eukaryotic microbial communities in an arable soil: a multiple barcoding approach. *Molecular Ecology* **23**(13): 3341-3355.

Li Y, Schuldt A, Ebeling A, Eisenhauer N, Huang Y, Albert G, Albracht C, Amyntas A, Bonkowski M, Bruelheide H, et al. 2024. Plant diversity enhances ecosystem multifunctionality via multitrophic diversity. *Nature Ecology and Evolution* **8**(11): 2037-2047.

Liang J, Crowther TW, Picard N, Wiser S, Zhou M, Alberti G, Schulze ED, McGuire AD, Bozzato F, Pretzsch H, et al. 2016. Positive biodiversity-productivity relationship predominant in global forests. *Science* **354**(6309): aaf8957.

Liu X, Lyu S, Zhou S, Bradshaw CJ. 2016. Warming and fertilization alter the dilution effect of host diversity on disease severity. *Ecology* **97**(7): 1680-1689.

Lopez-Garcia P, Moreira D. 2020. The Syntrophy hypothesis for the origin of eukaryotes revisited. *Nature Microbiology* **5**(5): 655-667.

Loreau M, Hector A. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**: 72–76.

Madaj A-M, Huang Y, Ebeling A, Ertel L, Gebler A, Gleixner G, Hines J, Roscher C, Weigelt A, Albracht C, et al. 2025. JenaTron - An Experimental Approach to Study the Effects of Plant History and Soil History on Grassland Ecosystem Functioning. *Journal of Visualized Experiments* **217**: e67496.

Maier S, Tamm A, Wu D, Caesar J, Grube M, Weber B. 2018. Photoautotrophic organisms control microbial abundance, diversity, and physiology in different types of biological soil crusts. *ISME Journal* **12**(4): 1032-1046.

Martin WF, Tielens AGM, Mentel M, Garg SG, Gould SB. 2017. The Physiology of Phagocytosis in the Context of Mitochondrial Origin. *Microbiology and Molecular Biology Reviews* **81**(3): e00008-00017.

Masigol H, Solbach MD, Pourmoghaddam MJ, Ahadi R, Mostowfizadeh-Ghalamfarsa R, Taheri SR, Tobias-Hünefeldt SP, Bonkowski M, Grossart HP. 2025. A glimpse into Oomycota diversity in freshwater lakes and adjacent forests using a metabarcoding approach. *Scientific Reports* **15**(1): 19124.

Mazor T, Doropoulos C, Schwarzmueller F, Gladish DW, Kumaran N, Merkel K, Di Marco M, Gagic V. 2018. Global mismatch of policy and research on drivers of biodiversity loss. *Nature Ecology and Evolution* **2**(7): 1071-1074.

Meng Y, Zhang Q, Ding W, Shan W. 2014. *Phytophthora parasitica*: a model oomycete plant pathogen. *Mycology* **5**(2): 43-51.

Meyer ST, Ebeling A, Eisenhauer N, Hertzog L, Hillebrand H, Milcu A, Pompe S, Abbas M, Bessler H, Buchmann N, et al. 2016. Effects of biodiversity strengthen over time as ecosystem functioning declines at low and increases at high biodiversity. *Ecosphere* **7**(12): e01619.

Mitchell CE, Tilman D, Groth JV. 2002. Effects of Grassland Plant Species Diversity, Abundance, and Composition on Foliar Fungal Disease. *Ecology* **83**(6): 1713-1726.

Mitchell RT, Deacon JW. 1986. Differential (Host-Specific) Accumulation of Zoospores of *Pythium* on Roots of Graminaceous and Non-Graminaceous Plants. *New Phytologist* **102**(1): 113-122.

Mommer L, Cotton TEA, Raaijmakers JM, Termorshuizen AJ, van Ruijven J, Hendriks M, van Rijssel SQ, van de Mortel JE, van der Paauw JW, Schijlen E, et al. 2018. Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. *New Phytologist* **218**(2): 542-553.

Money NP, Davis CM, Ravishankar JP. 2004. Biomechanical evidence for convergent evolution of the invasive growth process among fungi and oomycete water molds. *Fungal Genetics and Biology* **41**(9): 872-876.

