Functional Traits and Spatio-Temporal Structure of a Major Group of Soil Protists (Rhizaria: Cercozoa) in a Temperate Grassland

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Soil protists are increasingly appreciated as essential components of soil foodwebs; however, there is a dearth of information on the factors structuring their communities. Here we investigate the importance of different biotic and abiotic factors as key drivers of spatial and seasonal distribution of protistan communities. We conducted an intensive survey of a 10 m² grassland plot in Germany, focusing on a major group of protists, the Cercozoa. From 177 soil samples, collected from April to November, we obtained 694 Operational Taxonomy Units representing >6 million Illumina reads. All major cercozoan taxonomic and functional groups were present, dominated by the small flagellates of the Glissomonadida. We found evidence of environmental selection structuring the cercozoan communities both spatially and seasonally. Spatial analyses indicated that communities were correlated within a range of 3.5 m. Seasonal variations in the abundance of bacterivores and bacteria, followed by that of omnivores suggested a dynamic prey-predator succession. The most influential edaphic properties were moisture and clay content, which differentially affected each functional group. Our study is based on an intense sampling of protists at a small scale, thus providing a detailed description of the biodiversity of different taxa/functional groups and the ecological processes involved in shaping their distribution.

Keywords: biogeography, functional traits, soil ecology, protozoa, microbial assembly, environmental selection, dispersal limitation, soil protists

INTRODUCTION

Our understanding of soil ecosystem functioning relies on a clear image of the drivers of the diverse interactions occurring among plants and the components of the soil microbiome – bacteria, fungi, and protists. Protists are increasingly appreciated as important components of soil foodwebs (Bonkowski et al., 2019). Their varied and taxon-specific feeding habits differentially shape the communities of bacteria, fungi, algae, small animals, and other protists (Geisen, 2016;
factors, soil moisture (Bates et al., 2013; Lentendu et al., 2014), to protistan community assembly. In particular, among abiotic temporal abiotic and biotic processes significantly contributed communities in soil, we would demonstrate that spatial and hypothesized that by providing a thorough sampling of protistan characterized at large scales (Bates et al., 2013), some authors differences in soil organic matter quality (Christensen et al., 1992; trade-offs between r- and K-selected communities driven by selection of phylogenetically clustered microbiomes (Sapp et al., 2018), habitat filtering by root exudates resulting in N diversity of soil protists. We provided a functional trait-based classification of cercozoan and related endomyxan taxa found in our survey. We explored in a small, unfertilized grassland plot, how spatial distance, season, and edaphic parameters (abiotic and biotic) shape the diversity and dynamics of the cercozoan communities.

**MATERIALS AND METHODS**

**Study Site, Soil Sampling, and DNA Extraction**

The sampling site was located near the village of Wittlingen, Baden–Württemberg, in the Swabian Alb (“Schwäbische Alb”), a limestone middle mountain range in southwest Germany. Details of the sampling procedure are provided elsewhere (Regan et al., 2014, 2017; Stempfhuber et al., 2016). Briefly, a total of 360 samples were collected over a 6-month period from spring to late autumn in a 10 m² grassland plot within the site AEG31 of the Biodiversity Exploratory Alb (48.42 N; 9.5 E), with a minimum distance of 0.45 m between two adjacent samples (Supplementary Figure S1). For this study, we selected 180 samples, 30 samples from each sampling date (April, May, June, August, October, and November 2011). We were provided the soil DNA, extracted from duplicate homogenized soil subsamples (300 mg each) as described in Regan et al. (2017). Soil physicochemical parameters were determined as described in Regan et al. (2014, 2015); the parameters included in our analyses are listed in Supplementary Table S1, with their seasonal variation illustrated in Supplementary Figures S2, S3. Over this area, spatial variability was limited; only the proportion of clay content varied (indicated in Supplementary Figure S2 by high boxes). Soil moisture changed most dramatically over the sampling period, with a peak in April and lowest values in May and October (average = 40%, max = 63%, min = 23%, SD = 11). Microbial biomass-related carbon and nitrogen parameters peaked in April. Bacterial cell counts showed a distinct peak in April. Living plant and fungi-related parameters
followed the seasonal pattern of a minimum after winter, a maximum in summer, and a decrease in autumn (for the plant biomass, after mowing in August), with plant litter biomass following an inverse trend (Supplementary Figure S2).

