

This is a repository copy of *Impacts of tropospheric ozone exposure on peatland microbial consumers*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/120749/>

Version: Accepted Version

Article:

Payne, Richard John, Toet, Sylvia orcid.org/0000-0001-7657-4607, Ashmore, Michael Rutherford et al. (2 more authors) (2017) Impacts of tropospheric ozone exposure on peatland microbial consumers. *Soil Biology and Biochemistry*. 124–128. ISSN 0038-0717

<https://doi.org/10.1016/j.soilbio.2017.08.012>

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

1 Short Communication

2 **Impacts of tropospheric ozone exposure on peatland microbial consumers**

3 Richard J. Payne^{a,b}, Sylvia Toet^a, Mike Ashmore^c, Vincent E.J. Jassey^{d,e}, Daniel Gilbert^d

4 ^a Environment Department, University of York, Heslington, York YO10 5DD, United Kingdom.

5 ^b Department of Zoology and Ecology, Penza State University, Krasnaya str. 40, 440026 Penza, Russia.

6 ^c Stockholm Environment Institute, University of York, Heslington, York YO10 5DD, United Kingdom.

7 ^d Laboratoire Chrono-Environnement, UMR 6249 Université de Franche-Comté/CNRS, UFR Sciences et
8 Techniques, 16 route de Gray, 25030 Besançon, France.

9 ^e Université de Toulouse, INP, UPS, CNRS, Laboratoire d'Ecologie Fonctionnelle et Environnement
10 (Ecolab), 118 Route de Narbonne, 31062 Toulouse Cedex, France

11 ABSTRACT

12 Tropospheric ozone pollution is recognised as an important threat to terrestrial ecosystems but impacts
13 on peatlands are little understood despite the importance of peat as a global carbon store. Here we
14 investigate the impacts of three levels of elevated exposure to tropospheric ozone on peatland
15 microbial communities with a particular focus on testate amoebae, the dominant microbial consumers.
16 We found that in the intermediate (ambient + 25 ppb O₃) and high treatments (ambient +35 ppb
17 summer, +10 ppb year round) there were significant changes in testate amoeba communities, typified
18 by an increase in abundance of *Phyrganella* spp. and loss of diversity. *Phyrganella* is often suggested to
19 feed on fungi so the community change identified in our experiment might suggest that the testate
20 amoeba response is at least partially mediated by interactions with other microbial groups. We do not
21 find evidence for changes in numbers of undifferentiated microalgae, nematodes or rotifers but do find
22 weak evidence for an increase in flagellates and ciliates. Our results provide the first direct data to show
23 the impact of ozone on microbial consumers in peatlands.

24 KEYWORDS: Protists; Air pollution; Mire; Anthropocene

25 Tropospheric ozone (O₃) pollution is affecting an increasingly large proportion of the global land area
26 with widespread impacts on terrestrial ecosystems (Mills et al., 2011; Wilkinson et al., 2012; Fuhrer et
27 al., 2016). Through this century climate change is expected to increase the frequency of the intense
28 ozone events which lead to the most widespread damage (Royal Society, 2008). Ozone reduces soil
29 carbon sequestration and storage in forests (Talhelm et al., 2014) but there is considerable uncertainty
30 regarding impacts on the very large peatland carbon pool (c.600 GtC (Yu et al., 2010)). The limited
31 experimental evidence has shown changes in peatland plant communities and key carbon cycle
32 pathways but there is a lack of consistency between studies and the overall consequences for net
33 ecosystem carbon balance remain unclear (Morsky et al., 2008; Toet et al., 2009; Toet et al., 2011;
34 Williamson et al., 2016; Toet et al., 2017).