Neuhauser S, Kirchmair M, Bulman S, Bass D. 2014. Cross-kingdom host shifts of phytomyxid parasites. *BMC Evolutionary Biology* **14**: 33.

Nguyen BT, Dumack K, Trivedi P, Islam Z, Hu HW. 2023. Plant associated protists-Untapped promising candidates for agrifood tools. *Environmental Microbiology* **25**(2): 229-240.

Nowicki M, DeVries T, Siegel DA. 2022. Quantifying the Carbon Export and Sequestration Pathways of the Ocean's Biological Carbon Pump. *Global Biogeochemical Cycles* **36**(3): e2021GB007083.

Oburger E, Jones DL. 2018. Sampling root exudates – Mission impossible? *Rhizosphere* **6**: 116-133.

Ogden CG, Pitta P. 1990. Biology and ultrastructure of the mycophagus, soil testate amoeba, *Phryganella acropodia* (Rhizopoda, Protozoa). *Biology and Fertility of Soils* **9**: 101-109.

Old KM, Darbyshire JF. 1978. Soil fungi as food for giant amoebae. *Soil Biology and Biochemistry* **10**(2): 93-100.

Oliverio AM, Geisen S, Delgado-Baquerizo M, Maestre FT, Turner BL, Fierer N. 2020. The global-scale distributions of soil protists and their contributions to belowground systems. *Science Advances* **6**(4): eaax8787.

Ostfeld RS, Keesing F. 2000. Biodiversity and Disease Risk: the Case of Lyme Disease. *Conservation Biology* **14**(3): 722–728.

Pace ML, Lovett GM, Carey CC, Thomas RQ 2021. Chapter 2 - Primary Production: The Foundation of Ecosystems. In: Weathers KC, Strayer DL, Likens GE eds. *Fundamentals of Ecosystem Science (Second Edition)*. London: Academic Press, 29-53.

Pang Z, Chen J, Wang T, Gao C, Li Z, Guo L, Xu J, Cheng Y. 2021. Linking Plant Secondary Metabolites and Plant Microbiomes: A Review. *Frontiers in Plant Science* **12**: 621276.

Park M, Yun ST, Kim MS, Chun J, Ahn TI. 2004. Phylogenetic characterization of *Legionella*-like endosymbiotic X-bacteria in *Amoeba proteus*: a proposal for '*Candidatus* Legionella jeonii' sp. nov. *Environmental Microbiology* **6**(12): 1252-1263.

Partel M, Tamme R, Carmona CP, Riibak K, Moora M, Bennett JA, Chiarucci A, Chytry M, de Bello F, Eriksson O, et al. 2025. Global impoverishment of natural vegetation revealed by dark diversity. *Nature* **641**(8064): 917-924.

Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences of the United States of America* **110**(16): 6548-6553.

Petermann JS, Fergus AJ, Turnbull LA, Schmid B. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* **89**(9): 2399-2406.

Pieterse CMJ, de Jonge R, Berendsen RL. 2016. The Soil-Borne Supremacy. *Trends in Plant Science* **21**(3): 171-173.

Populer C 1981. Epidemiology of downy mildews. In: Spencer DM ed. *The downy mildews*. London: Academic Press, 57-105.

Randolph SE, Dobson AD. 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology* **139**(7): 847-863.

Rastas M, Latvala S, Hannukkala A. 2012. Occurrence of Plasmodiophora brassicae in Finnish turnip rape and oilseed rape fields. *Agricultural and Food Science* **21**(2): 141-158.

Reich PB, Tilman D, Isbell F, Mueller K, Hobbie SE, Flynn DFB, Eisenhauer N. 2012. Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades. *Science* **336**: 589-592.

Roger AJ, Munoz-Gomez SA, Kamikawa R. 2017. The Origin and Diversification of Mitochondria. *Current Biology* **27**(21): R1177-R1192.

Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, Schmid B, Schulze E-D. 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology* **5**(2): 107-121.

Rossmann M, Perez-Jaramillo JE, Kavamura VN, Chiaramonte JB, Dumack K, Fiore-Donno AM, Mendes LW, Ferreira MMC, Bonkowski M, Raaijmakers

JM, et al. 2020. Multitrophic interactions in the rhizosphere microbiome of wheat: from bacteria and fungi to protists. *FEMS Microbiology Ecology* **96**(4): fiaa032.