**Amplification, Library Preparation, and Sequencing**

Primer design, barcoding primers, amplification, library preparation, and Illumina sequencing have been described in detail (Fiore-Donno et al., 2018). The primers covered nearly the total diversity of Cercozoa, although they were biased against Endomyxa (mostly parasitic lineages). The primers amplified a fragment of 335–544 bp of the hypervariable region V4 of the SSU. Briefly, amplicons were obtained in two successive PCRs, the first using 1 µl of 1:10 soil DNA as template, the second using semi-nested primers and 1 µl of the first PCR as template. We employed the following final concentrations: GreenTaq polymerase (Fermentas, Canada) 0.01 units, buffer 1X, dNTPs 0.2 mM and primers 1 µM. The thermal program consisted of an initial denaturation step at 95°C for 2 min, 24 cycles at 95°C for 30 s, 50°C for 30 s, 72°C for 30 s; and a final elongation step at 72°C for 5 min. Barcoded primers were used in the second PCR to index samples. We pooled 15 µl of each of the successfully amplified samples (including the mock community, see below), then reduced the total volume to 80 µl; c. 800 ng of amplicons were used for the single library preparation as previously described (Fiore-Donno et al., 2018). The library concentration was 23 nM, of which 10 pM were used for the Illumina sequencing run. Sequencing was performed with a MiSeq v2 Reagent kit of 500 cycles on a MiSeq Desktop Sequencer (Illumina Inc., San Diego, CA, United States) at the University of Geneva (Switzerland), Department of Genetics and Evolution. To validate the bioinformatics pipeline (see below), we amplified DNA from a “mock community”, consisting of five categories (Supplementary Table S3), plus Cercomonas longicauda provided by S. Flues (GenBank DQ442884), totaling 11 species.

**Sequence Processing**

Paired reads were assembled following a published protocol (Lejzerowicz et al., 2014). The quality filtering discarded (i) all sequences with a mean Phred quality score < 30, shorter < 25 bases, with 1 or more ambiguities in the tag or in the sequence and with more than one ambiguity in the primers, and (ii) assembled sequences with a contig of < 100 bp and more than 10 mismatches (Table 1). Sequences were sorted by samples (“demultiplexing”) via detection of the barcodes (Supplementary Table S2; Fiore-Donno et al., 2018). The bioinformatics pipeline was optimized using the mock community. We first separated the sequences obtained from the 11 known taxa and ran the analysis with the steps listed in Table 1. The settings that made it possible to retrieve the expected 11 operational taxonomic units (hereafter OTUs) from the mock community were then applied to the environmental sequences.

Sequences were clustered into OTUs using vsearch v.1 (Rognes et al., 2016), with the abundance-based greedy clustering algorithm (agc) implemented in mothur v.3.9 (Schloss et al., 2009) with a similarity threshold of 97%. Using BLAST+ (Camacho et al., 2008) with an e-value of 1⁻⁵⁰ and keeping only the best hit, OTUs were identified using the PR2 database (Guillou et al., 2013); non-cercozoan OTUs were removed. Chimeras were identified using UCHIME (Edgar et al., 2011) as implemented in mothur v.3.9 as previously described (Fiore-Donno et al., 2018); chimeras and misaligned sequences were removed (Table 1).

