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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 in the intermediate (ambient + 25 ppb $O_3$) and high treatments (ambient +35 ppb summer, +10 ppb year round) there were 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 community change identified in our experiment might suggest that the testate amoeba response is at least partially mediated by interactions with 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.

KEYWORDS: Protists; Air pollution; Mire; Anthropocene

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; Williamson et al., 2016; Toet et al., 2017).

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; Jassey et al., 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 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 50mm 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 7-9 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 scaling (NMDS) ordination based on Bray-Curtis dissimilarity (Bray and Curtis, 1957) and tested for treatment effects in univariate data using ANOVA. We calculated testate amoeba relative abundance, concentration and biomass and conducted separate data analyses for each. Data analyses used PAST vers. 3.04 (Hammer et al., 2001) and the R-package vegan (Oksanen et al., 2007).

Results showed a significant difference in testate amoeba community structure between treatments for data based on biomass, concentration and relative abundance of all tests ($P \leq 0.03$; Table 1) and a clear treatment effect in the ordination plot (Fig. 1). These results were largely driven by a single taxon: *Phyrganella* spp. (Fig. 2) which was on average three times more abundant in the High treated samples; many analyses lost significance when this taxon was removed (Supplementary Table 1). Results were not significant for relative abundance and concentration based on live individuals only, most likely due to the low counts (Table 1). Testate amoeba species richness was significantly reduced compared to the control in Medium and High treatments (ANOVA: $F_{1,3}$=3.2, $P$=0.037, Fisher's LSD: $P$<0.05; Fig. 3). Mean testate amoeba biomass of the High treated samples was 50% greater than the control samples but the P-value was above the generally-accepted cut-off of P=0.05 (ANOVA: $F_{1,3}$=2.8, $P$=0.055; Fig. 3). We found no significant difference in abundance of the other groups of microorganisms quantified (Fig. 4) with the exception of grouped flagellate and ciliates (ANOVA: $F_3$=4.0, P=0.017) which were significantly more abundant than control in the Low and High treatments. However, counts were very low (mean=7.7

individuals per sample) so we cannot place strong weight on this result. In addition to treatment effects it is possible that the microbial communities of the mesocosms may have changed over the course of the experiment due to factors other than ozone; we have no data with which to test this.

Our results demonstrate clear changes in testate amoeba community due to ozone fumigation. Most changes start in the Medium treatment (ambient +25 ppb) and are highly significant with ozone leading to a community which is different in composition, less diverse and possibly of higher biomass. There are many plausible mechanisms for how ozone exposure could lead to changes in testate amoeba communities through both direct impacts (oxidation) and indirectly through changes in the peat physical environment, physiological change and community shifts in plant communities (Searles et al., 2001) or changes to microbial competitors, prey or predators (Li et al., 2015). As isotope tracer studies show that ozone only penetrates a few millimetres into peat soils (Toet et al., 2009) indirect impacts are more probable. Other results from this experiment have shown reduced pore-water ammonium and reduced methane emission but no evidence for impacts on sedge green leaf density, root biomass or dissolved organic carbon (Toet et al., 2017). These results do not directly imply a mechanism for the changes detected here. No other data on soil microbial communities are currently available for these mesocosms but there is data from other peatland studies. In a field mesocosm experiment Morsky et al. (2008) found that both the fungal PLFA 18:2ω6 and total PLFA concentration were enhanced by ozone exposure with no change in bacterial PLFAs. The increase in total PLFAs parallels the possible increase in testate amoeba biomass and ciliate+flagellate abundance here, potentially due to an increased food supply for protozoa. Our finding of increased testate amoeba biomass also parallels the results of Li et al. (2015) from mineral soils who found an increase in PLFAs linked to protozoa with ozone exposure. The finding of increased fungal PLFAs by Morsky et al. (2008) is particularly interesting given the increase in *Phryganella* spp (most likely predominantly *P. acropodia*) detected here. This taxon has been observed to feed on spores of a limited range of fungal species (Ogden and Pitta, 1990) and increase in abundance in response to increased fungal abundance (Coûteaux and Devaux, 1983; Coûteaux, 1985). The taxon is often considered to be mostly, or even exclusively mycophagous (Gilbert et al., 2000) but may primarily feed on saprophytic fungal exudates or exudate-feeding bacteria rather than fungi themselves (Vohník et al., 2011). The only study which has directly compared PLFA 18:2ω6c results with *P.acropodia* abundance did not find a correlation (Krashevska et al., 2008) but this was in a quite different ecosystem. We consider that an increased fungal abundance or changed fungal community structure in the ozone treated samples is one likely explanation for the testate amoeba changes detected.