Rüger L, Feng K, Dumack K, Freudenthal J, Chen Y, Sun R, Wilson M, Yu P, Sun B, Deng Y, et al. 2021. Assembly Patterns of the Rhizosphere Microbiome Along the Longitudinal Root Axis of Maize (*Zea mays* L.). *Frontiers in Microbiology* **12**: 614501.

Ruggiero MA, Gordon DP, Orrell TM, Bailly N, Bourgoin T, Brusca RC, Cavalier-Smith T, Guiry MD, Kirk PM. 2015. A higher level classification of all living organisms. *PLoS One* **10**(4): e0119248.

Salkeld DJ, Padgett KA, Jones JH. 2013. A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. *Ecology Letters* **16**(5): 679-686.

Samolov E, Baumann K, Budel B, Jung P, Leinweber P, Mikhailyuk T, Karsten U, Glaser K. 2020. Biodiversity of Algae and Cyanobacteria in Biological Soil Crusts Collected Along a Climatic Gradient in Chile Using an Integrative Approach. *Microorganisms* **8**(7).

Sasse J, Martinoia E, Northen T. 2018. Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in Plant Science* **23**(1): 25-41.

Schaap P, Schilde C. 2018. Encystation: the most prevalent and underinvestigated differentiation pathway of eukaryotes. *Microbiology* **164**(5): 727-739.

Schmid B, Joshi J, Schläpfer F 2002. Empirical evidence for biodiversity-ecosystem functioning relationships. In: Kinzig A, Tilman D, Pacala S eds. *Functional consequences of biodiversity: experimental progress and theoretical extensions*. Princeton: Princeton University Press, 120-150.

Schmid MW, van Moorsel SJ, Hahl T, De Luca E, De Deyn GB, Wagg C, Niklaus PA, Schmid B. 2021. Effects of plant community history, soil legacy and plant diversity on soil microbial communities. *Journal of Ecology* **109**(8): 3007-3023.

Schmidt KA, Ostfeld RS. 2001. Biodiversity and the Dilution Effect in Disease Ecology. *Ecology* **82**(3): 609–619.

Schnitzer SA, Klironomos J. 2011. Soil microbes regulate ecosystem productivity and maintain species diversity. *Plant Signaling & Behavior* **6**(8): 1240-1243.

Schultes SR, Ruger L, Niedeggen D, Freudenthal J, Frindte K, Becker MF, Metzner R, Pflugfelder D, Chlubek A, Hinz C, et al. 2025. Photosynthate distribution determines spatial patterns in the rhizosphere microbiota of the maize root system. *Nature Communications* **16**(1): 7286.

Schwelm A, Badstober J, Bulman S, Desoignies N, Etemadi M, Falloon RE, Gachon CMM, Legreve A, Lukes J, Merz U, et al. 2018. Not in your usual Top 10: protists that infect plants and algae. *Molecular Plant Pathology* **19**(4): 1029-1044.

Sekerci Y, Petrovskii S. 2015. Mathematical Modelling of Plankton-Oxygen Dynamics Under the Climate Change. *Bulletin of Mathematical Biology* **77**(12): 2325-2353.

Sherr EB, Sherr BF. 2002. Significance of predation by protists in aquatic microbial food webs. *Antonie Van Leeuwenhoek* **81**(1-4): 293-308.

Shirley MW, Smith AL, Tomley FM. 2005. The biology of avian *Eimeria* with an emphasis on their control by vaccination. *Advances in Parasitology* **60**: 285-330.

Singer D, Seppey CVW, Lentendu G, Dunthorn M, Bass D, Belbahri L, Blandenier Q, Debroas D, de Groot GA, de Vargas C, et al. 2021. Protist taxonomic and functional diversity in soil, freshwater and marine ecosystems. *Environment International* **146**: 106262.

Singhal RN, Jeet S, Davies RW. 1987. Experimental Transmission of *Saprolegnia* and *Achlya* to Fish. *Aquaculture* **64**(1): 1-7.