**Cercozoan Functional Traits**

We selected three categories of ecological relevance: feeding mode, morphology, and locomotion mode. For the feeding mode, we classified the cercozoan and endomyxan OTUs into bacterivores, eukaryvores (feeding on algae, fungi, other protists, and small animals but with no reports of feeding on bacteria) and omnivores, feeding on both bacteria and eukaryotes, according to available information in the literature. We applied two criteria for morphological classification: (i) the presence or absence of a shell (testate or naked); (ii) if the cell was an amoeba, a flagellate or an amoeboid flagellate. We retained existing combinations, consisting of five categories (Supplementary Table S3). We further distinguished two types of tests, organic or agglutinated – from those made of silica. The major difference in locomotion mode was set between cells creeping/gliding on

| TABLE 1 | Number of reads retrieved at each step of the bioinformatic pipeline. |
|---------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| **Mock community (11 taxa)**   | Unique              | Genuine Cercozoa    | Aligned             | Non-chimeric        | Clustered 97% sim.  |
| **Representative sequences**   | 8430                | 7582                | 7552                | 7319                | 182                 |
| **All sequences**              | 22818               | 14723               | 14693               | 14321               | 14321               |
| **% removed**                  | 0                   | 10                  | 0.2                 | 3                   | 0                   |
| **Environmental reads**        |                      |                     |                     |                     |                     |
| **Trimmed**                    |                      |                     |                     |                     |                     |
| **Representative sequences**   | 10052231            | 5556619             | 1324                | 694                 |
| **All sequences**              | 10052231            | 10029413            | 7856763             | 6225241             |
| **% removed**                  | 0                   | 22                  | 2                   |                     |

Initial number of paired reads = 15445807.
substrate (on soil particles or on roots) versus free-swimming ones (in interstices filled with water). The phytomyxean parasites (Endomyxa), due to their peculiar life cycle, were considered separately in each functional category. We assigned traits at different taxonomic levels, from order to genus (Supplementary Table S3), and provide the respective references (Supplementary Table S4).

**RESULTS**

**Sequencing Results**
We obtained over 15 million filtered, paired reads (Table 1). The rate of mis-tagging during the sequencing run (indicating

1http://sankey-diagram-generator.acquireprocure.com/ (accessed October 2018).
Supplementary Figure S4

sequence in the PR2 database (< 1000 sequences) contributed to only 3% of the total sequences. These 12 OTUs were attributed to five functional traits: Glissomonadida (mostly Sandonidae), Cryomonadida (mostly two different Rhogostoma lineages), Plasmodiophorida (mostly the parasitic Polymyxa graminis), Cercomonadida (Eocercomonas and Paracercomonas), and Spongomonadida. The phylogenetic tree (Supplementary Figure S5) obtained with 176 reference sequences and 694 OTUs was rooted with Phytomyxea (Endomyxa); the vampyrellids (Proteomyxidea) and the Novel Clades 10-11-12, were paraphyletic to the monophyletic Cercozoa (93%). In Cercozoa, the main clades were recovered, although with low support. We were able to recover environmental sequences from nearly every clade of the tree.

The rarefaction curve including all samples reached a plateau, suggesting that the global richness of 694 OTUs could have been obtained by c. 70000 sequences (Supplementary Figure S6A), and by only 15 samples (Supplementary Figure S6B), and that the observed distribution patterns would not have been influenced by undersampling. Most OTUs were present in all sites (only 8.15% of absences in Supplementary Table S2). Multiple site beta diversity, calculated with either presence-absence or abundance on both rarefied and relative data, showed minor variation between the six sampling dates, which was confirmed by a resampling approach of random subsets (average resampled Bray–Curtis distances comprised between 0.715 and 0.745) (Figure 3).

Diversity of Cercozoa

At a high taxonomic level, the majority of the 694 OTUs could be assigned to the phylum Cercozoa (91% of the sequences) (Figure 1), the remaining to Endomyxa (9%) and to the incertae sedis Novel clade 10 (Tremulida, 1%). Only 39% of the OTUs had 97-100% similarity to any known sequence in the PR2 database (Supplementary Figure S4). The 12 most abundant OTUs (>10000 sequences) accounted for 45% of the total sequences, while many low-abundance OTUs (243 < 1000 sequences) contributed to only 3% of the total sequences. These 12 OTUs were attributed to five orders: Glissomonadida (mostly Sandonidae), Cryomonadida (mostly two different Rhogostoma lineages), Plasmodiophorida (mostly the parasitic Polymyxa graminis), Cercomonadida (Eocercomonas and Paracercomonas), and Spongomonadida. The phylogenetic tree (Supplementary Figure S5) obtained with 176 reference sequences and 694 OTUs was rooted with Phytomyxea (Endomyxa); the vampyrellids (Proteomyxidea) and the Novel Clades 10-11-12, were paraphyletic to the monophyletic Cercozoa (93%). In Cercozoa, the main clades were recovered, although with low support. We were able to recover environmental sequences from nearly every clade of the tree.