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

49 Here we investigate the impact of ozone on testate amoebae and other peatland microorganisms using
50 a mesocosm experiment. Full details of the experimental set-up are described in Toet et al. (2017). In
51 brief, the experiment consisted of mesocosms (19 cm diameter, 35 cm depth) extracted from wet heath
52 peatland (UK NVC community M15: *Scirpus cespitosus-Erica tetralix*) and maintained with water table at
53 50mm depth. Mesocosms were exposed to one of: ambient O₃ (non-filtered air, c.25 ppb: 'control'),
54 ambient plus 10 ppb O₃ 24hrs/day ('low'), ambient plus 25 ppb O₃ 24hrs/day ('medium') and a high
55 summer exposure of ambient plus 35 ppb O₃ for the period April to September 8hrs/day and plus 10 ppb
56 for the remainder of the year ('high'). The upper 50 mm of 10-15 *Sphagnum papillosum* stems were
57 removed from 7-9 replicates after 3.5 years and stored refrigerated in glutaraldehyde (Mazei et al.,
58 2015). Microorganisms were separated by physical agitation and inspected microscopically at 400x
59 magnification with a minimum of 100 tests counted (Payne and Mitchell, 2009) and counts converted to
60 biomass following Gilbert et al. (1998a). In parallel with testate amoeba analyses, the abundance of
61 undifferentiated microalgae (principally desmids and diatoms), rotifers, nematodes, flagellates and
62 ciliates was recorded following the same method. We analysed multivariate data using one-way analysis
63 of similarity (ANOSIM: (Clarke, 1993)) and non-metric multi-dimensional scaling (NMDS) ordination
64 based on Bray-Curtis dissimilarity (Bray and Curtis, 1957) and tested for treatment effects in univariate
65 data using ANOVA. We calculated testate amoeba relative abundance, concentration and biomass and
66 conducted separate data analyses for each. Data analyses used PAST vers. 3.04 (Hammer et al., 2001)
67 and the R-package vegan (Oksanen et al., 2007).

68 Results showed a significant difference in testate amoeba community structure between treatments for
69 data based on biomass, concentration and relative abundance of all tests ($P \leq 0.03$; Table 1) and a clear
70 treatment effect in the ordination plot (Fig. 1). These results were largely driven by a single taxon:
71 *Phyrganella* spp. (Fig. 2) which was on average three times more abundant in the High treated samples;
72 many analyses lost significance when this taxon was removed (Supplementary Table 1). Results were not
73 significant for relative abundance and concentration based on live individuals only, most likely due to
74 the low counts (Table 1). Testate amoeba species richness was significantly reduced compared to the
75 control in Medium and High treatments (ANOVA: $F_{1,3}=3.2$, $P=0.037$, Fisher's LSD: $P < 0.05$; Fig. 3). Mean
76 testate amoeba biomass of the High treated samples was 50% greater than the control samples but the
77 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
78 no significant difference in abundance of the other groups of microorganisms quantified (Fig. 4) with the
79 exception of grouped flagellate and ciliates (ANOVA: $F_3=4.0$, $P=0.017$) which were significantly more
80 abundant than control in the Low and High treatments. However, counts were very low (mean=7.7

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

84 Our results demonstrate clear changes in testate amoeba community due to ozone fumigation. Most
85 changes start in the Medium treatment (ambient +25 ppb) and are highly significant with ozone leading
86 to a community which is different in composition, less diverse and possibly of higher biomass. There are
87 many plausible mechanisms for how ozone exposure could lead to changes in testate amoeba
88 communities through both direct impacts (oxidation) and indirectly through changes in the peat physical
89 environment, physiological change and community shifts in plant communities (Searles et al., 2001) or
90 changes to microbial competitors, prey or predators (Li et al., 2015). As isotope tracer studies show that
91 ozone only penetrates a few millimetres into peat soils (Toet et al., 2009) indirect impacts are more
92 probable. Other results from this experiment have shown reduced pore-water ammonium and reduced
93 methane emission but no evidence for impacts on sedge green leaf density, root biomass or dissolved
94 organic carbon (Toet et al., 2017). These results do not directly imply a mechanism for the changes
95 detected here. No other data on soil microbial communities are currently available for these mesocosms
96 but there is data from other peatland studies. In a field mesocosm experiment Morsky et al. (2008)
97 found that both the fungal PLFA 18:2 ω 6 and total PLFA concentration were enhanced by ozone
98 exposure with no change in bacterial PLFAs. The increase in total PLFAs parallels the possible increase in
99 testate amoeba biomass and ciliate+flagellate abundance here, potentially due to an increased food
100 supply for protozoa. Our finding of increased testate amoeba biomass also parallels the results of Li et
101 al. (2015) from mineral soils who found an increase in PLFAs linked to protozoa with ozone exposure.
102 The finding of increased fungal PLFAs by Morsky et al. (2008) is particularly interesting given the
103 increase in *Phryganella* spp (most likely predominantly *P. acropodia*) detected here. This taxon has been
104 observed to feed on spores of a limited range of fungal species (Ogden and Pitta, 1990) and increase in
105 abundance in response to increased fungal abundance (Coûteaux and Devaux, 1983; Coûteaux, 1985).
106 The taxon is often considered to be mostly, or even exclusively mycophagous (Gilbert et al., 2000) but
107 may primarily feed on saprophytic fungal exudates or exudate-feeding bacteria rather than fungi
108 themselves (Vohník et al., 2011). The only study which has directly compared PLFA 18:2 ω 6c results with
109 *P. acropodia* abundance did not find a correlation (Krashevskaya et al., 2008) but this was in a quite
110 different ecosystem. We consider that an increased fungal abundance or changed fungal community
111 structure in the ozone treated samples is one likely explanation for the testate amoeba changes
112 detected.

