Soil Biology & Biochemistry 94 (2016) 10–18

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

The soil food web revisited: Diverse and widespread mycophagous soil protists

Stefan Geisen [a, *], Robert Koller [a, 1], Maike Hünninghaus [a], Kenneth Dumack [a], Tim Urich [b, 2], Michael Bonkowski [a]

[a] Department of Terrestrial Ecology, Institute of Zoology, University of Cologne, Germany
[b] Department of Ecogenomics and Systems Biology, University of Vienna, Austria

ARTICLE INFO

Article history:
Received 3 October 2015
Received in revised form
9 November 2015
Accepted 11 November 2015
Available online 27 November 2015

Keywords:
Soil protists
Soil food web
Trophic interactions
Fungi
Soil metatranscriptomes
Microcosm studies

ABSTRACT

Soil protists are commonly suggested being solely bacterivorous, serving together with bacterivorous nematodes as the main controllers of the bacterial energy channel in soil food webs. In contrast, the fungal energy channel is assumed to be controlled by arthropods and mycophagous nematodes. This perspective accepted by most soil biologists is, however, challenged by functional studies conducted by taxonomists that revealed a range of mycophagous protists. In order to increase the knowledge on the functional importance of mycophagous protists we isolated and initiated cultures of protist taxa and tested eight for facultative feeding on diverse fungi in microcosm experiments. Two different flagellate species of the genus *Cercomonas*, the testate amoeba *Cryptodifflugia operculata* and four genera of naked amoebae (*Acanthamoeba* sp., *Leptomyxa* sp., two *Mayorella* spp. and *Thecamoeba* spp.) fed and grew on yeasts with four taxa (*Cercomonas* sp., *Leptomyxa* sp., *Mayorella* sp., and *Thecamoeba* sp.) also thriving on spores of the plant pathogenic hyphal-forming fungus *Fusarium culmorum*.

To identify the potential importance of mycophagous protists in the environment we applied a data-mining approach targeting small subunit (SSU) rRNA data obtained in metatranscriptomes of five fundamentally different terrestrial samples. We focused our analyses on the distribution and relative abundances of two well-studied mycophagous protist groups, vampyrellid amoebae and grossglockneriid ciliates. Both groups were detected in all of the highly contrasting terrestrial samples, comprising up to 3% of all protist SSU rRNA transcripts. SSU transcripts of these two groups, in contrast to all remaining protist SSU transcripts, showed strong correlations with the relative abundance of fungal sequences indicating close direct trophic interactions.

Taken together, this study provides evidence that mycophagy among soil protists is common and might be of substantial but hitherto overlooked ecological importance in terrestrial ecosystems. Future studies should aim at evaluating taxon-specific (facultative) mycophagy, decipher changes caused in the fungal community and quantitatively evaluate the functional importance of this trophic position in soil ecosystems.

1. Introduction

Soil biologists generally discriminate the nutrient flows in soil food webs into a bacterial and a fungal-based energy channel (e.g. Moore and Hunt, 1988; Holtkamp et al., 2011). Heterotrophic protists are considered as major consumers of bacterial biomass supplementing higher trophic levels with nutrients bound in these microbes; in contrast, protists are supposed to be of marginal importance in the fungal energy channel where microarthropods and mycophagous nematodes are suggested to be the predominant consumers (Hunt et al., 1987; de Ruiter et al., 1995; Bonkowski, 2004).

A major reason why soil biologists largely treat protists as bacterivores derived from traditional extraction and cultivation methods that select for bacterivorous protists (Page, 1988; Berthold and Palzenberger, 1995; Ekelund, 1998). Protist taxonomists have, however, long realized that diverse facultative and obligate mycophagous protist taxa are common in soils (Old and Darbyshire, 1978; Petz et al., 1985; Ekelund, 1998). For example, all described