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Functional Diversity

More than half of the soil cercozoan OTUs were considered to be bacterivores (57%), followed by omnivores (21%), and eukaryvores (4%). Plant parasites (3%) and parasites of Oomycota (1%) were only marginally represented (Figure 2). Naked flagellates (36%) or amoeboflagellates (34%) together
constituted the majority of the morphotypes, whereas testate cells (organic/agglutinated or siliceous) were less frequent, 12 and 7%, respectively. Naked amoebae were only marginally represented (1%). The dominant locomotion mode was creeping/gliding on substrate (86%), with only 1% free-swimming.

**Spatial Structuring and Seasonal Variation**

The spatial distribution of the cercozoan communities was non-random for all OTUs (Mantel $R = 0.1661$, $p = 0.0001$), and also for all functional groups considered (Table 2). Mantel correlograms indicated that similarities among communities decreased with distance, although the coefficients were low (Mantel correlograms, Figure 3 and Table 2). At distances between 0.45 to 3.9 m, cercozoan communities showed positive autocorrelations, whereas communities at distances between 5.5 and 12 m were more dissimilar (i.e., negatively correlated). No spatial autocorrelations were observed at distances ranging from 4 to 5.5 m (Figure 3). These results were reproducible at different distance classes, i.e., at intervals of 0.25, 0.5, and 1 m (Figure 3A). Similar results were obtained when functional traits were considered (Figure 3B).

In contrast to the overall homogeneity of beta diversity, cercozoan community structure changed significantly over time (anosim: $R = 0.22$, $p < 0.001$; PERMANOVA: $F_{5−170} = 5.01$, $p < 0.001$; MRPP: $p < 0.001$), although ordination (PCoA) suggested that the community turnover between April and May was most influential (Supplementary Figure S3). The cercozoan communities, binned by families or functional groups, showed distinct seasonal patterns of relative abundance (Figure 4). While the bacterivorous families peaked in April and decreased in May (except Spongomonadidae), the omnivorous, shell bearing Euglypha, Rhogostomidae, and Trinematidae exhibited an opposite pattern, increasing from April to May. The relative abundance of endomyxan plant parasites (Polymyxa and Spongospora) did not differ over the sampling season.

**The Main Driver of Cercozoan Species Turnover: Season, Distance, or Soil Parameters?**

Cercozoan beta diversity was influenced by soil parameters (shown in Supplementary Table S1) (RDA: $F_{11−164} = 3.3301$, $p = 0.001$), spatial distance (RDA: $F_{6−169} = 3.0202$, $p < 0.001$), and seasonality (RDA: $F_{3−172} = 3.389$, $p < 0.001$). Variance partitioning among the four predictors indicated that biotic and abiotic soil parameters, spatial distance, and seasonality together accounted for 18.32% (adjusted $R^2$) of the total variation in cercozoan beta diversity (Figure 5). Spatial distance explained 4.4% of the variation, followed by abiotic soil parameters (3.5%), seasonality (1.2%), and biotic soil parameters (0.4%). Different combinations of the predictors would explain the remaining variation, notably spatial distance/abiotic soil parameters (3%) and abiotic/biotic soil parameters (2.3%).