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

118 ACKNOWLEDGEMENTS

119 RJP was supported by a fellowship from the Conseil Régional de Franche-Comté during the course of this
120 research. Data analysis, interpretation and publication were supported by the Russian Science
121 Foundation (14-14-00891). We gratefully acknowledge the support of staff at Newcastle University
122 in providing access to the Close House experimental facility and maintaining the experimental

123 mesocosms and ozone treatments. The experiment was funded by the Natural Environment Research
124 Council through grant NE/E015700/1.

125 REFERENCES

- 126 Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin.
 127 Ecological Monographs 27, 325-349.
- 128 Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Australian
 129 Journal of Ecology 18, 117-143.
- 130 Coûteaux, M.-M., 1985. Relationships between testate amoebae and fungi in humus microcosms. Soil
 131 Biology and Biochemistry 17, 339-345.
- 132 Coûteaux, M.-M., Devaux, J., 1983. Effet d'un enrichissement en champignons sur la dynamique d'un
 133 peuplement thécamoebien d'un humus. Revue d'Ecologie et de Biologie du Sol 20, 519-545.
- 134 Fuhrer, J., Val Martin, M., Mills, G., Heald, C.L., Harmens, H., Hayes, F., Sharps, K., Bender, J., Ashmore,
 135 M.R., 2016. Current and future ozone risks to global terrestrial biodiversity and ecosystem processes.
 136 Ecology and Evolution 6, 8785-8799.
- 137 Gilbert, D., Amblard, C., Bourdier, G., André-Jean, F., Mitchell, E.A., 2000. Le régime alimentaire des
 138 thécamoebiens (Protista, Sarcodina). L'Année Biologique 39, 57-68.
- 139 Gilbert, D., Amblard, C., Bourdier, G., Francez, A.-J., 1998a. Short-term effect of nitrogen enrichment on
 140 the microbial communities of a peatland, In: Amiard, J.C., Le Rouzic, B., Berthet, B., Bertru, G. (Eds.),
 141 Oceans, Rivers and Lakes: Energy and Substance Transfers at Interfaces: Proceedings of the Third
 142 International Joint Conference on Limnology and Oceanography held in Nantes, France, October 1996.
 143 Springer Netherlands, Dordrecht, pp. 111-119.
- 144 Gilbert, D., Amblard, C., Bourdier, G., Francez, A.J., 1998b. The microbial loop at the surface of a
 145 peatland: structure, function, and impact of nutrient input. Microbial Ecology 35, 83-93.
- 146 Hammer, Ø., Harper, D., Ryan, P., 2001. PAST-palaeontological statistics, ver. 1.89. Palaeontologia
 147 Electronica 4.
- 148 Jassey, V.E.J., Chiapusio, G., Binet, P., Buttler, A., Laggoun-Défarge, F., Delarue, F., Bernard, N., Mitchell,
 149 E.A., Toussaint, M.L., Francez, A.J., 2013a. Above-and belowground linkages in Sphagnum peatland:
 150 climate warming affects plant-microbial interactions. Global Change Biology 19, 811-823.
- 151 Jassey, V.E.J., Meyer, C., Dupuy, C., Bernard, N., Mitchell, E.A.D., Toussaint, M.-L., Metian, M., Chatelain,
 152 A.P., Gilbert, D., 2013b. To what extent do food preferences explain the trophic position of
 153 heterotrophic and mixotrophic microbial consumers in a Sphagnum peatland? Microbial Ecology 66,
 154 571-580.
- 155 Jassey, V.E.J., Shimano, S., Dupuy, C., Toussaint, M.-L., Gilbert, D., 2012. Characterizing the feeding
 156 habits of the testate amoebae *Hyalosphenia papilio* and *Nebela tinctoria* along a narrow "fen-bog" gradient
 157 using digestive vacuole content and 13 C and 15 N isotopic analyses. Protist 163, 451-464.
- 158 Jassey, V.E.J., Signarbieux, C., Hättenschwiler, S., Bragazza, L., Buttler, A., Delarue, F., Fournier, B.,
 159 Gilbert, D., Laggoun-Défarge, F., Lara, E., 2015. An unexpected role for mixotrophs in the response of
 160 peatland carbon cycling to climate warming. Scientific Reports 5.
- 161 Karimi, B., Meyer, C., Gilbert, D., Bernard, N., 2016. Air pollution below WHO levels decreases by 40 %
 162 the links of terrestrial microbial networks. Environmental Chemistry Letters 14, 467-475.
- 163 Krashevskaya, V., Bonkowski, M., Maraun, M., Ruess, L., Kandeler, E., Scheu, S., 2008. Microorganisms as
 164 driving factors for the community structure of testate amoebae along an altitudinal transect in tropical
 165 mountain rain forests. Soil Biology and Biochemistry 40, 2427-2433.
- 166 Li, Q., Yang, Y., Bao, X., Liu, F., Liang, W., Zhu, J., Bezemer, T.M., van der Putten, W.H., 2015. Legacy
 167 effects of elevated ozone on soil biota and plant growth. Soil Biology and Biochemistry 91, 50-57.
- 168 Mazei, Y., Chernyshov, V., Tsyganov, A.N., Payne, R.J., 2015. Testing the Effect of Refrigerated Storage on
 169 Testate Amoeba Samples. Microbial Ecology 70, 861-864.

