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Effects of rotifers, copepods and chironomid larvae on microbial communities in peatlands

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Abstract

Interactions between the microbial loop and the classical grazing food chain are essential to ecosystem ecology. The goal of the present study was to examine the impact of chironomid larvae, rotifers and copepods on the major components of the microbial food web (algae, bacteria, heterotrophic flagellates, testate amoebae and ciliates) in peatlands. Two enclosure experiments were carried out in a *Sphagnum* peatland. In the experiments we manipulated rotifers, copepods and macroinvertebrates, i.e. chironomid larvae (*Psectrocladius sordidellus* gr). During the experiments variation was observed in the abundance of potential predators. The beginning of the first experiment was distinguished by dominance of rotifers, but five days later copepods were dominant. In the second experiment copepods dominated. The results of this study are the first to suggest a substantial impact of chironomid larvae, rotifers and copepods on microorganism communities in peatland ecosystems. The impact is reflected by both a decrease in the abundance and biomass of testate amoebae and ciliates and a transformation of the size structure of bacteria. Heterotrophic flagellates (HNF) were not controlled by metazoans, but rather by testate amoebae and ciliates, as HNF were more abundant in the control treatment.

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Introduction

Peatlands are generally characterized by rich biodiversity and play an important role in global C sequestration (Bragazza et al. 2006). Furthermore, they belong to those ecosystems in Europe that disappear fastest and are endangered most, which is particularly alarming in the context of progressive climate warming (Robson et al. 2005). Research has largely focused on the effect of climate change and anthropogenic disturbances on the conversion of stored

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organic carbon to CO₂ and CH₄ in the peatland plant community (Bragazza et al. 2006). Plant communities in *Sphagnum*-dominated peatlands are adequately documented (Bragazza et al. 2006). Little is known about how short-term (days to months) variability of potential predators may influence microbial communities (Brown et al. 2011; Mieczan and Tarkowska-Kukuryk 2013). 'Trophic cascade theory' describes direct and indirect impacts of predation on productivity, biomass and composition of lower trophic levels in freshwater ecosystems (Carpenter et al. 1985; Shurin et al. 2002; Miyashita and Niwa 2006). Various mechanisms have been suggested that may reduce or amplify the cascading effects, including nutrient regeneration by predators, omnivory, or the emergence of inedible species (Carpenter

et al. 1985; Woodward and Hildrew 2002; Hoekman 2010). In freshwater ecosystems the microbial loop is inseparable from the grazing food chain (Azam et al. 1983). The microbial loop is a model of the pathway of carbon flow and nutrient cycling through microbial components of aquatic communities. In addition to bacterial uptake of non-living organic matter, many direct links exist between algae, bacteria, and other heterotrophic microbes. By these numerous pathways fixed organic carbon can be retrieved and is often used by larger-sized microorganisms that may be more available for consumption by larger organisms (Azam et al. 1983; Amalfitano et al. 2015). Thus the controlling factors in microbial communities and the relationships between bacteria and protozoa are important for understanding the functioning of the peatland ecosystem.

