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# Effects of experimental addition of nitrogen and phosphorus on microbial and metazoan communities in a peatbog

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### Abstract

Interactions between microbial communities and the classical grazing food chain are essential for understanding the functioning of peatbog ecosystems. However, little is known of how short-term intensification of eutrophication processes may influence microbial and metazoan communities in transitional peatbog. We addressed the hypothesis that an increase in the concentration of nutrients will affect the species richness and abundance of microorganisms and small metazoans and cause changes in the food web structure in a peatbog. The experiments were performed in a transitional peatbog. Four experimental variants were conducted (control and nutrient-enriched: +P, +N and P + N). Increased habitat fertility was found to modify the taxonomic composition and functioning of microbial communities. We observed a strong reduction in the species richness of testate amoebae—top predators, and a substantial increase in the abundance of bacteria, flagellates and ciliates. A better understanding of which parameters regulate microbial populations in peatbogs is critical for more accurate prediction of how peatbogs will respond to global climate change or anthropogenic disturbances.

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## Introduction

Peatbog ecosystems have recently received attention because of their unique flora and fauna, including many rare and endemic species. They are also of key importance for maintaining stable ecological relationships in individual areas, but due to increasing human impact (changes in land use structure, atmospheric pollution or drainage) they are among the most threatened ecosystems (Bragazza et al. 2012). Eutrophication is one of the main factors

http://dx.doi.org/10.1016/j.ejop.2017.03.001 0932-4739/© 2017 Elsevier GmbH. All rights reserved. contributing to the loss of biodiversity in peatbogs in Europe, because increased nutrients are related to increased productivity and the competitive exclusion of shorter species (Middleton 2002). Peatbogs are highly sensitive to deposition of nutrients and other pollutants. Nitrogen and phosphorus deposition affects many peatbogs in industrialised regions and may transform them from carbon sinks into carbon sources, with important consequences for the global carbon cycle (Bragazza et al. 2012; Gilbert et al. 1998a; Wardle et al. 2012). Nitrogen addition can affect directly and indirectly CH<sub>4</sub> emission. Direct effects include both inhibition of methanogenesis (decreasing emission) by nitrate and inhibition of oxidation (increase emission) by ammonium

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(Bragazza et al. 2012). Indirect effects primarily concern the alternation of vascular plant cover due to increased nutrient availability (Nykanen et al. 2002; Wardle et al. 2012). The addition of limiting nutrients to ecosystems often results in enhanced productivity of vegetation and stimulation of the microbial community. However, the microbial community may be more sensitive or respond more readily to changes in trophic status than vegetation. Changes in vegetation patterns due to nutrient loading may take years to be observed (Berendse et al. 2001; Bragazza et al. 2005, 2012; Middleton 2002), while microbial processes display effects after a short exposure to elevated nutrient levels (Mieczan et al. 2015a). Thus, microbial processes and patterns may be used as sensitive indicators of eutrophication or changes in environmental conditions (Wright et al. 2009). As yet no studies have been carried out on the effect of an experimental increase in habitat fertility on microbial communities in transitional bogs. Transitional bogs cover a considerably small area and usually occur near dystrophic lakes and small ponds and in the marginal parts of peatbogs. They are overgrown by turfs and moss-sedge plants, which also form floating blankets of vegetation, the so-called "floating islands". They occur in basin-shaped depressions without outflow, often in the immediate surroundings of dystrophic lakes (Bałaga 2007). A transitional bog is not a uniform ecosystem. The dominant area of a peatbogs is the open bog, mainly occupied by Sphagnum and other peat-forming vegetation (Herbichowa and Potocka 2004). Little is known of how short-term variability of concentrations of nutrients may influence protistan and metazoan community composition (Jiroušek et al. 2013; Mieczan and Tarkowska-Kukuryk 2013; Mitchell et al. 2003). Abiotic factors, however, can change the strength of biotic interactions, with wide-ranging consequences for biological communities. Among microbial organisms, protists have proved to be useful bioindicators of anthropogenic pollution in peatbogs and soil (Andersen et al. 2013; Mieczan 2007, 2009; Mieczan et al. 2012, 2015b; Wardle et al. 2012). Only a few studies have monitored the effects of simulated addition of nitrogen and phosphorus on microbial communities of peatbogs (Gilbert et al. 1998a,b; Mieczan et al. 2015a; Payne et al. 2013). It has been demonstrated that eutrophication has led to serious economic and environmental problems (Buosi et al. 2011; Gilbert et al. 1998b; Wright et al. 2009). The research conducted so far mainly concerned the effect of increased content of biogenic compounds on primary producers and protists in the Pradeaux raised bog in France (Gilbert et al. 1998b). The addition of PKCa and NPKCa was shown to increase the biomass of heterotrophic bacteria and ciliates and to decrease the contribution of testate amoebae and microalgae. A study by Payne et al. (2013) in an ombrotrophic peatland showed that an increase in ammonia concentration leads to an increase in the proportion of autotrophic organisms (algae) and a decrease in that of heterotrophic organisms (bacteria and fungi) in the total microbial biomass. Mieczan et al. (2015a) suggested that nutrient addition greatly destabilises Sphagnum peatland and carbonate fen function by altering the interactions between microbial and macrobial communities in these ecosystems. There are still very little data describing the impact of simulated eutrophication of transitional peatbog on the functioning of microorganisms forming a microbial loop and on the trophic relationships in these ecosystems. Knowledge of these scenarios is extremely important in predicting the response of ecosystems to increasing human pressure, including climate change.

We tested the hypothesis that: (1) due to their faster rate of multiplication, the response of bacteria, algae and ciliates to the increased concentration of biogenic compounds will be much faster than that of testate amoeba and small metazoa and an increase in the concentrations of nutrients will mediate changes in species richness and abundance of microbial community, (2) an increased concentration of biogenic compounds causes changes in the significance and strength of relationships between dominant microbial consumers and food web structure.

#### **Material and Methods**

#### Study site

The study was performed in the Moszne peatbog located in the western part of Polesie Lubelskie (eastern Poland,  $51^{\circ}N$ ,  $23^{\circ}E$ ) (Fig. 1). 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  $14.1 \pm 3^{\circ}C$  and  $17.9 \pm 4^{\circ}C$ , respectively. The mean monthly rainfall ranged from 551 mm in May to 420 mm in July and peat porosity ranged from 94% to 96%.

#### **Experimental design**

In investigated peatbog, the experiment consisted of two groups of treatments (fertilised and control), with three replicates each. 'Mesocosm' experimental studies were conducted 3 times a year, in the spring, summer and autumn of 2014, for a period of 21 days in each season. Microbial communities were examined in situ in polyethylene enclosures (80 L each,  $45 \text{ cm} \times 45 \text{ cm}, 40 \text{ cm}$  deep). In each season (spring, summer and autumn) 12 enclosures were created for all experimental treatments (3 for each of the 4 treatments). In order to maintain habitat conditions similar to natural ones, in each of the three seasons Sphagnum moss was also included in each experimental treatment-Sphagnum angustifolium, which is dominant in these microhabitats. Mosses were added to the samples in the amount of 10 g w. m. Three enclosures were gently filled with surface water (control treatment) and peat mosses. Each experimental variant was prepared in 3 enclosures. Enrichment with biogenic compounds was carried out

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