170 Meyer, C., Gilbert, D., Gillet, F., Moskura, M., Franchi, M., Bernard, N., 2012. Using “bryophytes and their
171 associated testate amoeba” microsystems as indicators of atmospheric pollution. *Ecological Indicators*
172 13, 144-151.

173 Mills, G., Hayes, F., Simpson, D., Emberson, L., Norris, D., Harmens, H., Büker, P., 2011. Evidence of
174 widespread effects of ozone on crops and (semi-) natural vegetation in Europe (1990–2006) in relation
175 to AOT40-and flux-based risk maps. *Global Change Biology* 17, 592-613.

176 Morsky, S.K., Haapala, J.K., Rinnan, R., Tiiva, P., Saarnio, S., Silvola, J., Holopainen, T., Martikainen, P.J.,
177 2008. Long-term ozone effects on vegetation, microbial community and methane dynamics of boreal
178 peatland microcosms in open-field conditions. *Global Change Biology* 14, 1891-1903.

179 Nguyen-Viet, H., Bernard, N., Mitchell, E.A., Cortet, J., Badot, P.-M., Gilbert, D., 2007. Relationship
180 between testate amoeba (Protist) communities and atmospheric heavy metals accumulated in *Barbula*
181 *indica* (Bryophyta) in Vietnam. *Microbial Ecology* 53, 53-65.

182 Nguyen Viet, H., Gilbert, D., Bernard, N., Mitchell, E.A., Badot, P.-M., 2004. Relationship between
183 atmospheric pollution characterized by NO₂ concentrations and testate amoebae density and diversity.
184 *Acta Protozoologica* 43, 233-239.

185 Ogden, C., Pitta, P., 1990. Biology and ultrastructure of the mycophagus, soil testate amoeba,
186 *Phryganella acropodia* (Rhizopoda, Protozoa). *Biology and Fertility of Soils* 9, 101-109.