The classical grazer food chain and the microbial food web are linked by several direct and indirect interactions (Rooney and McCann 2012; Karus et al. 2014). Grazing by rotifers and copepods is important for the recycling of nutrients and production of dissolved organic substrates for bacteria, but is also a controlling factor for protozoan community structure (Miyashita and Niwa 2006). Rotifer and copepod predation on ciliates is well documented in lakes (Arndt 1993; Jack and Gilbert 1997). As revealed in studies by Francez (1986) and Błędzki and Ellison (2003), both rotifers and copepods may reach a relatively high abundance in peatland ecosystems. Thus they seem to play a significant role in controlling the number of protozoa. However, our knowledge of the food web in peatlands remains fragmentary (Gilbert et al. 1998; Mieczan and Tarkowska-Kukuryk 2013). There is still no well-recognized effect of macroinvertebrates on the structure of lower trophic levels in peatland ecosystems. Most widely distributed in Sphagnum peatlands are dipterans of the Orthocladiinae (Tarkowska-Kukuryk and Mieczan 2014). Larvae of midges are well adapted to survive in a variety of environmental rigours, such as desiccation, anoxia and low temperature (Lencioni and Rossaro 2005). Due to their high densities, rapid growth and high grazing rates, chironomid larvae have great potential to regulate the abundance of lower trophic levels. Chironomids are able to ingest a variety of food types - algae, detritus and associated microorganisms, woody debris, and invertebrates (Pinder 1986; Hirabayashi and Wotton 1999). The most commonly reported ingested food in freshwater ecosystems is detritus with associated protozoans and algae (Lawrence and Gresens 2004; Tarkowska-Kukuryk and Mieczan 2008; Maasri et al. 2010). The larvae of chironomids have a wide food spectrum, usually do not adhere to one feeding mode, and commonly exhibit food switching. Many taxa can be classified as both collector/grazers and filtrators. Diet composition of larvae usually depends on available food resources and phenology (Pinder 1986; Enterkin et al. 2007). However, the impacts of the introduction of chironomid larvae need to be explicitly examined because chironomids exert bottom-up controls as well as top-down controls through excretion and regeneration of nutrients (Davine and Vanni 2002; Wickham et al. 2004).

The objective of this study was to test the following two hypotheses: (i) chironomid larvae prey on rotifers and copepods, which results in reduced grazing/predation pressure on algae, protozoa and bacteria (top-down control); and (ii) introduction of chironomid larvae increases the biomass of algae via effective nutrient regeneration (bottom-up effect).

Material and Methods

Study site

The study was performed in the Moszne *Sphagnum* peatland located in the western part of Polesie Lubelskie (eastern Poland, 51° N, 23° E). The vegetation of this area is dominated by *Eriophorum vaginatum* (L.), *Carex acutiformis* Ehrhart., *Carex gracilis* Curt., *Sphagnum angustifolium* (C.C.O. Jensen ex Russow), *Sphagnum cuspidatum* Ehrh. ex Hoffm., and *Polytrichum* sp. The mean air temperatures in May and July were 14.1 ± 3 °C and 17.9 ± 4 °C, respectively. The mean monthly rainfall ranged from 551 mm in May to 420 mm in July and peat porosity ranged from 94% to 96%.

Abiotic variables

At the start and end of the experiments, temperature, electrolytic conductivity (EC), pH, dissolved oxygen (DO), chlorophyll *a*, soluble reactive phosphorus (P-PO₄³⁻), ammonia nitrogen (N-NH₄) and total organic carbon (TOC) were measured. Physical and chemical analyses were performed according to standard methods for hydrochemical analyses (Golterman 1969). Temperature, EC, pH and DO were assessed at the sites with a multiparametric probe (Hanna Instruments), P-PO₄³⁻ was determined by the colorimetric method, and N-NH₄ by Kjeldahl's method. Chlorophyll *a* concentration was determined by spectrophotometric analysis of acetone extracts of algae retained on Whatman GF/F filters (0.7 μm, 2.5 cm diameter) according to Golterman (1969). Total organic carbon (TOC) analysis was performed with an OI Corporation Model 700 TOC analyzer.

Field experiments

Field experiments using intact natural communities can help to identify the various direct and indirect effects of trophic levels on microbial populations and communities. In this experiment we manipulated rotifers and copepods – RC, and macroinvertebrates (chironomid larvae) – MA. Microcosm experiments were performed during two periods, in May and July 2013. The first experiment was conducted from 7 to 20 May (Exp. 1) and the second from 14 to 27 July (Exp. 2) 2013. Twelve 30 l polyethylene enclosures (15 cm in depth), closed at the bottom and fixed to a wooden frame, were placed in *Sphagnum* hollows. Samples were collected from a total of twelve experimental enclosures (six control

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