Soil Biology & Biochemistry 112 (2017) 68–76

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

# Distribution patterns of soil microbial eukaryotes suggests widespread algivory by phagotrophic protists as an alternative pathway for nutrient cycling

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## ARTICLE INFO

*Article history:*
Received 2 December 2016
Received in revised form
26 April 2017
Accepted 2 May 2017
Available online 8 May 2017

*Keywords:*
Protist
Eukaryotic micro-algae
Phagotrophic protists
Carbon cycling
V9 region of the SSU rRNA gene
High-throughput sequencing

## ABSTRACT

High-throughput sequencing (HTS) of soil environmental DNA (eDNA) allows assessing the full diversity of soil micro-eukaryotes. The resulting operational taxonomic units (OTUs) can be assigned to potential taxonomic and functional identities using increasingly complete reference databases. HTS of soil eDNA is revealing a high diversity and abundance of potential eukaryovorous protists, thus challenging the paradigm of the predominantly bacterivorous function of soil phagotrophic protists (i.e. microbial loop).

Using Illumina sequencing of soil eDNA and targeting the V9 region of the SSU rRNA gene, we investigated the taxonomic and functional diversities, distribution and co-occurrence patterns of soil micro-eukaryotes in three land-use categories: forests, meadows and croplands located in Switzerland. Each OTU was assigned to a broad functional category (phototrophs, phagotrophs, osmotrophs, or parasites).

Total OTU richness was similar in the three land-use categories, but community composition differed significantly between forests and other land-uses. The proportion of fungal sequences (especially Basidiomycota) was highest, and phototroph (i.e. soil microalgae) sequences least abundant in forests. Seven OTUs representing phagotrophic protists, together accounting for >25% of all phagotroph sequences, were significantly correlated to the total number of phototroph sequences, thus suggesting algivory. At least three of these OTUs corresponded to known algal predators.

These results suggest that beyond plants, soil microalgae represent a functionally significant but rarely considered input of carbon in soils that should be taken into account when modelling soil nutrient cycling.

## 1. Introduction

Our perception of the diversity and functional roles of protists is rapidly changing due mainly to the application of high-throughput sequencing (HTS) of environmental DNA (eDNA). HTS has revealed the extent of the huge unknown protist diversity in the photic zone of the world's oceans and shown that a large fraction of this diversity corresponded to mutualistic and parasitic symbionts (de Vargas et al., 2015). Likewise, studies performed on terrestrial habitats are revealing similarly high diversity of protists with a dominance of saprotrophs and parasites (Dupont et al., 2016; Geisen et al., 2014, 2015a). These studies also revealed that many protists feed on eukaryotes, thus questioning the long-held view that soil phagotrophs fed mainly on bacteria (i.e. soil microbial loop) (Dumack et al., 2016a,b; Geisen et al., 2015b, 2016; Geisen, 2016).

Soil microbial eukaryotes, including protists and fungi, are involved in numerous biotic interactions and recognised as key actors of biogeochemical cycling (Verni and Gualtieri, 1997; van der Wal et al., 2013), and are thus considered a key element in soil fertility. However, the first (and still often the only) recognised

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http://dx.doi.org/10.1016/j.soilbio.2017.05.002

functional role of soil protists was grazers of bacteria leading to the ”soil microbial loop” paradigm, according to which phagotrophic grazing on soil bacteria releases labile compounds such as ammonium that stimulate plant growth (Bonkowski and Clarholm, 2012; Clarholm, 1985). Although feeding on bacteria is unquestionably widespread in phagotrophic microbial eukaryotes, there is increasing evidence that eukaryovory (i.e. the act of feeding partially or exclusively on other eukaryotes) is also common (Dumack et al., 2016a,b; Geisen et al., 2016). This implies that soil nutrient cycles are likely more complex than generally assumed.

Recent studies focusing on soil invertebrates have also questioned the origin of the carbon source feeding the soil communities, suggesting that very few soil invertebrates depend on litter (Pollierer et al., 2009) and suggesting that soil algae represent a functionally relevant source of soil carbon (Schmidt et al., 2016). The latter experimental study showed that autotrophic microbes contributed up to 17% of the body carbon of collembolan and 3% of earthworms over one week. However it is yet unclear to what extent this input is direct or if algae are first ingested by microbial grazers such as soil phagotrophs.

Several soil protists are known to be highly specialized predators of eukaryotes. For example, grossglockneriid ciliates feed exclusively on fungi (Petz et al., 1985). Parasitoids are also frequent in soils, including the widespread but still poorly studied *Rozella* group (also known as “Rozellida”; Lara et al., 2010 or Cryptomycota Jones et al., 2011) which prey on chytrids, oomycetes and green algae and also include endo-nuclear parasites of Amoebozoa that ultimately cause cell death and lysis (Corsaro et al., 2014). In those cases, nutrient release by protists does not rely on bacterivory, implying pathways for nutrient cycling alternative to the microbial loop. It is unclear how quantitatively relevant this pathway is but one way to assess this is to study the diversity and abundance of taxa involved in these trophic relationships using the now available data from massive sequencing of soil environmental DNA.