187 Oksanen, J., Kindt, R., Legendre, P., O’Hara, B., Stevens, M.H.H., Oksanen, M.J., Suggests, M., 2007. The
188 vegan package. *Community ecology package* 10.

189 Payne, R., Gauci, V., Charman, D.J., 2010. The impact of simulated sulfate deposition on peatland testate
190 amoebae. *Microbial Ecology* 59, 76-83.

191 Payne, R.J., Mitchell, E.A.D., 2009. How many is enough? Determining optimal count totals for ecological
192 and palaeoecological studies of testate amoebae. *Journal of Paleolimnology* 42, 483-495.

193 Payne, R.J., Thompson, A.M., Standen, V., Field, C.D., Caporn, S.J.M., 2012. Impact of simulated nitrogen
194 pollution on heathland microfauna, mesofauna and plants. *European Journal of Soil Biology* 49, 73-79.

195 Royal Society, 2008. Ground-level ozone in the 21st century: future trends, impacts and policy
196 implications. Royal Society, London.

197 Searles, P.S., Kropp, B.R., Flint, S.D., Caldwell, M.M., 2001. Influence of solar UV-B radiation on peatland
198 microbial communities of southern Argentina. *New Phytologist* 152, 213-221.

199 Talhelm, A.F., Pregitzer, K.S., Kubiske, M.E., Zak, D.R., Company, C.E., Burton, A.J., Dickson, R.E.,
200 Hendrey, G.R., Isebrands, J.G., Lewin, K.F., Nagy, J., Karnosky, D.F., 2014. Elevated carbon dioxide and
201 ozone alter productivity and ecosystem carbon content in northern temperate forests. *Global Change*
202 *Biology* 20, 2492-2504.

203 Toet, S., Ineson, P., Peacock, S., Ashmore, M., 2011. Elevated ozone reduces methane emissions from
204 peatland mesocosms. *Global Change Biology* 17, 288-296.

205 Toet, S., Oliver, V., Ineson, P., McLoughlin, S., Helgason, T., Peacock, S., Stott, A.W., Barnes, J., Ashmore,
206 M., 2017. How does elevated ozone reduce methane emissions from peatlands? *Science of The Total*
207 *Environment* 579, 60-71.

208 Toet, S., Subke, J.-A., D’Haese, D., Ashmore, M.R., Emberson, L.D., Crossman, Z., Evershed, R.P., Barnes,
209 J.D., Ineson, P., 2009. A new stable isotope approach identifies the fate of ozone in plant–soil systems.
210 *New Phytologist* 182, 85-90.

211 Vohník, M., Burdík, Z., Vyhna, A., Koukol, O., 2011. Interactions between testate amoebae and
212 saprotrophic microfungi in a Scots pine litter microcosm. *Microbial Ecology* 61, 660-668.

213 Wilkinson, D.M., Mitchell, E.A., 2010. Testate amoebae and nutrient cycling with particular reference to
214 soils. *Geomicrobiology Journal* 27.

215 Wilkinson, S., Mills, G., Illidge, R., Davies, W.J., 2012. How is ozone pollution reducing our food supply?
216 *Journal of Experimental Botany* 63, 527-536.

217 Williamson, J.L., Mills, G., Hayes, F., Jones, T., Freeman, C., 2016. How do increasing background
218 concentrations of tropospheric ozone affect peatland plant growth and carbon gas exchange?
219 Atmospheric Environment 127, 133-138.
220 Yu, Z., Loisel, J., Brosseau, D.P., Beilman, D.W., Hunt, S.J., 2010. Global peatland dynamics since the Last
221 Glacial Maximum. Geophysical Research Letters 37.

