Pedobiologia 62 (2017) 1–8

Contents lists available at ScienceDirect

Pedobiologia - Journal of Soil Ecology

journal homepage: www.elsevier.de/pedobi

Changes in bacterial community composition and soil respiration indicate rapid successions of protist grazers during mineralization of maize crop residues

Maike Hünninghaus[a], Robert Koller[a,1], Susanne Kramer[b], Sven Marhan[b], Ellen Kandeler[b], Michael Bonkowski[a,*]

[a] Terrestrial Ecology, Institute of Zoology, University of Cologne, Zülpicher Str. 47b, 50674 Köln, Germany
[b] Institute of Soil Science and Land Evaluation, Soil Biology Department, University of Hohenheim, Emil-Wolff-Str, 27, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 1 December 2016
Received in revised form 9 March 2017
Accepted 15 March 2017

Keywords:
Plant litter mineralization
Protists
Soil microorganisms
Grazer species richness
PLFA
Succession

ABSTRACT

Decomposition of organic matter is crucial for ecosystem functioning. Microorganisms, which are responsible for the mineralization of organic matter, are usually treated as a homogeneous functional guild, despite mineralization capacity can differ profoundly between taxa. In addition, a significant part of the microbial community is top-down controlled by microbial grazers, such as protist. Since protist grazing is selective, and selectivity differs among species, we hypothesized that protist taxa complement each other in grazing intensity and thereby affect bacterial community structure and mineralization rate. In a laboratory experiment the species richness of protist communities was manipulated in an arable field soil and the mineralization rate of maize litter residues followed during the decomposition of the labile (4 days) and recalcitrant (3 weeks) carbon (C) fractions. Mineralization rate overall increased in the presence of protists. Changes in microbial function could be related to changes in microbial community composition (measured by phospholipid fatty acids pattern). During microbial decomposition, different protist grazers gained influence on mineralization rates over consecutive time intervals, indicating that a succession of protists caused an enhanced bacterial C-mineralization of plant detritus. Protist identity and species richness affected the microbial community composition, but not the magnitude of its mineralization function. In general, protist identity appeared to be more relevant for the composition of the microbial communities at the beginning of decomposition while the protist species richness appeared to be more critical in the later, slow phase of decomposition. This study provides an example that the overall outcome of ecosystem processes, such as mineralization rate is regulated by the sum of positive and negative effects of complex species interactions operating at a very fine spatial and temporal scales.

1. Introduction

The mineralization and transfer of belowground carbon (C) back to the atmosphere is a key process of terrestrial ecosystems and has been estimated to be an order of magnitude larger than anthropogenic CO_2 emissions (Nielsen et al., 2011). About 90% of plant biomass enters the soil system as detritus, driving the belowground food webs (Gessner et al., 2010). Soil microbes catalyse the mineralization of C from detritus, and recycle nutrients for primary production (Billings et al., 2015; Gunina et al., 2014). Accordingly, microorganisms determine a critical balance between C mineralization and sequestration (Bardgett, 2005; Gessner et al., 2010).

The microbial decomposer community is often treated as a single functional guild, despite ample evidence that its composition plays critical roles in determining ecosystem process rates (Reed and Martiny, 2007; Schimel and Gulledge, 1998; Strickland et al., 2009). The huge species richness of soil communities has led to the hypothesis that functional redundancy is great among soil organisms (Andrén et al., 1999; Bengtsson, 1998; Setälä et al.,

* Corresponding author at: Department of Terrestrial Ecology, Institute of Zoology, University of Cologne, Zülpicher Str. 47b, 50674 Köln, Germany.
E-mail address: m.bonkowski@uni-koeln.de (M. Bonkowski).
[1] Now at: Forschungszentrum Jülich, Institute of Bio- and Geosciences, IBG-2: Plant Sciences, Jülich, Germany.

http://dx.doi.org/10.1016/j.pedobi.2017.03.002
0031-4056/

2005), but there are clear examples that species richness affects functional stability of ecosystem processes (Griffiths et al., 2000, 2001a; Saleem et al., 2013) and specific aspects of C cycling (Nielsen et al., 2011).

Protist are assumed to be the major consumers of bacterial biomass in soils (Brussaard, 1998; Crotty et al., 2011; De Ruiter et al., 1993; Trap et al., 2016) and hold a key role in the balance between C loss from the ecosystem by respiratory CO_2 release, and conservation through sequestration in living biotic particulate fractions (Anderson, 2011). Given that natural communities typically have multiple predators feeding on most prey, understanding emergent multiple predator effects on detritus decomposition is a crucial issue for community ecology (Sih et al., 1998). Protists strongly control bacterial populations (Glücksman et al., 2010) and have been shown to reduce bacterial biomass by as much as 60% (Clarholm et al., 1981). Selective grazing of bacterial predators is known to influence the growth conditions of individual bacteria taxa (Bjørnlund et al., 2006; Hahn and Höfle, 1999; Saleem et al., 2012), can substantially enhance bacterial production (Bonkowski et al., 2000; Sundin et al., 1990), and alter microbial community dynamics (Kreuzer et al., 2006; Marschner et al., 2011; Rosenberg et al., 2009).