Solbach MD, Albracht C, Dumack K, Eisenhauer N, Fiore-Donno AM, Heck N, Vogel A, Wagg C, Bonkowski M. 2025a. Legacies of consecutive summer droughts on soil-borne plant parasitic protists (Oomycota: Stramenopila and Phytomyxea: Rhizaria) and protistan consumers (Cercozoa: Rhizaria) along an experimental plant diversity gradient. *New Phytologist* **Early view**.

Solbach MD, Bonkowski M, Dumack K. 2021. Novel Endosymbionts in Rhizarian Amoebae Imply Universal Infection of Unrelated Free-Living Amoebae by Legionellales. *Frontiers in Cellular and Infection Microbiology* **11**: 642216.

Solbach MD, Bonkowski M, Dumack K. 2025b. *Katarium polorum* n. sp., n. g., a novel thecofilosean amoeba (Cercozoa, Rhizaria) from the polar oceans. *Journal of Eukaryotic Microbiology* **72**(2): e13071.

Spehn EM, Hector A, Joshi J, Scherer-Lorenzen M, Schmid B, Bazeley-White E, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, et al. 2005. Ecosystem Effects of Biodiversity Manipulations in European Grasslands. *Ecological Monographs* **75**(1): 37–63.

Stat M, Morris E, Gates RD. 2008. Functional diversity in coral–dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* **105**(27): 9256-9261.

Strydom RF, Wilson CR, Tegg RS, Balendres MA, van der Waals JE. 2024. Advancements in Spongospora subterranea: Current Knowledge, Management Strategies, and Research Gaps. *Potato Research* **67**(4): 1497-1537.

Stuart K, Brun R, Croft S, Fairlamb A, Gurtler RE, McKerrow J, Reed S, Tarleton R. 2008. Kinetoplastids: related protozoan pathogens, different diseases. *Journal of Clinical Investigation* **118**(4): 1301-1310.

Svoboda J, Mrugala A, Kozubikova-Balcarova E, Petrusek A. 2017. Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. *Journal of Fish Diseases* **40**(1): 127-140.

Tamada T, Baba T. 1973. Beet Necrotic Yellow Vein Virus from Rizomania-Affected Sugar Beet in Japan. *Japanese Journal of Phytopathology* **39**(4): 325-332.

Thines M, Choi YJ. 2016. Evolution, Diversity, and Taxonomy of the Peronosporaceae, with Focus on the Genus *Peronospora*. *Phytopathology* **106**(1): 6-18.

Thines M, Kamoun S. 2010. Oomycete-plant coevolution: recent advances and future prospects. *Current Opinion in Plant Biology* **13**(4): 427-433.

Thines M, Voglmayr H 2009. An Introduction to the White Blister Rusts (Albuginales). In: Lamour K, Kamoun S eds. *Oomycete Genetics and Genomics*. Hoboken, NJ: John Wiley & Sons, Inc., 77–92.

Tilman D. 1996. Biodiversity: Population versus ecosystem stability. *Ecology* **77**(2): 350-363.

Tilman D. 2001. An evolutionary approach to ecosystem functioning. *Proceedings of the National Academy of Sciences of the United States of America* **98**(20): 10979-10980.

Tilman D, Cassman K, Matson P, Naylor R, Polasky S. 2002. Agricultural sustainability and intensive production practices. *Nature* **418**: 671–677.

Tilman D, Isbell F, Cowles JM. 2014. Biodiversity and Ecosystem Functioning. *Annual Review of Ecology, Evolution, and Systematics* **45**(1): 471-493.

Tilman D, Reich PB, Isbell F. 2012. Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. *Proceedings of the National Academy of Sciences of the United States of America* **109**(26): 10394-10397.

Tilman D, Reich PB, Knops J, Wedin D, Mielke T, Lehman C. 2001. Diversity and productivity in a long-term grassland experiment. *Science* **294**(5543): 843-845.

Tilman D, Reich PB, Knops JM. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* **441**(7093): 629-632.

Tsyganov AN, Nijs I, Beyens L. 2011. Does climate warming stimulate or inhibit soil protist communities? A test on testate amoebae in high-arctic tundra with free-air temperature increase. *Protist* **162**(2): 237-248.

Turkensteen LJ, Flier WG, Wanningen R, Mulder A. 2008. Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology* **49**(6): 688-696.