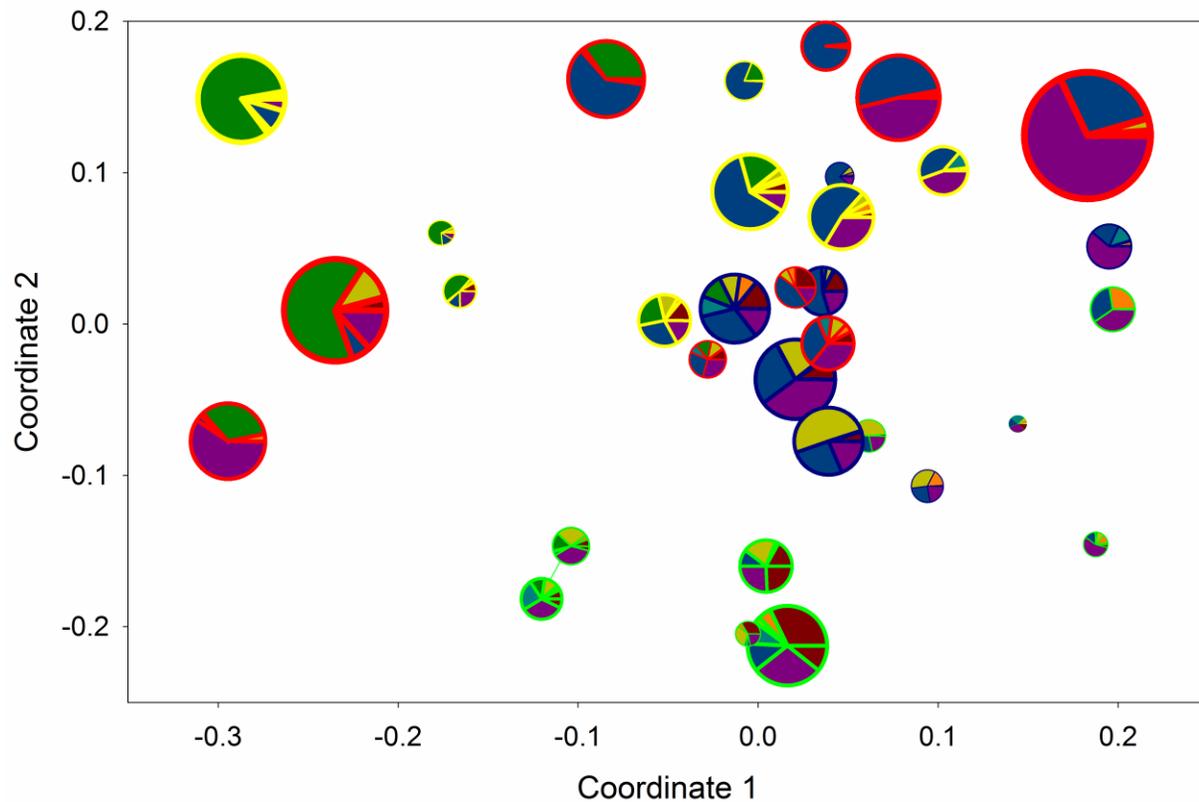
222

223

224

225 FIGURES and TABLES

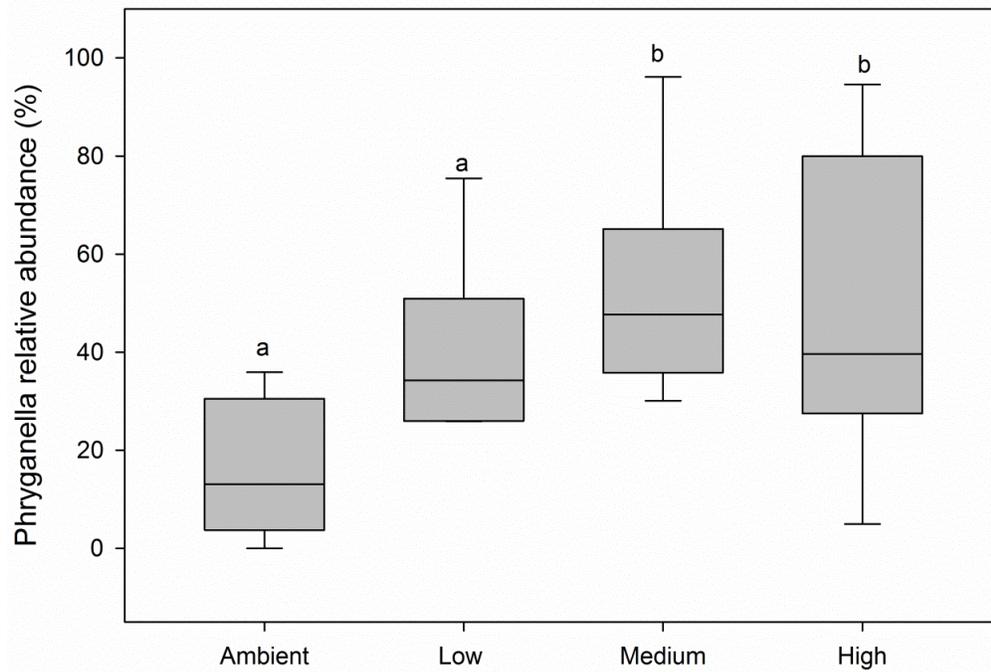
226 Figure 1. Non-metric multidimensional scaling (NMDS) ordination of testate amoeba data based on
227 biomass represented by all tests. Symbols sized in proportion to total biomass with pies showing
228 proportions of selected major species. Stress is relatively high (0.25) so patterns should be interpreted
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