**Edaphic Parameters Influencing Cercozoan Communities**

Among soil abiotic factors, soil moisture was most important (ANOVA: $F_{1,163} = 11.546$, $p < 0.001$), followed by clay, organic C, pH, total N, and NO$_3$~content (Table 3). Biotic parameters (microbial biomass C and N contents, number of bacteria, litter, archaeal 16S, plant biomass and fungal PLFAs) explained a significant but lower amount of the variation in cercozoan beta diversity (Table 3). Using linear mixed effect models, we specified which abiotic and biotic factors affected the 12 most abundant cercozoan families (Supplementary Table S6). Two-thirds of the cercozoan/endomyxan families (8 of 12) were
positively affected by soil moisture \( (p < 0.001) \), but not the Spongomonadidae, the Allapsidae, or the parasitic Spongospora nasturtii and Polymyxa lineages. The relative abundances of the testate amoebae, i.e., Euglyphidae and unclassified Euglyphida, Trinematidae, and Rhogostomidae, responded negatively to soil moisture. In contrast, naked flagellates and amoeboflagellates were positively correlated with increasing soil moisture. Flagellates correlated negatively and amoeboflagellates positively with clay content. Flagellates, Spondonidae, and plant parasitic Polymyxa were positively correlated with pH, while testate amoebae (Euglyphidae) were negatively associated with pH (Supplementary Table S6).

**DISCUSSION**

**Environmental Selection or Random Distribution?**

Using Cercozoa and Endomyxa as models of very diverse and abundant soil protists, we demonstrated that their community composition in a small grassland site was non-random, but instead was spatially and temporally structured. With an average of an estimated 1000 OTUs per gram of soil, high local species richness was a striking characteristic of cercozoan communities, with however small changes in beta diversity across space and time (Supplementary Table S5). Our results align with the consistent patterns of high alpha - and low beta diversity which have been found for protists in grasslands (Fiore-Donno et al., 2016) and, including specifically Cercozoa, in tropical forests (Lentendu et al., 2018). Sufficient sampling, attested by our saturation curves (Supplementary Figure S4), allowed us to further partition the beta diversity suggesting that deterministic processes drive the observed cercozoan community assembly. Nonetheless, our design did not allow us to exclude the influence of neutral dynamics and competitive interactions that may lead to similar distribution patterns (Dini-Andreote et al., 2015). Spatial distance (4.4%) and soil biotic and abiotic factors (2.9%) explained substantial variation indicating that cercozoan communities were significantly influenced by spatial gradients in the edaphic parameters (Figure 5 and Supplementary Figure S2).

On a small spatial scale, our results correspond to large-scale patterns of protistan distribution as described by Lentendu et al. (2018), who established environmental selection as the main process driving protistan spatial patterns. This is in striking contrast to two recent studies. Bahram et al. (2016) reported a random spatial distribution of small soil eukaryotes, including protists, in boreal forests at a range of 0.01 to 64 m.
They explained the observed distribution by invoking drift and homogenizing dispersal. The most striking difference compared with our study is the low efficiency of their ITS2 primers for the retrieval of protists (66 rhizarian OTUs, including the Cercozoa). This is at least one order of magnitude lower than in our data, possibly indicating a non-thorough sampling of the rhizarian communities in their study, which could in turn have hampered a robust assessment of the observed distribution patterns. In the second study, Zinger et al. (2018) suggested the absence of dispersal limitation and a stochastic distribution of protists in a tropical forest, using a 10 m-resolution sampling grid. This might have been too coarse to detect spatial patterns of protists: according to our results, only negative correlations could be detected at such a distance (Figure 3).

Although we established the importance of environmental selection, homogenizing processes such as neutral assembly mechanisms may have contributed to the observed community assembly, as suggested by the positive autocorrelation of cercozoan communities up to a distance of 3.9 m (Figure 3). However, 18.32% of explained variance (Figure 5) highlighted the importance of microhabitats for the non-random distribution of the cercozoan communities.