* Corresponding author. Current address: Department of Terrestrial Ecology, Netherlands Institute for Ecology (NIOO-KNAW), Wageningen, The Netherlands. Tel.: +31 (0) 317 473 613.
E-mail address: S.geisen@nioo.knaw.nl (S. Geisen).
[1] Current address: Institute of Bio- and Geosciences, IBG-2: Plant Sciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany.
[2] Current address: Institute for Microbiology, Ernst-Moritz-Arndt University Greifswald, Germany.

http://dx.doi.org/10.1016/j.soilbio.2015.11.010
0038-0717/

ciliates of the family Grossglockneriidae are obligatory mycophagous (Foissner and Didier, 1983; Petz et al., 1985, 1986). Facultative mycophagous protists (omnivores) that feed on a range of soil eukaryotes are vampyrellid amoebae (Old and Darbyshire, 1978; Hess et al., 2012), *Thecamoeba* spp. (Bamforth, 2004), few testate amoebae (Coûteaux and Darbyshire, 1998; Mitchell et al., 2008; Wilkinson, 2008; Wilkinson and Mitchell, 2010) and eumycetozoans (Stephenson and Feest, 2012). All of the aforementioned taxa are usually large (often >100 μm), but mycophagy has even been reported in small flagellates (Hekman et al., 1992; Flavin et al., 2000). Still, detailed knowledge on diversity and functional importance of mycophagous protists in terrestrial ecosystems remains limited, despite the fact that mycophagous protists can reach biomasses similar to those of bacterivorous protists (Ekelund, 1998).

Recent methodological advances using molecular techniques provided means of investigating the largely unknown diversity of soil protists. These sequence based studies have revealed that cultivable protists only represent a small fraction of the total protist community in soils (Foissner, 1999b; Bates et al., 2013; Kamono et al., 2013; Geisen et al., 2015b). The ecological function of uncultivated protists remains, however, largely unknown, as it is usually impossible to reliably link molecular phylogenetic information with realized ecological functioning. Molecular sequence-information needs to be supplemented with functional information on ecological traits of the respective taxon, which relies mainly on *in-vitro* studies on cultivated protists. Experiments on cultivated species revealed not only a variety of mycophagous protists, but also that even closely related protists differentially feed on bacterial prey (e.g. Böhme et al., 2009; Glücksman et al., 2010; Saleem et al., 2012). Mycophagous protists might also feed differentially on fungi, which could lead to shifts in fungal communities in soils. Some evidence for this hypothesis came from former studies showing that yeasts are a preferred food source for protists, while hyphae forming fungi were a less suitable prey (Heal, 1963; Bunting et al., 1979; Allen and Dawidowicz, 1990).

In order to increase the knowledge on the diversity and importance of mycophagy among soil protists we (1) cultivated soil protists and tested if facultative mycophagy is common for "bacterivorous" protists. Further (2) we used a data mining approach on SSU rRNA sequences obtained in metatranscriptomes of five highly diverse soils (Geisen et al., 2015b) to investigate the presence and relative abundance of mycophagous protists.

2. Materials and methods

2.1. Isolation of protists from soil and microscopic observation on facultative mycophagy

Soil samples were taken in Pulheim Stommeln (Germany; 51°01′N, 6°45′E); Müncheberg (Germany; 52°30′N, 14°07′E), in Les Verrines (France; 46°25′N, 0°7′E) and Cologne (Germany; 50°55′N, 6°55′E). The organic soil horizon was sampled in three locations (upper 2 cm in Pulheim Stommeln, 10 cm in Müncheberg and 10 cm in Cologne). Additionally, soil from earthworm burrows (2 mm around burrows) was sampled at Les Verrines (Table 1).