Our results clearly demonstrate that ozone exposure leads to a significant change in testate amoeba community, likely to be mediated by interactions with other microbial groups. The loss of diversity and increased dominance by a single taxon suggest a potential loss of functional redundancy and degradation of resilience. It seems clear that ozone exposure can be added to the increasingly-long list of global change factors which are known to influence peatland microbial consumers.

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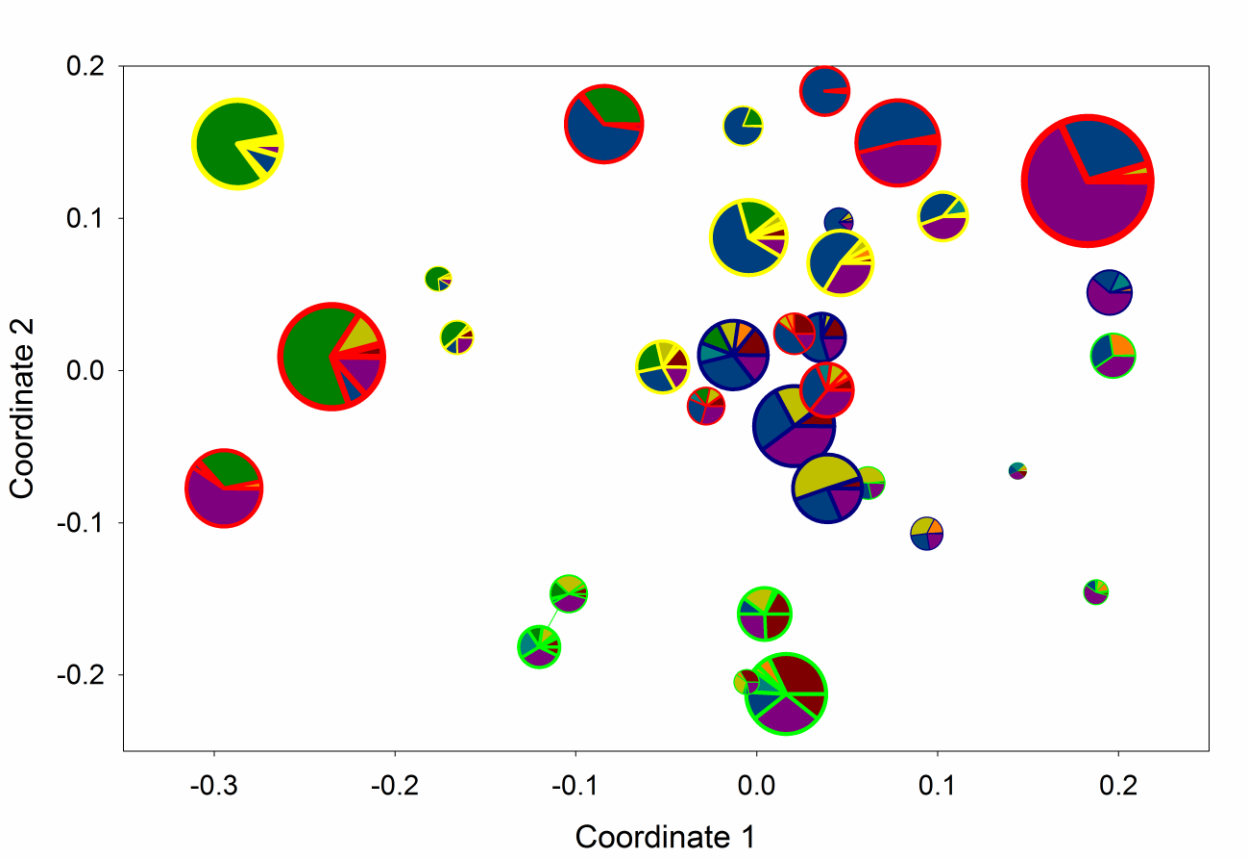
224