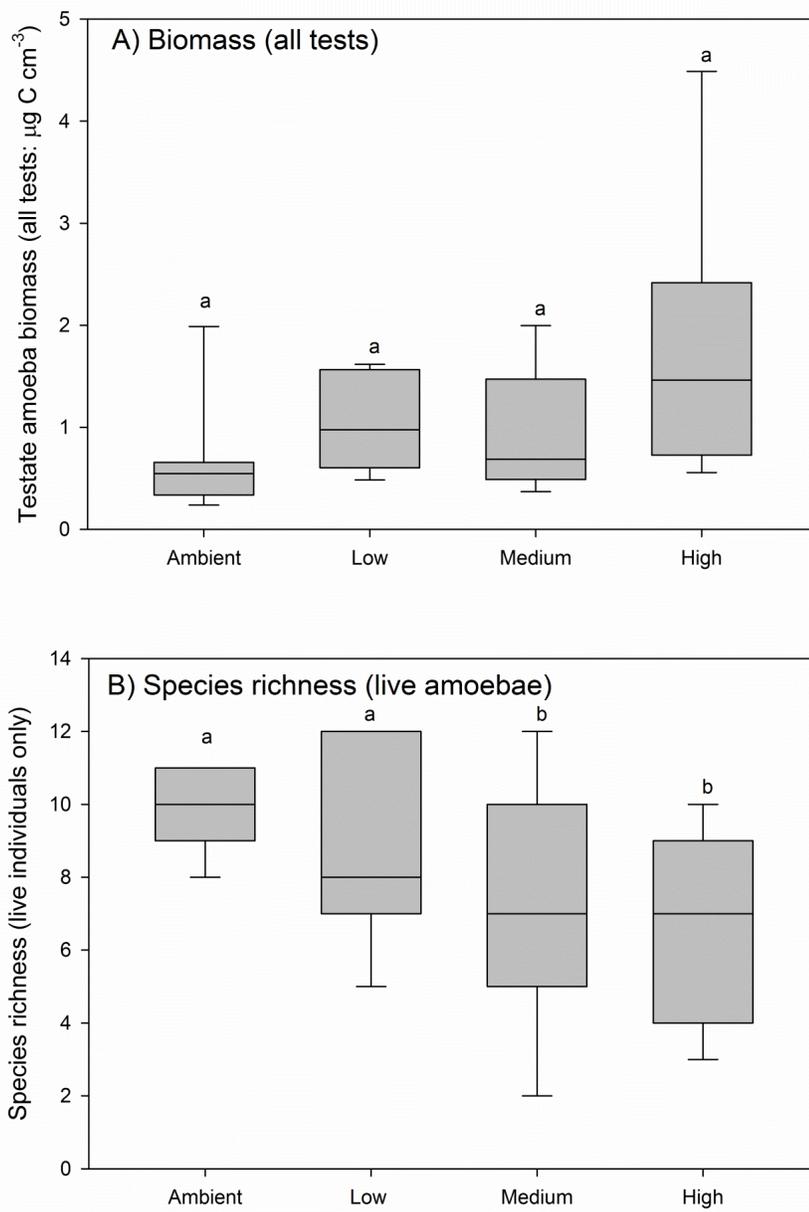
237 significant ($P < 0.01$).