The true diversity of soil protists has long been poorly known, mainly due to methodological limitations for their isolation, culture and subsequent identification (Ekelund and Ronn, 1994; Foissner, 1999). Metabarcoding (environmental DNA amplicon based identification) of high-throughput sequencing data is now the golden standard for environmental screening of microbial diversity (Pawlowski et al., 2016). HTS data may also inform on the functioning of ecosystems based on the genetic identification of the organisms and knowledge on their lifestyles (de Vargas et al., 2015; Lara et al., 2015; Massana et al., 2014). The next step is to infer the biotic relationships between these organisms, which can be hypothesized when OTUs co-occur systematically across many samples, as can now be assessed by HTS. In practice, the nature of these relationships (i.e. trophic, but also symbiosis, competition, etc.) is not known, and co-occurrence data can thus be difficult to interpret in biological terms. Examples of known relationships taken from the literature can however illustrate well-supported co-occurrence and clarify the true nature of these relationships between organisms. Examples are manifold: predation of ciliates on fungi (Petz et al., 1985), of cercozoa on chlorophytes (Dumack et al., 2016a; Hess et al., 2012; Hess and Melkonian, 2013) but also symbioses, like between trebouxiophytes and testate amoebae (Gomaa et al., 2013). Putative relationships inferred from metabarcoding studies can also be explored by conducting new observations and experiments.

Phototrophic protists (i.e. eukaryotic algae) in soils include mostly exclusive free-living phototrophs (e.g. Bacillariophyta, Chrysophyceae, Xanthophyceae) and photosymbionts as in lichens (e.g. Trebouxiophyceae). Soil eukaryotic algae constitute an important part of the so-called cryptogamic crusts, which represent a significant carbon input in arid ecosystems (Elbert et al., 2012; Freeman et al., 2009; Frey et al., 2013). They are however also widespread in more humid soils but their functional role there is less well known and, consequently, has not been considered in the classical model of the soil microbial loop (Berard et al., 2005).

In order to assess the patterns of micro-eukaryotic taxonomic and functional diversities and address questions such as the possible role of soil algae as a carbon source it is useful to compare contrasted terrestrial ecosystems. Here we describe and compare the overall diversity and community structure of soil micro-eukaryotes in forest, meadow and cropland soils from 44 sites in Switzerland based on Illumina sequencing of the V9 region of SSU rRNA gene. Based on these data, we explored more specifically the abundance patterns of phototrophs and the co-occurrence patterns with their potential phagotroph predators. This trophic link was also explored by direct microscopic observations.

## 2. Material and method

### 2.1. Sampling

We collected 44 soil samples in permanent plots of the Swiss Biodiversity Monitoring program which aims to assess biodiversity all over Switzerland (BDM http://www.biodiversitymonitoring.ch/en/home.html). The sites included three land-uses which cover most of the Swiss territory (16 forests, 16 meadows and 12 croplands) (Fig. 1, Table S1) and spanned a diversity of soil types that could be arguably considered as representative of the entire country. Likewise, samples were collected in a range of altitudes covering most of the Swiss territory (excepted alpine sites). We expect therefore to cover a significant part of the microeukaryotic diversity present in Swiss soils. In this purpose, each sample was characterized using the typology of Swiss natural habitats (Delarze et al., 2015) (Table S1). Forests included both coniferous (e.g. *Picea abies*), or broadleaved trees (e.g. *Fagus sylvatica*). Most meadows were amended and used to produce fodder. Croplands were used for maize, cereals or tobacco cultivation. Meadows and croplands were designed as open habitats as much more light reach their soil surface than in forests. Sampling was performed over one month between September 27th, 2012 and October 31st, 2012. At each site, three topsoil cores (5 cm diameter x 5 cm depth) were taken along a circle of 1 m radius in the same land-use and pooled. Soil samples were kept cool (in an icebox) and DNA was extracted within 2–3 days.

### 2.2. DNA extraction, amplification and sequencing

DNA was extracted using the MoBio PowerSoil extraction kit (Carlsbad, CA, USA) according to the manufacturer instructions. The SSU rRNA V9 region was amplified using the broad spectrum

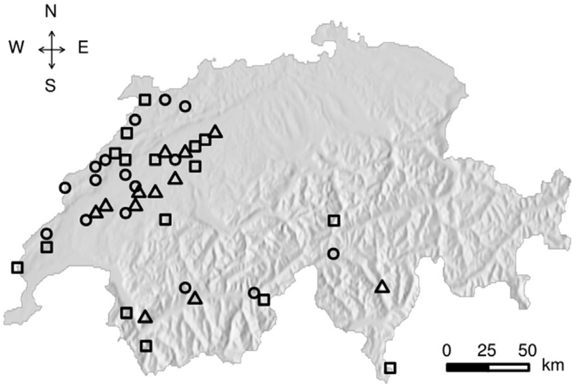

**Fig. 1.** Location of the 44 sampling sites in Switzerland. Squares, circles and triangles indicate forests, meadows, and croplands, respectively.