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Short Communication

Impacts of tropospheric ozone exposure on peatland microbial consumers

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ABSTRACT

Tropospheric ozone pollution is recognised as an important threat to terrestrial ecosystems but impacts on peatlands are little understood despite the importance of peat as a global carbon store. Here we investigate the impacts of three levels of elevated exposure to tropospheric ozone on peatland microbial communities with a particular focus on testate amoebae, the dominant microbial consumers. We found that intermediate (ambient + 25 ppb O_3) and high ozone treatments (ambient +35 ppb summer, +10 ppb year round) led to significant changes in testate amoeba communities, typified by an increase in abundance of *Phyrganella* spp. and loss of diversity. *Phyrganella* is often suggested to feed on fungi so the observed community change in testate amoeba could be due to ozone-induced changes in the abundance of fungi or other microbial groups. We do not find evidence for changes in numbers of undifferentiated microalgae, nematodes or rotifers but do find weak evidence for an increase in flagellates and ciliates. Our results provide the first direct data to show the impact of ozone on microbial consumers in peatlands.

Tropospheric ozone (O_3) pollution is affecting an increasingly large proportion of the global land area with widespread impacts on terrestrial ecosystems (Mills et al., 2011; Wilkinson et al., 2012; Fuhrer et al., 2016). Through this century climate change is expected to increase the frequency of the intense ozone events which lead to the most widespread damage (Royal Society, 2008). Ozone reduces soil carbon sequestration and storage in forests (Talhelm et al., 2014) but there is considerable uncertainty regarding impacts on the very large peatland carbon pool (c.600 GtC (Yu et al., 2010)). The limited experimental evidence has shown changes in peatland plant communities and key carbon cycle pathways but there is a lack of consistency between studies and the overall consequences for net ecosystem carbon balance remain unclear (Morsky et al., 2008; Toet et al., 2009; Toet et al., 2011, 2017; Williamson et al., 2016).

A key mediator of change in the peatland carbon cycle is the microbial foodweb comprised of prokaryotes (bacteria, archaea), micro- and macroeukaryotes including phototrophs (e.g. chrysophytes, diatoms), fungi, protozoa (e.g. ciliates, flagellates, testate amoebae) and micrometazoa (nematodes, rotifers) (Gilbert et al., 1998b; Jassey et al., 2013a). A particular focus of this paper is testate amoebae which are the most abundant group of eukaryotic microorganisms in peatlands (<50% of extractable non-fungal biomass (Gilbert et al., 1998b)). Testate amoebae play important roles in ecosystem processes such as primary production through C assimilation by mixotrophs (Jassey et al., 2015) and decomposition through top-down control on the microbial foodweb (Wilkinson and Mitchell, 2010; Jassey et al., 2012, 2013b). Peatland testate amoebae are known to be sensitive to pollutants including sulphur (Payne et al., 2010), nitrogen (Nguyen Viet et al., 2004; Payne et al., 2012), heavy metals (Nguyen-Viet et al., 2007) and particulate matter (Meyer et al., 2012) and changes in testate amoebae due to pollution have been linked to re-structuring of overall microbial foodweb structure (Karimi et al., 2016). The impact of ozone on testate amoebae and other microbial consumers has not been

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Table 1
ANOSIM tests of differences in testate amoeba community structure between experimental O_3 treatments. ns = non-significant. A version of this table with the abundant Phryganella spp. excluded is presented as Supplementary Table 1.

Analysed data	Tests included	R_{ANOSIM} and *P*-value
Relative abundance	All	0.10 ($P = 0.03$)*
	Live individuals only	ns
Concentration	All	0.10 ($P = 0.03$)*
	Live individuals only	ns
Biomass	All	0.14 ($P = 0.004$)*
	Live individuals only	0.12 ($P = 0.01$)*

*In post-hoc testing Bonferroni corrected *P*-values are significant for comparison of control with high treatment and control with medium treatment only.

addressed in any previous peatland studies and is an important knowledge gap.

Here we investigate the impact of ozone on testate amoebae and other peatland microorganisms using a mesocosm experiment. Full details of the experimental set-up are described in Toet et al. (2017). In brief, the experiment consisted of mesocosms (19 cm diameter, 35 cm depth) extracted from wet heath peatland (UK NVC community M15: *Scirpus cespitosus-Erica tetralix*) and maintained with water table at 50 mm depth. Mesocosms were exposed to one of: ambient O_3 (non-filtered air, c.25 ppb: 'control'), ambient plus 10 ppb O_3 24hrs/day ('low'), ambient plus 25 ppb O_3 24hrs/day ('medium') and a high summer exposure of ambient plus 35 ppb O_3 for the period April to September 8hrs/day and plus 10 ppb for the remainder of the year ('high'). The upper 50 mm of 10–15 *Sphagnum papillosum* stems were removed from 7 to 9 replicates after 3.5 years and stored refrigerated in glutaraldehyde (Mazei et al., 2015). Microorganisms were separated by physical agitation and inspected microscopically at 400x magnification with a minimum of 100 tests counted (Payne and Mitchell, 2009) and counts converted to biomass following Gilbert et al. (1998a). In parallel with testate amoeba analyses, the abundance of undifferentiated microalgae (principally desmids and diatoms), rotifers, nematodes, flagellates and ciliates was recorded following the same method. We analysed multivariate data using one-way analysis of similarity (ANOSIM: (Clarke, 1993)) and non-metric multi-dimensional