Enrichment cultures were established to isolate (facultative) mycophagous protists. From each soil sample, 1 g dry weight of soil was suspended in 250 ml Neff's Modified Amoeba Salina (NMAS) according to Page (1988). After shaking on an orbital shaker (Köttermann, Uetze, Germany) at 100 rpm for 10 min and fourfold dilution with NMAS, 20 μl of the suspension was added to wells of a 24 multiwell-plate (Sarstedt, Nümbrecht, Germany). A mixed fungal inoculum of 80 μl of a 0.4 g l^{-1} NMAS solution of dried *Saccharomyces cerevisiae* (Ruf, Quakenbrück, Germany) and 160 μl of a *Fusarium culmorum* spore solution with a concentration of four spores * μl^{-1} were added to each well. Plates were sealed with Parafilm and stored at 15 °C in the dark. These enrichment cultures were examined microscopically for mycophagous protists, i.e. growth on fungi and ingestion of fungal material, 7 and 21 days after incubation using an inverted microscope (Nikon Eclipse TS-100, Japan) at 100× and 400× magnification.

Subsequently, enrichment cultures with fungal growth medium were initiated using malt extract agar (MEA; 1.5%). MEA plates were prepared by adding malt extract (1.5%; AppliChem, Darmstadt, Germany) and agarising it by adding 0.5% non-nutrient agar followed by autoclaving (122 °C, 20 min). MEA plates were inoculated with 100 μl suspension of *F. culmorum* spores and hyphae in H_2O_{dest} to establish active fungal cultures. Cultures of the yeast *Cryptococcus laurentii* were incubated on potato glucose agar (1.5%; Sigma–Aldrich, St. Louis, USA) supplemented with yeast extract (0.5%; Oxoid Limited; Hampshire, England).

Amoebae and amoeboflagellates that showed indications of facultative mycophagy (Table 1) were cultivated monoxenically on bacteria for subsequent microcosm experiments. For that, individual protists were transferred from enrichment cultures to 60 mm Petri dishes filled with NMAS using a tapered glass pipette under an inverted phase-contrast microscope (Nikon TS-100, Japan). These monoclonal protist cultures were incubated at room temperature. Observations and microphotographs of protists were performed on a Nikon Eclipse 90i (Japan) equipped with phase contrast and Differential Interference Contrast optics at 100–400× magnification.

Protist cultures obtained were tested for their feeding preferences on three fungal taxa, i.e. two yeasts *C. laurentii* and *S. cerevisiae* and spores of the hyphal-forming fungus *F. culmorum*. A low-density fungal suspension (100 μl fungal suspension) with a concentration of 300 cells μl^{-1} (*S. cerevisiae* and *C. laurentii*) or 40 cells μl^{-1} (*F. culmorum*) was directly added to protist cultures. The protist cultures were grown on accompanying bacteria in a 60 mm petridish for one week. The cultures were microscopically investigated for uptake of fungal material 2 and 24 h after inoculation, and microphotographs of protists ingesting fungal material were recorded.

2.2. Facultative mycophagy of the bacterivorous protist Acanthamoeba castellanii

Further microcosm tests were conducted using the model protist *Acanthamoeba castellanii* Neff strain to test potential for facultative mycophagy on four different fungi. Two strains of the single celled yeasts *S. cerevisiae* and two filamentous fungi, *Neurospora crassa* and *Coprinus cinerea* were presented as potential prey for *A. castellanii* grown axenically (proteose peptone–yeast extract–glucose medium, 4:2:1 mixture, respectively) (Rosenberg et al., 2009). The experiment was run in 96 well-plates (flat-bottom; Sarstedt, Nümbrecht, Germany), filled with a 150 μl sterile mixture of NMAS enriched with nutrient broth (Merck, Darmstadt, Germany) at 1:9 v/v (NB-NMAS). 100 spores of all four fungi were inoculated either alone or together with *A. castellanii*. Control treatments contained only NB-NMAS or *A. castellanii* in NB-NMAS. All treatments were replicated eightfold. Before use, *A. castellanii* cultures were washed three times with sterile NMAS after centrifugation at 800 rpm for 3 min and 100 amoebae were added to each well of the *A. castellanii* treatments. Control plates received equivalent amounts of NMAS. Plates were sealed with Parafilm and directly placed in an automated microplate reader (Varioscan, Thermo Scientific, Waltham, USA) at room temperature. Optical density (OD) as an estimate of changes in fungal density/growth was measured every hour for a total of four days. Plates were additionally examined microscopically for amoebae growth and potential contamination every second day.