225 FIGURES and TABLES

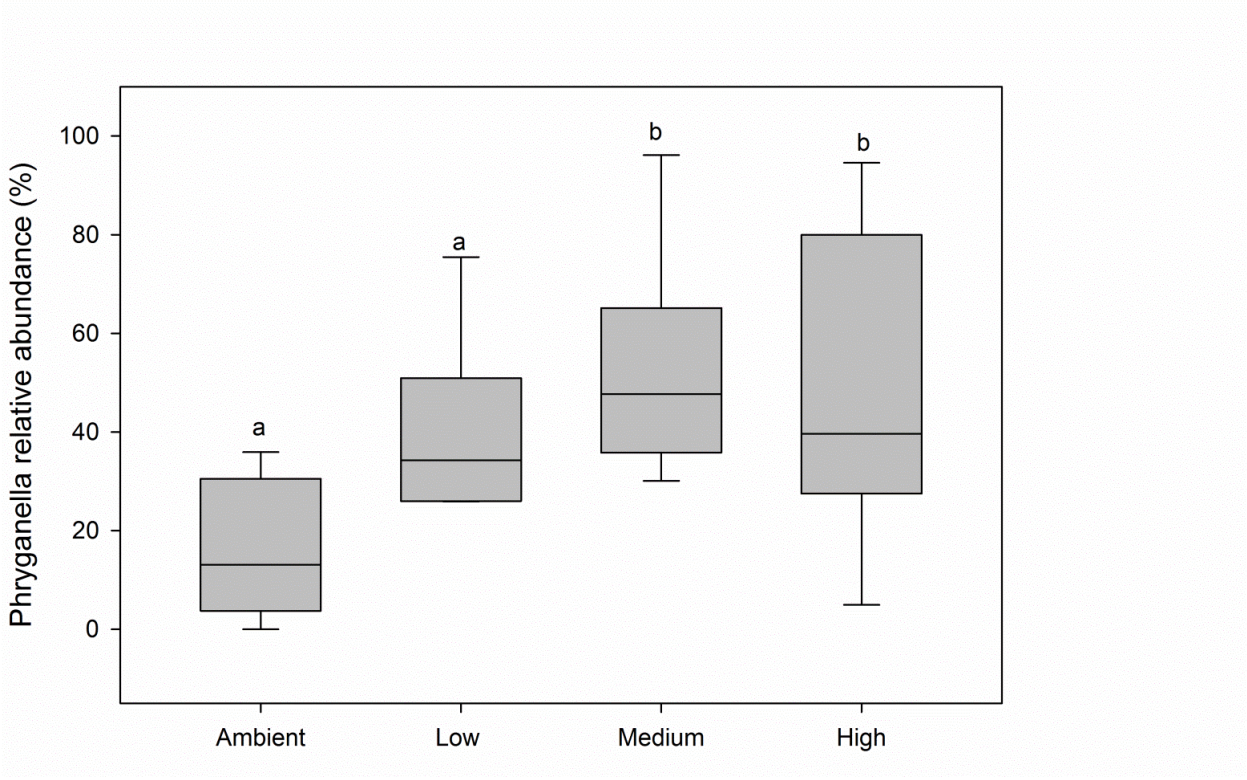
226 Figure 1. Non-metric multidimensional scaling (NMDS) ordination of testate amoeba data based on
227 biomass represented by all tests. Symbols sized in proportion to total biomass with pies showing
228 proportions of selected major species. Stress is relatively high (0.25) so patterns should be interpreted
229 with caution. There is an overall significant difference between treatments (ANOSIM, $P<0.01$), with
230 significant differences between control and both high and medium treatments when tested individually.
231 Different treatments are marked by differently coloured outlines and enclosing polygons (green=
232 ambient, blue=low, yellow=medium and red=high).




233

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

237 significant (P<0.01).




238

239 Figure 3. A) Total testate amoeba biomass based on all tests. B) Species richness based on live
240 individuals. Boxes show the median (central line), first and third quartiles (grey box) and tenth and
241 ninetieth percentiles ('whiskers'). Significant differences are marked by differing letters. Differences

between treatments for biomass are marginally non-significant (P=0.55).