### Environmental Selection and Patch Dynamics Selected for Specific Functional Traits

Significant relationships between functional traits and soil abiotic and biotic factors (Supplementary Table S6) indicated that the distribution of protistan traits was driven by environmental selection in our study site. Some of these environmental filters showed marked spatial gradients (clay content, pH, total N), while other showed more temporal variation (moisture, NH$_4^+$, total plant biomass) (Supplementary Figure S2). Soil moisture is well known to influence microbial activity (Tecon and Or, 2017). The spatial variation in clay content together with the temporal variation in soil moisture triggered opposite responses from specific lineages or functional groups (Supplementary Table S6 and Figure 6).

Soil moisture was a major abiotic predictor in our study site, although with different effects according to taxonomic and functional groups. While flagellates and amoeboflagellates were favored by moisture, the cercozoan testate cells were correlated with drier conditions, suggesting patch dynamic processes connected to different living modes. In accordance with our model for Euglyphididae, Ehrmann et al. (2012) reported a preference of testate amoebae for relatively dry soils and low pH in forests. We can conclude that in grasslands, testate amoebae exhibit drought resistance, in contrast with naked cells and cells covered with scales (i.e., Thaumatomonadidae), suggesting a protective role of the shell (Supplementary Table S4). Building a shell, however, slows down the reproduction rate (Schönborn, 1992), and thus generates a trade-off between protection and reproductive fitness. In contrast, amoeboflagellates and flagellates, with their faster reproduction rate (Ekelund and Rønn, 1994), would have an increased fitness in moist conditions.

The spatial distribution of soil clay content was an important structuring factor in our study site. Experimentally increasing clay content has been shown to improve water retention capacity and favor soil bacteria (Heijnen et al., 1993). In addition, it leads to a reduction of habitable pore space, which in our study seemed to favor the amoeboflagellates (Paracercomonadidae and Cercomonadidae) and the naked flagellates (Allapsidae, but not Sandonidae) (Figure 6 and Supplementary Table S6). The small percentage of free-swimming protists was negatively correlated with bulk density (Supplementary Table S6), suggesting their preference for larger soil pores. Another important structuring factor was pH, despite its low variability in our study site.
Schematic illustration showing the positive (+) or negative (–) interactions between the four most influential soil physicochemical parameters (based on the ANOVA, Table 3), and the relative abundances of the major families or morphotypes (details of the models in Supplementary Table S6).

(Supplementary Figure S2 and Supplementary Table S1 – pH 6.08–7.23). Globally, cercozoans have been shown to prefer neutral or basic soils (Dupont et al., 2016) and their relative abundance was seen to increase significantly along a pH gradient from c. 4 to 6.5 (Shen et al., 2014). Over a narrower gradient, we showed that Sandonidae and the endomyxan parasitic Spongospora nasturtii lineage were positively correlated with a slightly basic pH, and the Euglyphaidae with a slightly more acidic one (Figure 6 and Supplementary Table S6). Our results thus suggest that different taxa could have different pH preferences. Soil organic carbon had a negative effect only on the Euglyphaidae, in conjunction with nitrogen content, while the C/N ratio had a positive effect, suggesting their preference for low-nutrient soils with a relatively higher C than N content.

Bacterial cell counts were a major explanatory biotic factor, negatively correlated with endomyxan plant parasites. This correlation was robust, also when taxonomy, nutrition or locomotion modes, and morphology, were considered. This illustrates the fundamental role of biotic interactions shaping microbial community structure and ultimately plant health (Hassani et al., 2018) and confirms the filtering effect on the cercozoan/endomyxan community structure revealed in the rhizosphere of Arabidopsis thaliana (Sapp et al., 2018). In our study, cercozoan taxa that positively correlated with bacterial numbers were identified as creeping/gliding on substrate. This is in accordance with soil protists feeding mostly on bacterial biofilms (Böhme et al., 2009). Interestingly, there was no positive correlation between bacterial cell counts and bacterivores. Most bacterivorous families, however, were strongly and positively correlated with soil moisture (Supplementary Table S6), which shared a strong peak in April with bacterial cell counts, potentially masking the effect of bacterial cell numbers (although there was no co-correlation between those variables across the year). Contrary to other bacterivores, Spongomonadidae did not follow the seasonal variation of the bacteria (Figure 4). This may be explained by their living modes, mostly in substrate-attached colonies (Strüder-Kypke and Hausmann, 1998). It has been suggested that the colonial species may also feed by saprotrophy (Strüder-Kypke and Hausmann, 1998), a hypothesis supported by our observed positive correlation with extractable organic carbon (Supplementary Table S6). We know very little about the interactions of archaea with soil protists. Thus it is worth pointing out the to date unexplained significant negative effect of archaeal abundance on Paracercomonadidae (Supplementary Table S6).
Seasonal Variability Affected the Trophic Structure of Cercozoan Communities

