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Soil water availability strongly alters the community composition of soil protists

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ABSTRACT

Drought and heavy rainfall are contrasting conditions expected to result from increasingly extreme weather during climate change; and both scenarios will strongly affect the functioning of soil systems. However, little is known about the specific responses of soil microorganisms, whose functioning is intimately tied to the magnitude of the water-filled pore space in soil. Soil heterotrophic protists, being important aquatic soil organisms are considered as key-regulators of microbial nutrient turnover. We investigated the responses of distinct protist taxa to changes in soil water availability (SWA) using a modified enumeration technique that enabled quantification of protist taxa up to genus level. Our study revealed a non-linear shift of protist abundance with decreasing SWA and this became apparent at a maximum water-filled pore size of $\leq 40\,\mu m$. Generally, taxa containing large specimen were more severely affected by drought, but responses to either drought or rewetting of soils were not uniform among taxa. Changes in water availability may thus affect the functioning of key taxa and soil ecosystems long before aboveground "drought" effects become apparent.

Introduction

Global temperature has increased and is expected to further increase in the coming century, with annual daily maximum temperatures rising by about 3 °C by mid-21st century and by about 5 °C by the late 21st century, resulting in more frequent and extreme drought events in many parts of the world (IPCC 2012; Sherwood et al. 2013). The term 'drought' is generally associated with the damage of plants due to the lack of soil water (Kramer 1983), but limited soil water availability (SWA) can impair the function of soil ecosystems long before symptoms become visible aboveground. This is especially true for processes performed by microbial soil organisms, whose functions are intimately tied to the magnitude and connectivity of water films around soil particles. We are, however, still largely ignorant of specific responses of soil organisms to these global change phenomena (de Vries et al. 2012a,b; Bradford 2013). However, this topic has gained more interest in the last decade and soil microbial communities with altered SWA were investigated, mostly showing changes in bacterial and fungal communities (Evans and Wallenstein 2012, 2014; Fierer et al. 2003; Fuchslueger et al. 2014).

In contrast to bacteria and fungi, their microbial predators, i.e. soil protists are largely ignored in these studies. Due to their high biomass and with estimated annual production rates of more than 100 kg ha^{-1} (Bouwman and Zwart 1994), protists are assumed to play a key role in carbon (C) and nutrient cycling in soils (Schröter et al. 2003; Christensen et al. 2007; Crotty et al. 2012b). Protists are the most basal microbial consumers, being a fundamental source for C transfer to higher trophic levels in the soil food web (Crotty et al. 2012a). Direct effects of protists result from their high grazing impact on microbial communities, but more important appear to be indirect effects of protists that lead to a stimulation of microbial turnover and respiration (Anderson 2008; Bonkowski 2004; Clarholm 1985) and plant performance (Koller et al. 2013). For example laboratory experiments with planted soil have shown that consumption of microbial biomass by protists led to a 20–40% increased microbial activity and CO_2—C release (Alphei et al. 1996; Rosenberg et al. 2009) and microbial liberation of CO_2 from decomposing plant litter increased up to 100% in presence of protists (Bonkowski et al. 2000).

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Despite living in soil, protists are aquatic organisms, and their function ultimately depends on the availability of water in the three-dimensional pore space (Anderson 2000; Griffiths et al. 2001). Decreasing SWA has been shown to reduce protist replication rates due to limited mobility of protist grazers in the microvolumes of soil water and hence reduced accessibility to bacterial prey (Darbyshire 1976). This can result in significantly negative effects on soil nutrient cycling and plant growth (Kuikman et al. 1991). Protists, are extremely diverse and individual taxa differ fundamentally based on phylogenetic relatedness, morphology and behaviour (e.g. Cavalier-Smith 1998; Adl et al. 2012; Saleem et al. 2012). The size of individual taxa can differ by as much as three orders of magnitude in soil (Finlay 2002; Foissner 1998; Glücksman et al. 2010). Consequently, taxon-specific dependencies on SWA are likely, simply because large, free-swimming taxa will be more vulnerable to desiccation than small, surface-associated forms. However, it is largely unknown how complex, natural protist communities respond to altered soil moisture regimes.