Tyler BM, Tripathy S, Zhang X, Dehal P, Jiang RH, Aerts A, Arredondo FD, Baxter L, Bensasson D, Beynon JL, et al. 2006. *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* **313**(5791): 1261-1266.

Uren NC 2000. Types, Amounts, and Possible Functions of Compounds Released into the Rhizosphere by Soil-Grown Plants. In: Willig S, Varanini Z, Nannipieri P eds. *The Rhizosphere - Biochemistry and Organic Substance at the Soil-Plant Interface*. Boca Raton: CRC Press, 1-22.

Uzuhashi S, Kakishima M, Tojo M. 2010. Phylogeny of the genus *Pythium* and description of new genera. *Mycoscience* **51**(5): 337-365.

van der Heijden MG, Bardgett RD, van Straalen NM. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**(3): 296-310.

van der Heijden MG, Schlaeppi K. 2015. Root surface as a frontier for plant microbiome research. *Proceedings of the National Academy of Sciences of the United States of America* **112**(8): 2299-2300.

van der Plaats-Niterink J. 1981. Monograph of the genus *Pythium*. *Studies in Mycology* **21**: 1-244.

van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, et al. 2013. Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology* **101**(2): 265-276.

van West P, Appiah AA, Gow NAR. 2003. Advances in research on oomycete root pathogens. *Physiological and Molecular Plant Pathology* **62**(2): 99-113.

Verheyen K, Ceunen K, Ampoorter E, Baeten L, Bosman B, Branquart E, Carnol M, De Wandeler H, Grégoire J-C, Lhoir P, et al. 2013. Assessment of the functional role of tree diversity: the multi-site FORBIO experiment. *Plant Ecology and Evolution* **146**(1): 26-35.

Visvesvara GS, Moura H, Schuster FL. 2007. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. *FEMS Immunology and Medical Microbiology* **50**(1): 1-26.

Vogel A, Ebeling A, Gleixner G, Roscher C, Scheu S, Ciobanu M, Koller-France E, Lange M, Lochner A, Meyer ST, et al. 2019. A new experimental approach to test why biodiversity effects strengthen as ecosystems age. In: Eisenhauer N, Bohan DA, Dumbrell AJ eds. *Mechanisms underlying the relationship between biodiversity and ecosystem function*: Academic Press, 221-264.

Vogel A, Eisenhauer N, Weigelt A, Scherer-Lorenzen M. 2013a. Plant diversity does not buffer drought effects on early-stage litter mass loss rates and microbial properties. *Global Change Biology* **19**(9): 2795-2803.

Vogel A, Fester T, Eisenhauer N, Scherer-Lorenzen M, Schmid B, Weisser WW, Weigelt A. 2013b. Separating drought effects from roof artifacts on ecosystem processes in a grassland drought experiment. *PLoS One* **8**(8): e70997.

von Gersdorff Jørgensen L. 2017. The fish parasite Ichthyophthirius multifiliis - Host immunology, vaccines and novel treatments. *Fish & Shellfish Immunology* **67**: 586-595.

Wagg C, Roscher C, Weigelt A, Vogel A, Ebeling A, de Luca E, Roeder A, Kleinspehn C, Temperton VM, Meyer ST, et al. 2022. Biodiversity-stability relationships strengthen over time in a long-term grassland experiment. *Nature Communications* **13**(1): 7752.

Wakelin SA, Armstrong C, Ganasamurthy S, Wigley K, Mercier C. 2025. Comparison of primer sets for short-read NGS-based characterisation of oomycete communities. *Journal of Microbiological Methods* **239**: 107293.

Walden S, Jauss RT, Feng K, Fiore-Donno AM, Dumack K, Schaffer S, Wolf R, Schlegel M, Bonkowski M. 2021. On the phenology of protists: recurrent patterns reveal seasonal variation of protistan (Rhizaria: Cercozoa and Endomyxa) communities in tree canopies. *FEMS Microbiology Ecology* **97**(7): fiab081.

Walker CA, van West P. 2007. Zoospore development in the oomycetes. *Fungal Biology Reviews* **21**(1): 10-18.

Wallenhammar A-C. 1996. Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. *Plant Pathology* **45**: 710–719.