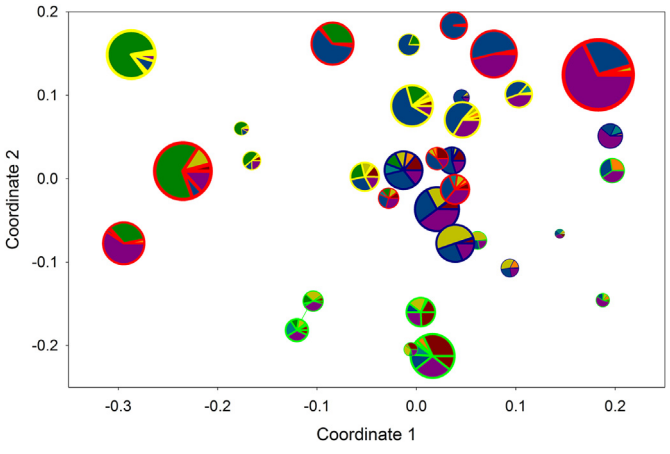

Fig. 1. Non-metric multidimensional scaling (NMDS) ordination of testate amoeba data based on biomass represented by all tests. Symbols sized in proportion to total biomass with pies showing proportions of selected major species. Stress is relatively high (0.25) so patterns should be interpreted with caution. There is an overall significant difference between treatments (ANOSIM, $P < 0.01$), with significant differences between control and both high and medium treatments when tested individually. Different treatments are marked by differently coloured outlines (green = ambient, blue = low, yellow = medium and red = high). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

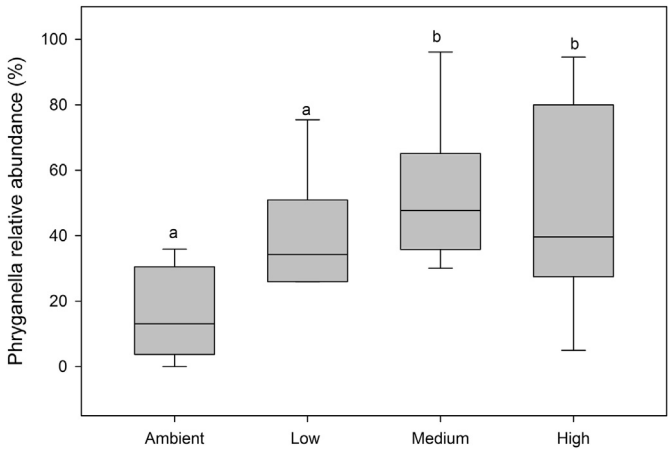

Fig. 2. Differences in relative abundance of *Phryganella* between treatments. Boxes show the median (central line), first and third quartiles (grey box) and tenth and ninetieth percentiles ('whiskers'). Significant differences between treatments are marked by differing letters. Overall differences are highly significant (P < 0.01).

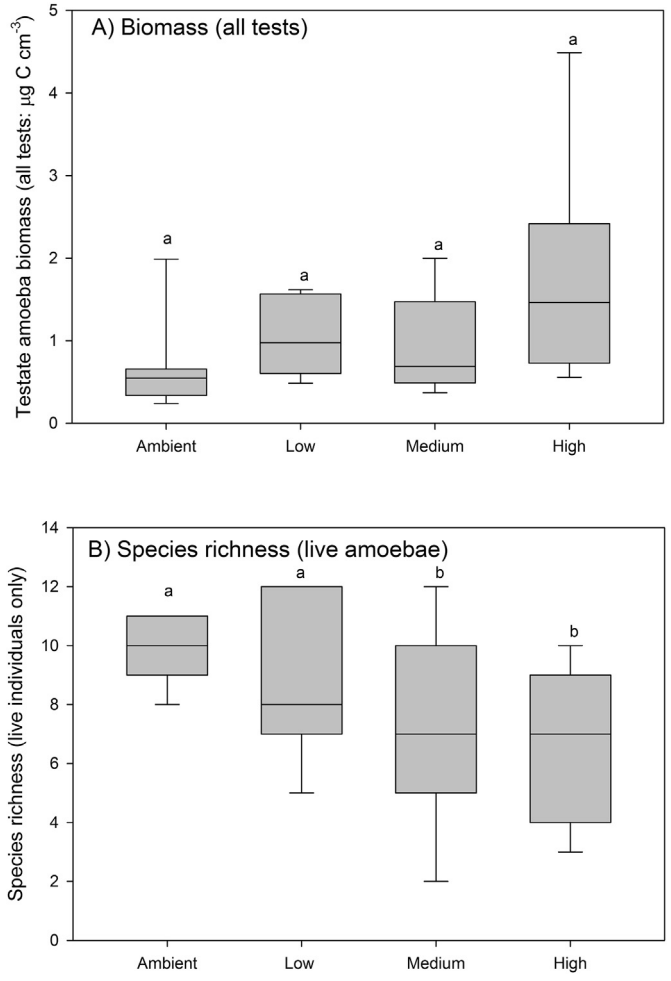

Fig. 3. A) Total testate amoeba biomass based on all tests. B) Species richness based on live individuals. Boxes show the median (central line), first and third quartiles (grey box) and tenth and ninetieth percentiles ('whiskers'). Significant differences are marked by differing letters. Differences between treatments for biomass are marginally non-significant (P = 0.55).