Taxonomic studies on natural populations of soil protists have mainly been restricted to groups with larger specimens that dominate the upper humus layers and share fixed, readily determinable morphological characters, such as testate amoebae and ciliates (e.g. Bamforth 1971; Foissner 1987, 1999; Bamforth 2007; Krashevska et al. 2007). In comparison, knowledge on the taxonomic composition of communities of flagellates and naked amoebae is extremely limited, despite these groups containing a huge diversity of soil species, and vastly outnumbering other protist groups in the mineral soil horizons (e.g. Elliott and Coleman 1977; Finlay et al. 2000; Scharroba et al. 2012; Domonell et al. 2013). Unlike suspension and filter feeders such as many ciliates, and free-swimming flagellates, naked amoebae and amoeboid flagellates directly graze on bacterial colonies and biofilms attached to substrates (Darbyshire et al. 1989; Parry et al. 2004; Weitere et al. 2005; Böhme et al. 2009), and their flexible bodies seem particularly suited to survive in the tiny water films around mineral particles. It has been shown that they still can access prey in water-filled soil pores of only 2 μm in diameter with help of their elongate pseudopodia (Elliott et al. 1980; Darbyshire 2005), but their overall activity is expected to decline concomitantly as the connectivity between soil aggregates decreases at reduced SWA (Ritz and Young 2011). Profoundly rapid changes in abundance of flagellates and naked amoebae were reported with increasing SWA using cultivation-based enumeration studies (Clarholm 1981; Anderson 2000; Bischoff 2002), but none of these studies aimed at resolving the taxonomic composition of the protist communities. Recent high-throughput soil surveys confirmed strong impacts of moisture on the community composition of protists in various soils investigated (Baldwin et al. 2013; Bates et al. 2013), but these sequence based approaches generally fail to provide quantitative information on protist abundance (Medinger et al. 2010; Pawlowski et al. 2011; Weber and Pawlowski 2013; Stoeck et al. 2014). Further, it is unclear if protist abundance will decline in a linear manner. Since the individual size of different protist species in soil commonly spans over three orders of magnitude, a linear decline of total protist abundance with pore space might be assumed. Therefore, detailed quantitative studies distinguishing the responses of specific protist taxa to changes in SWA regimes are needed.

The purpose of our study was to obtain quantitative estimates on the abundance of flagellate and amoeboid soil protists with high taxonomic resolution, and to relate the expected changes in community composition to specific moisture conditions. The study was performed as part of a controlled semi-field experiment in Terrestrial Model Ecosystems (TME) (Knacker et al. 2004). We expected higher abundances of protists at higher SWA as habitat space and connectivity between particles increases with increasing water films. Further, we hypothesized stronger changes in community composition and shifts towards smaller species at decreasing SWA.

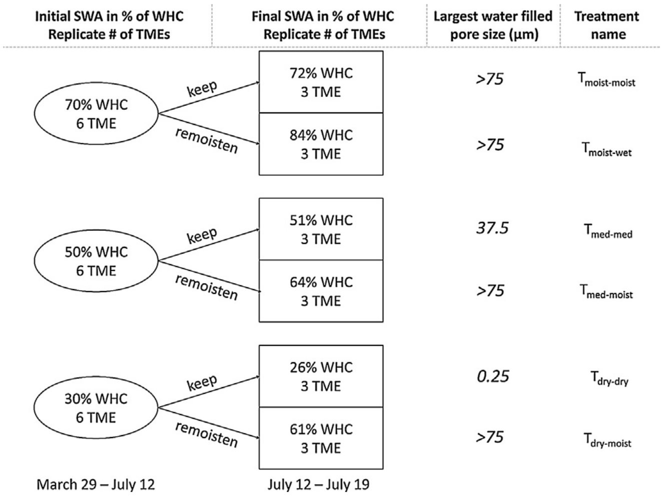

Fig. 1. Left: Schematic diagram of the experimental design (SWA: soil water availability; TME: terrestrial model ecosystem; WHC: water holding capacity); largest water filled pore sizes (μm) estimated according to SPAW graphical interface; treatment names reflecting SWA during the first 15 weeks – the last week of the experiment.

Materials and methods

Study site and experimental setup

A controlled semi-field experiment in terrestrial model ecosystems (TME) consisting of undisturbed soil cores was set up close to Flörsheim, Germany (N50°04′; E8°40′) in order to manipulate SWA and to evaluate effects on soil meso- and microfauna (www.bik-f.de). The soil cores (30 cm diameter, 40 cm depth) were excavated from a meadow on alluvial clay. Soil texture was a silty clay with 9.9% sand, 41.9% clay and 28.2% silt, that contained 2.93% organic matter (pH ($CaCl_2$) = 6.9) and had a water holding capacity of 58.7%.

After the TME cores had been excavated in the field they were incubated on temperature-controlled carts under laboratory conditions (see Knacker et al. 2004 for details of the TME approach). The temperature of the soil cores was kept at 18–24 °C and light intensity was $300 \pm 50\ \mu\text{E}\ \text{m}^{-2}\ \text{s}^{-1}$. The TME cylinders could be drained at the bottom and the volumetric SWA of each TME was monitored in the upper 6 cm using hydra probes (accuracy ±3% (v/v), ecotech Umwelt-Meßsysteme GmbH, Bonn, Germany). This monitoring was used to adjust the daily irrigation volumes individually for each TME replicate. Depending on the watering volume either a pump disperser or "rain heads" (i.e. acrylic glass vessels with micropipettes at their bottom) were used for irrigation (Knacker et al. 2004). Moisture manipulation started at the beginning of the experiment (start: March 29th, 2013) in all TMEs, aiming for SWA of 30%, 50% and 70% of the water holding capacity (WHC). The desired WHC levels were reached after 31, 9, and 2 days after starting the study, respectively. One week before harvest (harvest: July 19th, 2013), the irrigation scheme was changed in half of the TMEs in order to simulate heavy rain events. All individual moisture treatments were replicated three times (Fig. 1).

To estimate the diameter of water filled pores at a given moisture level, actual WHC values were converted into water potentials using the SPAW graphical interface (Saxton and Rawls, 2006). The pore neck diameter of the largest water-filled pores (μm) was then calculated using the formula $D = 300*P^{-1}$, where D is the pore neck diameter of the largest water-filled pores (μm), and P is the