238

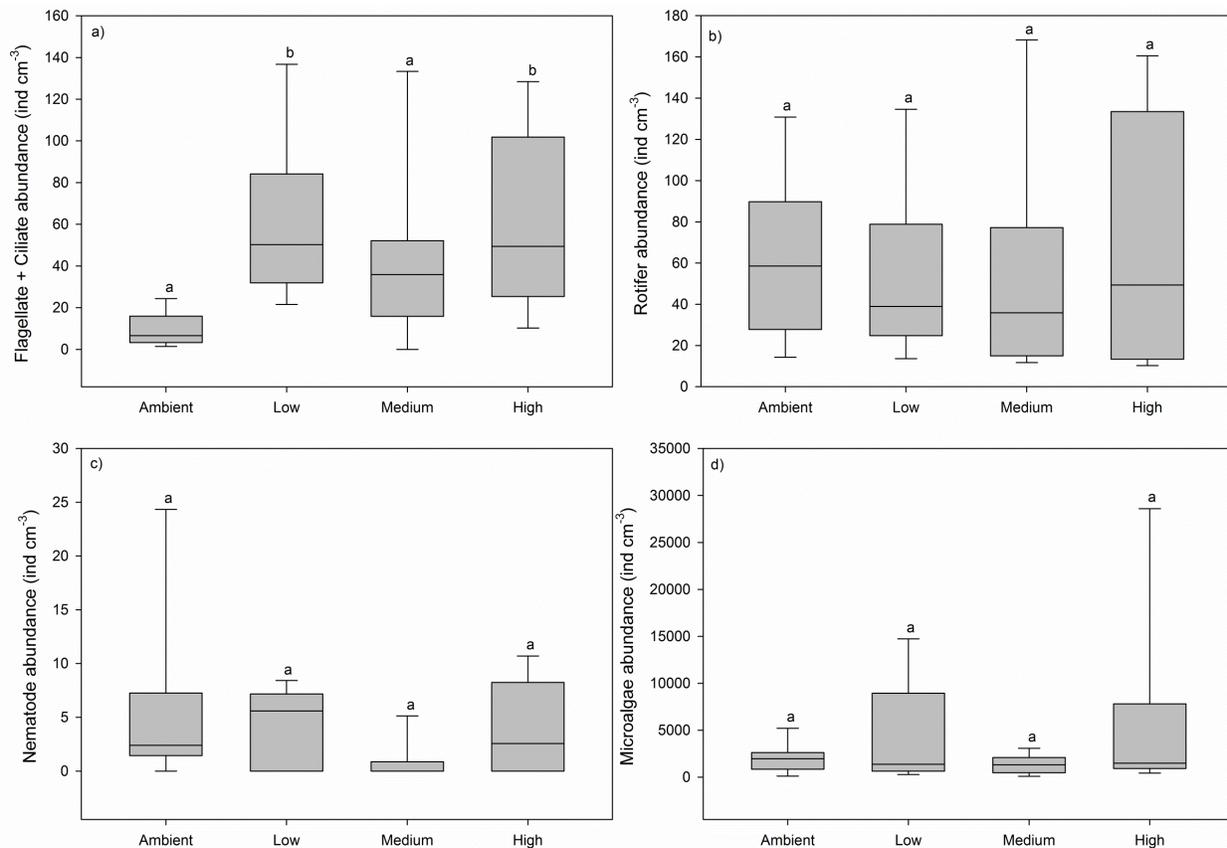
239 Figure 3. A) Total testate amoeba biomass based on all tests. B) Species richness based on live
240 individuals. Boxes show the median (central line), first and third quartiles (grey box) and tenth and
241 ninetieth percentiles ('whiskers'). Significant differences are marked by differing letters.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

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



243

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



250

251 Table 1. ANOSIM tests of differences in testate amoeba community structure between experimental O₃
 252 treatments. ns=non-significant. A version of this table with the abundant Phryganella spp. excluded is
 253 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)*

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

256

257 Supplementary Table 1. ANOSIM tests of differences in testate amoeba community structure between
 258 experimental O₃ treatments with *Phyrrgranella* 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)*

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

260