Research paper

Taxonomic and functional traits responses of *Sphagnum* peatland testate amoebae to experimentally manipulated water table

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ABSTRACT

Biomonitoring tools are useful to assess the impact of environmental changes on the functioning of ecosystems. Existing tools mostly require species identification, thus allowing to estimating changes in biodiversity, and possibly inferring ecosystem functioning, using functional diversity and traits based approaches.

Testate amoebae are good indicators of surface moisture conditions in *Sphagnum* peatlands and are routinely used in palaeoecology. Their shells (tests), on which identification is based, can also be used to define functional traits and thus to infer changes in ecosystem functioning.

We investigated the response of testate amoeba communities to manipulated water table depth (wet: −4 cm, intermediate: −15 cm, and dry: −25 cm) over time (seven time points, 19 months) using mesocosms by comparing two approaches: community structure and functional traits responses, using a combination of morphological (biovolume, length, aperture size and position) and physiological (mixotrophy/heterotrophy, shell material) traits.

This is the first study investigating the effect of water table depth on testate amoeba assemblages over time using a mesocosm approach. Taxonomical and functional approaches showed similar response patterns, confirming that water level acted as a strong environmental filter. After one year *Hyalosphenia papilio* decreased in the dry treatment, and the community structure shifted towards a dominance of dry indicators (*Nebela tincta* complex, *Corythion dubium, Euglypha compressa*) and the selected functional traits (smaller, heterotrophic, compressed species, with a ventral aperture) corresponded to drought adaptations.

In line with recent observational and transfer function studies exploring the use of testate amoebae functional traits, our experimental results illustrate how well-selected traits could be used to monitor the impact of present and past climatic changes on *Sphagnum* peatlands.

1. Introduction

Biomonitoring tools are useful to assess the impact of environmental changes on the functioning of ecosystems. Finding and testing relevant proxies to monitor the evolution of ecosystems are therefore priorities in applied ecological research (Bartkowski et al., 2015; Laurila-Pant et al., 2015). In addition to describing biodiversity itself, good bioindicators should also inform about ecosystem services. Biomonitoring approaches are now accepted as standard tools to assess environmental quality (European Commission, 1999; Finlayson, 1994).

Functional diversity (FD) is a useful tool to model ecosystem functioning. Based on the relationship between environmental constraints and community structure, through functional traits, FD allows assessing the impact of environmental changes on both community structure and ecosystem functioning (Bockstael et al., 2000; Cadotte et al., 2011; Keddy, 1992; Woodward and Diament, 1991). Species traits may explain both the effects of environmental changes on community structure, (response traits) and the feedback from species presence on ecosystem functioning (effect traits) (Lavorel and Garnier, 2002). Traits used for FD analysis should ideally relate to niche characteristics and/ or directly or indirectly to ecosystem processes (Mlambo, 2014; Violle et al., 2007). The FD approach was initially developed for macroscopic organisms such as plants, fishes and soil invertebrates, for which different sets of traits have been validated and more recently for

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microorganisms (Arrieira et al., 2015; Barnett et al., 2007; Fournier et al., 2016, 2015, 2012; Jassey et al., 2016; Lamentowicz et al., 2015; Marcisz et al., 2016; van Bellen et al., 2017; Violle et al., 2011). Our focus here is on the impact of climate change on testate amoebae living in *Sphagnum* peatlands.

Sphagnum-dominated peatlands are key ecosystems in the global carbon cycle as C stores and sinks (Frokling and Roulet, 2007; Turunen et al., 2002), but are threatened by direct human impact (peat harvesting, drainage) and climatic change (warming, drought) (Belyea and Malmer, 2004; Bonn et al., 2016; Briones et al., 2014; MEA, 2005). Northern peatlands represent an important carbon stock of 500 ± 100 gigatons, representing ca. ⅓ of all soil carbon trapped in ca. 3% of total land area (Gorham, 1991; Yu, 2012). The key to peat accumulation is the low decomposition rate, which is driven by the anoxic conditions in the usually water-logged soils (Belyea and Malmer, 2004; Rydin and Jeglum, 2006). *Sphagnum*-dominated peatlands, are primarily situated in high latitudes where documented and predicted climate warming are greatest (Hassol, 2004; IPCC, 2013). Understanding their response to climatic change is therefore a research priority (Belyea and Malmer, 2004; Pachauri and Mayer, 2015; Rydin and Jeglum, 2006; Yu, 2006), for their potential impact on carbon cycle at the global scale as well as for biodiversity conservation at the regional scale, especially in marginal climatic setting for their development at mid-latitudes.