The diversity of protists is huge, both in morphology and phylogeny (Adl et al., 2012; Ruggiero et al., 2015), but it is still an open debate whether protist function differs across morphotypes (Crotty et al., 2012a), and even more whether the identity and diversity within a functional group matters to ecosystem functioning (Glücksman et al., 2010; Reed and Martiny, 2007; Saleem et al., 2012). Since predation modifies bacterial turnover and community structure, thereby also affecting the mobilization of different C sources, specific effects of different protist species or morphotypes would have important consequences for the entire C flow through the microbial compartment of the soil food web. Identifying key players during the dynamics of detritus decomposition is of pivotal significance as it is essential to assess the consequences of biodiversity loss and subsequent effects on C and nutrient cycles (Gessner et al., 2010). Further, the assumption that shared characteristics of species reflect similar functions is seldom tested (Verhoef and Olff, 2010). A "one-taxonomic group-one-diet" hypothesis is generally applied to generalize soil ecology (Eggers and Jones, 2000), but has very limited scope for furthering the understanding of food-web interactions, or the impact of linkages between organisms and nutrient cycling within the soil (Crotty et al., 2012b).

Using vastly different protist taxa and morphotypes in combinations of 1, 2, 4 and 6 species we hypothesized overall detritus C mineralization rate, as a measure of ecosystem function, to increase with increasing species richness of protist grazers due to mechanisms of niche complementarity (Saleem et al., 2012). This hypothesis was based on the fact that different protist species exhibit distinct grazing preferences, and different morphotypes fundamentally differ in their grazing strategies (Boenigk and Arndt, 2002; Parry, 2004). The grazing preferences should lead to grazer-specific changes in bacterial community composition, and this can be measured by analyzing patterns of microbial phospholipid fatty acids (PLFA). However, decomposition is temporarily dynamic with readily decomposing substrates being mineralized very quickly, followed by a slow and gradual decline of the more resistant fractions (Marschner et al., 2012; Poll et al., 2010, 2008; Schaefer et al., 2009). This changing substrate availability will thus directly influence the growth rates of microbial decomposers and top-down control by the associated food webs, thereby likely influencing the degree of complementarity between grazers (Kramer et al., 2016). To account for these dynamics we set up two laboratory experiments, one short-term experiment (4 days) and one 'long'-term experiment (3 weeks) to monitor the effects of species richness and functional group richness of protist grazers on overall microbial mineralization of carbon from soil and decomposing litter.

2. Material and methods

2.1. Experimental set-up

A protist-free bacteria inoculum was produced according to the protocol of Kreuzer et al. (2006). Briefly, 50 g of air-dried grassland soil (0–10 cm, Cologne, Germany) were mixed on a shaker (Köttermann Typ 4020, Uetze, Germany) at 90 rpm for 20 min in 100 ml Neff's modified amoebae saline (NMAS, (Page, 1976)). After 2.5 h of sedimentation the supernatant was passed twice through a filter (Rundfilter 595 $^1/_2$, Schleicher and Schuell, Dassel, Germany) and the flow through was subsequently filtered through a 3 μm sterile syringe filter (Whatman, Buckinghamshire, England) and through a 1.2 μm sterile syringe filter (Sartorius Minisart, Göttingen, Germany), respectively.

The protist-free bacteria filtrate was then added to a sterilized (autoclaving 30 min, 130 °C) soil-sand mixture (1:1) and incubated in darkness at 20 °C. The filtrate and two subsamples of the incubated soil were checked for protist contamination at 100× magnification with phase contrast (Nikon Eclipse, Japan) on every second day during this incubation time. After two weeks of incubation with bacteria, 40 g of the soil-sand mixture were diluted in 60 ml NMAS and shaken for 30 min at 90 rpm to produce the final inoculum. With this suspension autoclaved soil (originating from an agricultural field site near Göttingen, Germany, see Kramer et al. (2012) for further information) was inoculated with 25 μl bacteria suspension per g soil.

Two sets of 124 microcosms were prepared: 50 ml centrifugation tubes (Falcon, Oneonta, NY, USA) were filled with 20 g ± 0.4 g of the autoclaved and microbially inoculated soil for the 3 week long-term experiment and 2 g ± 0.04 g soil were filled into plastic 6 ml screw cap vessels for a parallel 4 day-short term experiment. All soil treatments were incubated at 15% water-content (i.e. 38% water holding capacity) for nine days to ensure establishment of the bacterial community in the soil.

Protist cultures were transferred to 15 ml plastic tubes and washed three times to remove nutrient broth by centrifugation at 700 rpm for 3 min and resuspending the pellet in NMAS. Protist densities were microscopically estimated using a Neubauer counting chamber.

About 500 protists g^{-1} dw soil were added as single species or in combination (Table 1) and incubated for 9 days to establish stable protist and bacteria communities. Controls contained bacteria-inoculated soil without protists.

For the short term experiment 0.02 ± 0.0005 g sterilized powdered maize litter (43.45% C, 1.11% N) and 200 μl sterile distilled water were mixed into the soil immediately before the start of the respiration measurements. Respiration was measured at hourly intervals over the first 88 h after detritus inoculation (ADI) using a microrespirometer (Griffiths et al., 2001b). After termination at 4 days ADI the soil was frozen for subsequent PLFA-extraction.

In the long term experiment 0.2 ± 0.005 g sterilized powdered maize litter and 2 ml distilled water were added into each experimental tube, relatively the same amount (5 mg C per g soil DW) as in the short term experiment. Open experimental tubes and a test tube containing 1 ml KOH (1N), to absorb produced CO_2, were placed in 1 l amber bottles for three weeks. Titrimetric determination of the CO_2 production with $BaCl_2$ and HCl was performed after 2, 4, 7, 10, 14 and 21 days. CO_2-content was