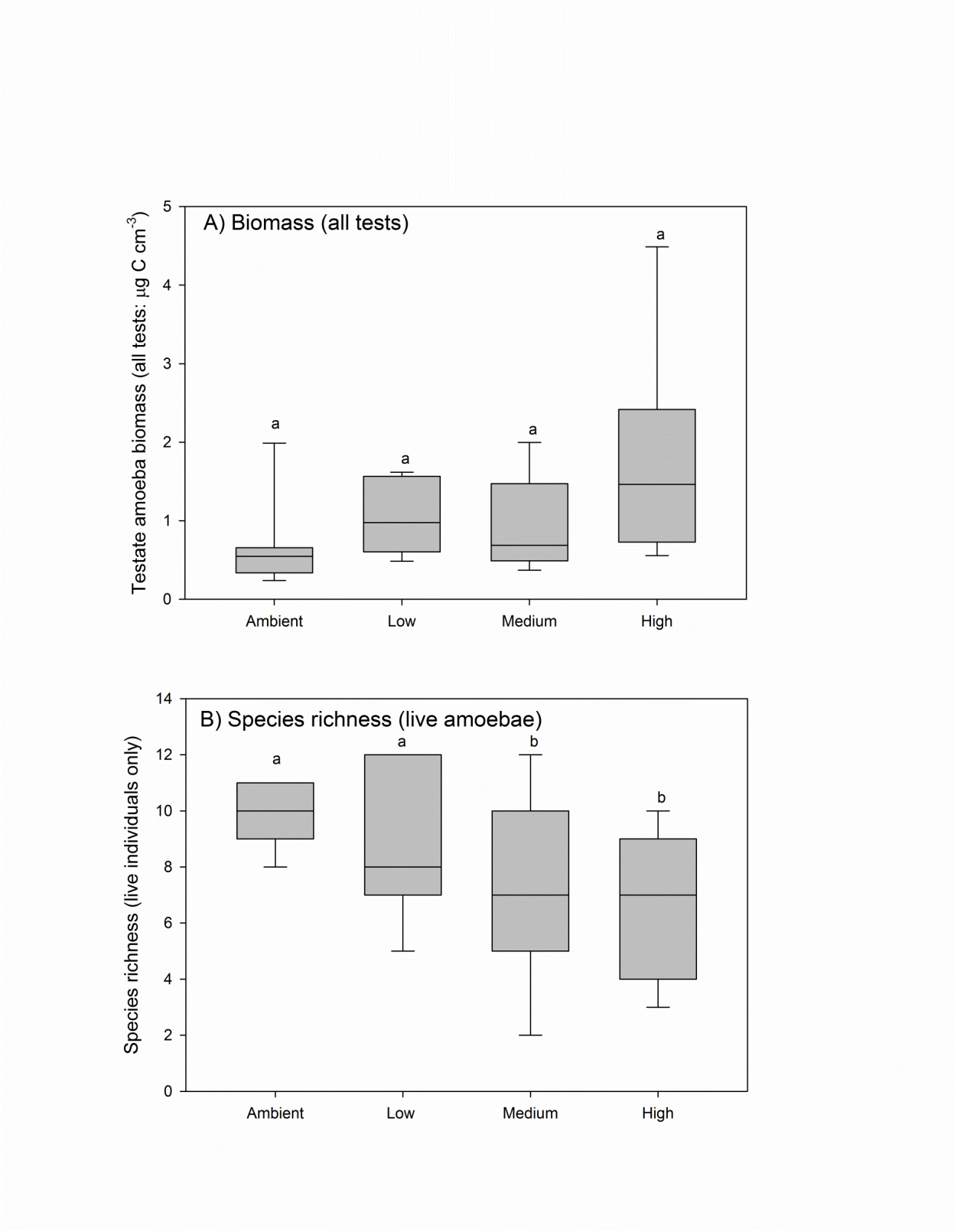


Figure 4. Box plots showing difference in abundance of quantified microbial groups in experimental mesocosms. A) Flagellates and ciliates, B) Rotifers, C) Nematodes, D) Microalgae. Boxes show the median (central line), first and third quartiles (grey box) and tenth and ninetieth percentiles ('whiskers'). Significant differences are marked by differing letters (significant differences were only found for flagellates and ciliates). Note that for all the groups other than microalgae absolute numbers of individuals counted were low.