Wang L, Liu Z, Bres C, Jin G, Fanin N. 2025. Exploring microbial ubiquity across different plant functional groups and organs. *Plant and Soil* **513**: 2869–2885.

Wang MC, Bartnicki-Garcia S. 1974. Mycolaminarans: storage (1→3)-β-D-glucans from the cytoplasm of the fungus *Phytophthora palmivora*. *Carbohydrate Research* **37**(2): 331-338.

Wang Y, Koopmann B, von Tiedemann A. 2022. Methods for Assessment of Viability and Germination of *Plasmodiophora brassicae* Resting Spores. *Frontiers in Microbiology* **12**: 823051.

Wannenmacher M, Haberstroh S, Kreuzwieser J, Doan TH, Niederberger J, Prietzel J, Lang F, Werner C. 2025. Spatio-Temporal Plasticity of Root Exudation in Three Temperate Tree Species: Effects of Season, Site, and Soil Characteristics. *Physiologia Plantarum* **177**(6): e70681.

Wardle DA. 1999. Is "Sampling Effect" a Problem for Experiments Investigating Biodiversity-Ecosystem Function Relationships? *Oikos* **87**(2): 403-407.

Weber A, Zwillenberg L, van der Laan P. 1952. A Predacious Amœboid Organism destroying Larvæ of the Potato Root Eelworm and other Nematodes. *Nature* **169**: 834–835.

Weigelt A, Weisser WW, Buchmann N, Scherer-Lorenzen M. 2009. Biodiversity for multifunctional grasslands: Equal productivity in high-diversity low-input and low-diversity high-input systems. *Biogeosciences* **6**: 1695–1706.

Weiland JJ, Bornemann K, Neubauer JD, Khan MFR, Bolton MD. 2019. Prevalence and Distribution of Beet Necrotic Yellow Vein Virus Strains in North Dakota and Minnesota. *Plant Disease* **103**(8): 2083-2089.

Weisser WW, Roscher C, Meyer ST, Ebeling A, Luo G, Allan E, Beßler H, Barnard RL, Buchmann N, Buscot F, et al. 2017. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic and Applied Ecology* **23**: 1-73.

Whittaker RH. 1969. New Concepts of Kingdoms of Organisms: Evolutionary relations are better represented by new classifications than by the traditional two kingdoms. *Science* **163**(3863): 150-160.

Win-Tin, Dick MW. 1975. Cytology of Oomycetes: Evidence for Meiosis and Multiple Chromosome Associations in Saprolegniaceae and Pythiaceae, with an Introduction to the Cytotaxonomy of *Achlya* and *Pythium*. *Archives of Microbiology* **105**: 283–293.

Xiong W, Jousset A, Guo S, Karlsson I, Zhao Q, Wu H, Kowalchuk GA, Shen Q, Li R, Geisen S. 2018. Soil protist communities form a dynamic hub in the soil microbiome. *ISME Journal* **12**(2): 634-638.

Xiong W, Song Y, Yang K, Gu Y, Wei Z, Kowalchuk GA, Xu Y, Jousset A, Shen Q, Geisen S. 2020. Rhizosphere protists are key determinants of plant health. *Microbiome* **8**(1): 27.

Xue P, Minasny B, McBratney A, Jiang Y, Luo Y. 2023. Land use effects on soil protists and their top-down regulation on bacteria and fungi in soil profiles. *Applied Soil Ecology* **185**: 104799.

Yelenik SG, D'Antonio CM. 2013. Self-reinforcing impacts of plant invasions change over time. *Nature* **503**(7477): 517-520.

Zhang Q, Yang R, Tang J, Yang H, Hu S, Chen X. 2010. Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. *PLoS One* **5**(8): e12380.

Zhao J, Fan D, Guo W, Wu J, Zhang X, Zhuang X, Kong W. 2023. Precipitation Drives Soil Protist Diversity and Community Structure in Dry Grasslands. *Microbial Ecology* **86**(4): 2293-2304.

Zuppinger-Dingley D, Schmid B, Petermann JS, Yadav V, De Deyn GB, Flynn DF. 2014. Selection for niche differentiation in plant communities increases biodiversity effects. *Nature* **515**(7525): 108-111.