Cercozoan communities showed seasonal oscillations, in accordance with results from bacteria in grasslands (Muller Barboza et al., 2018). The most significant community turnover occurred between April and May (Supplementary Figure S3A), when a series of concomitant changes in edaphic factors was observed (Supplementary Figure S2). The high soil moisture in April favored bacterial activity and proliferation. At the same time, high levels of extractable organic C indicated abundant root exudates or decomposition at the onset of the plant growing season, when plant total biomass was still low. We hypothesized that this triggered a series of events: the bacteria, usually C limited, were stimulated by this C input. Since fungi were not yet abundant, bacteria were mostly responsible for the high nitrogen content in the microbial biomass. Consequently, the release of NH$_4^+$ (also peaking in April) may be best explained by protistan predation on bacteria (Bonkowski, 2004). This was confirmed by the high abundances of five bacterivorous families (Figure 4). In May, bacteria, bacterivores, and nitrogen-related parameters all decreased, together with soil moisture. In sharp contrast, all three omnivorous families increased in May. We hypothesized that predation by omnivores could have contributed to the decline of the bacterivores, in addition to the negative effect of declining soil moisture (Supplementary Figure S2). Our study, based on DNA, does not make it possible to distinguish between active and resting stages, but we probably also amplified extracellular DNA from recently deceased cells. Thus, the seasonal variation we observed was probably an underestimate.

Our hypothesis that the abundance of plant parasites would follow the annual plant cycle was not supported, since they showed no seasonal variation (Supplementary Figure S2). Phytophymesans are known to form resistant cysts in plant root hairs that remain for years in the soil after plant decay (Dixon, 2009).

Number of OTUs, Diversity, and Functional Traits

Cercozoan diversity was in accordance with previous studies, which established Sarcomonadea (Glissomonadina and Cerconadina) as the dominant class in different terrestrial habitats (Howe et al., 2009; Geisen et al., 2015; Fiore-Donno et al., 2018): more specifically in feces (Bass et al., 2016), in the soil of neotropical forests (Lentendu et al., 2018), on the leaves of Brassicaceae (Ploch et al., 2016), in heathlands (Bugge Harder et al., 2016), and in German grasslands, including the site studied here (Venter et al., 2017). Especially the genus Rhogostoma is very common in soil (Fiore-Donno et al., 2018).

In conclusion, we showed that environmental selection driven by abiotic and biotic edaphic factors significantly determined community assembly of Cercozoa and Endomyxa. Considering functional traits and their trade-offs, we were able to highlight the importance of environmental selection and patch dynamics as underlying processes. We believe that our study has bearing for other soil protists and soil ecosystems beyond the limits of this small grassland plot. Once the patterns underlying the small-scale distribution of protists are detected, they can be upscaled and contribute to understanding global protistan biogeographies. This is a prerequisite for predicting effects of human-induced changes (i.e., land management or global warming) on these widespread and functionally important soil organisms.

AUTHOR CONTRIBUTIONS

EK and SM developed the design of the SCALEMIC experiment. KMR performed the DNA extractions. KMR and RSB analyzed the soil properties. AMF-D conducted the amplifications, Illumina sequencing, and bioinformatics pipeline. TR-H, FD, and AMF-D performed the statistics. AMF-D, TR-H, and MB interpreted the data. AMF-D wrote the manuscript. All authors participated in the revisions and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01332/full#supplementary-material
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