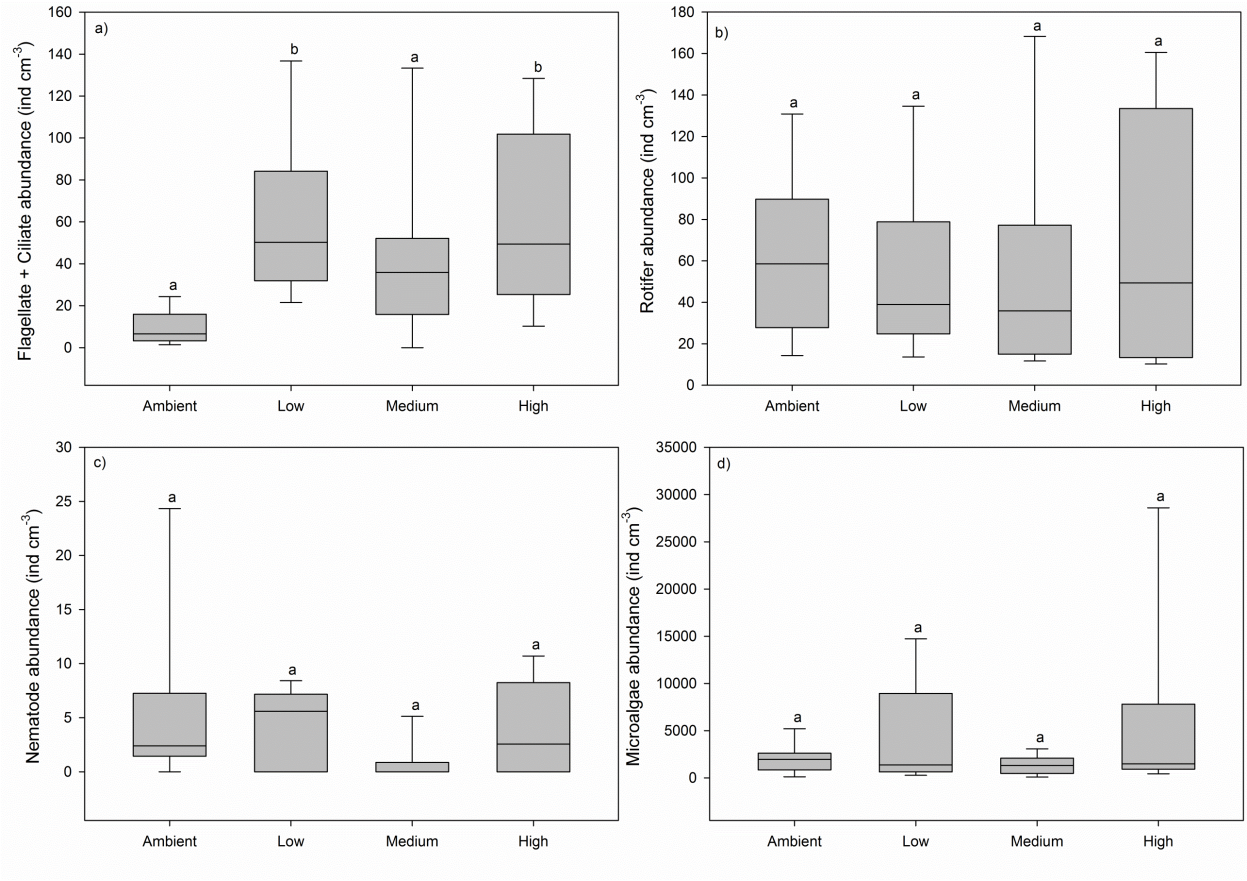



Table 1. ANOSIM tests of differences in testate amoeba community structure between experimental $O_3$ treatments. ns=non-significant. A version of this table with the abundant Phryganella spp. excluded is presented as Supplementary Table 1.

| 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.

Supplementary Table 1. ANOSIM tests of differences in testate amoeba community structure between experimental $O_3$ treatments with *Phyrgranella* spp. excluded. ns=non-significant.

| Analysed data | Tests included | $R_{ANOSIM}$ and *P*-value |
|---|---|---|
| Relative abundance | All | ns |
| | Live individuals only | ns |
| Concentration | All | ns |
| | Live individuals only | ns |
| Biomass | All | ns |
| | Live individuals only | 0.09 (*P*=0.03)* |

* In post-hoc testing Bonferroni corrected *P*-values show no significant difference between any of the treatments.