As the vegetation of peatlands is dominated by perennial plants, shifts in vegetation in response to environmental changes are slow. By contrast, soil microorganisms, including protists such as testate amoebae, have short generation times and are thus well adapted to being used as early warning indicators of environmental changes (Buttler et al., 1996; Gilbert et al., 1998; Laggoun-Defarge et al., 2008). Even within stable conditions such as found in raised bogs, testate amoebae were shown to be better indicators of micro-environmental gradient (hummock-pool) than plants (Koenig et al., 2015). Testate amoebae are a polyphyletic group of protists building a shell (i.e. "test"). They are known to be well correlated with environmental gradients like soil moisture, water table depth, pH, or nutrients content (Booth and Zygmunt, 2005; Jassey et al., 2011; Swindles et al., 2009). Their decay-resistant shell, well preserved in peat and sediments after the organisms' death, allows past climates to be reconstructed by inferring ancient local conditions through testate amoeba community structures (Chambers et al., 2012; Talbot et al., 2010). In addition, testate amoebae functional traits can also be defined in accordance with functional diversity assumptions and be used for biomonitoring or paleoreconstruction (Fournier et al., 2015; Jassey et al., 2016; Kajukało et al., 2016; van Bellen et al., 2017).

The structure of testate amoeba communities living in peatlands has long been shown to be well correlated to factors such as water table depth and pH (Amesbury et al., 2013; Jassey et al., 2011; Marcisz et al., 2014; Mazei et al., 2007; Mieczan, 2009; Swindles et al., 2009; Wilken et al., 2013). The informative power of testate amoebae relies strongly on the accuracy of identification. However, due to taxonomic uncertainties (Booth, 2008; Mitchell et al., 2013, 2008; Payne et al., 2012), a significant yet unknown fraction of the data used in ecological studies contain errors. Although numerical experiments showed that such taxonomic inconsistencies generally have little effect on ecological interpretation, (Payne et al., 2011), it is nevertheless desirable to develop bioindication tools that are robust to potential identification biases.

The approach we are developing is to analyse the patterns of testate amoeba functional traits along environmental gradients and how they respond to experimental manipulations of key environmental factors, which were identified as major potential drivers of community structure based on observational studies. In a bioindication perspective, it is essential to understand the dynamic processes involved in environmental filtering in order to test the causality of relationships and determine at which temporal scale organisms can be used as bioindicators

– and this can only be achieved using experimental approaches (Marcisz et al., 2014).

The key driver – and environmental filter – we selected is water table depth, which generally emerges as the strongest explanatory variable in ecological studies of testate amoebae communities (Mitchell et al., 2008) and is also one of the main factors controlling the vegetation composition and functioning of peatland ecosystems (Rydin and Jeglum, 2006).

We monitored the temporal pattern of the response of *Sphagnum* peatland testate amoeba communities and corresponding variations in traits to artificially manipulated water table. Our hypothesis was that the testate amoeba community weighted mean of traits (CWM: the abundance-weighted mean of the species trait values within a community) and community structure would exhibit similar responses to water level manipulation, but that the CWM would be more directly ecologically informative than community structure in understanding ecosystem functioning. The traits include shell and aperture size, which were shown to determine the trophic position of testate amoebae (Jassey et al., 2013a), with feedback on C and nutrient cycling, and mixotrophy, which was shown to relate to ecosystem carbon balance (Jassey et al., 2015).

2. Material and methods

2.1. Experimental design

We assessed the response of testate amoeba communities to water table depth using a mesocosm experiment. The mesocosms were plastic tanks filled with water (online supplementary material). In the centre of each tank a PVC tube (45 cm high, 12 cm diameter with regularly-spaced lateral holes to allow water movement) contained a peat core collected from a cutover bog using a large-diameter peat corer, topped with a 15 cm thick carpet of *Sphagnum fallax* (including the living mosses and underlying litter), collected from another peatland (Creux de l'Epral, 47°12′18.3″N; 006°56′05.83″E; altitude: 990 m) with an extensive homogeneous cover of this moss (see Mulot et al., 2015 for a complete description of the experiment setup). The water level in each mesocosm was adjusted to maintain three average water table depths (− 4 cm, − 15 cm, and − 25 cm, hereafter referred to as "wet", "intermediate" and "dry" treatments). Each treatment was replicated five times, giving a total of 15 mesocosms. Each carpet was seeded with a water extract from pool, hummock, and lawn ("bog microbial cocktail") to provide the full community potential at the beginning of the experiment (Mulot et al., 2015). Therefore, the communities should be undistinguishable at T0 in all mesocosms. For the extraction of testate amoebae, the top three centimetres of *Sphagnum* mosses were first collected in August 2nd 2012 (T0), then after two month (T1; October 4th), four months (T2; November 29th), seven months (T3; March 3rd, 2013), twelve months (T4; August 11th), sixteen months (T5; December 15th), and eighteen months (T6; March 27th, 2014). Water levels were recorded twice a day using automatic custom piezometers as described in Mulot et al. (2015).

2.2. Testate amoeba isolation and characterisation

Testate amoebae were extracted by sieving and back-sieving through mesh filters (Booth et al., 2010; Jassey et al., 2011). A minimum of 100 individuals (living + dead) per sample were counted. The rationale for including living as well as dead individuals was that this corresponds to the community that eventually will be preserved in the peat and recovered in palaeoecological studies. Our focus here is thus not specifically on the living community and thus a potential limitation inherent to this choice is that by including the dead amoebae we expect some lag in the response to the experimental treatment. In some late dry samples, testate amoeba density was very low and only 50 individuals could be found. As the diversity in these samples was low