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1 Short Communication

2 **Ammonia exposure promotes algal biomass in an ombrotrophic peatland**

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17 ABSTRACT

18 Nitrogen pollution affects many peatlands with consequences for their biodiversity and ecosystem
19 function. Microorganisms control nutrient cycling and constitute most of the biodiversity of peatlands
20 but their response to nitrogen is poorly characterised and likely to depend on the form of deposition.
21 Using a unique field experiment we show that ammonia exposure at realistic point source levels is
22 associated with a general shift from heterotrophic (bacteria and fungi) to autotrophic (algal) dominance
23 and an increase in total biomass. The biomass of larger testate amoebae increased, suggesting increased
24 food supply for microbial predators. Results show the widespread impacts of N pollution and suggest
25 the potential for microbial community-based bioindicators in these ecosystems.

26 KEYWORDS: Nitrogen; Pollution impact; Bioindication; Euglenids; Desmids; Testate amoebae

27 Ombrotrophic peatlands, which receive their nutrients from the atmosphere, are naturally
28 oligotrophic and highly sensitive to atmospheric deposition of acids, nutrients, and other pollutants.
29 Nitrogen deposition affects many peatlands in industrialised regions and may shift peatlands from
30 carbon sinks to sources with important consequences for the global carbon cycle (Aerts et al., 1992,
31 Bragazza et al., 2006). Such processes are microbially-mediated but the overall microbial community
32 response to N is poorly characterised. Microbes constitute most of the biodiversity of peatlands (Gilbert
33 and Mitchell 2006) but studies of the biodiversity impacts of N pollution in terrestrial ecosystems are
34 almost entirely restricted to plants. The form of N deposition is an important determinant of ecosystem
35 impacts (Stevens et al., 2011; Liu and Greaver 2009), with evidence that the impacts of gaseous
36 ammonia can be particularly acute (Sheppard et al., 2011). Ammonia is a significant threat to peatlands
37 in areas of intensive agriculture (Sutton et al., 2011) but existing research on the microbial response to N
38 has been limited to wet NH_4NO_3 application (Gilbert et al., 1998). In this study, we use a morphological,
39 functional group approach to investigate the response of micro-organisms to ammonia dry deposition in
40 a field experiment.

41 The Whim Moss experiment is a globally-unique pollution experiment on a *Calluna vulgaris*-
42 *Eriophorum vaginatum* blanket bog in southern Scotland (3° 16'W, 55° 46'N: Leith et al., 2004). NH_3 is
43 released from a pipe 1 m above the ground when air temperature exceeds 0°C, wind speed exceeds 2.5
44 m s^{-1} and wind direction is within the range 180-215°. Monthly average NH_3 concentrations are
45 determined using passive ALPHA samplers (Sutton et al., 2001) positioned at 0.1 m above the vegetation
46 along a downwind transect (Sheppard et al., 2011). After nine years of treatment (September 2011), the
47 upper 50mm of ten stems of *Sphagnum capillifolium* (Ehrh.) Hedw. were extracted at seven locations
48 along this transect and placed in 10% formaldehyde. Additional samples were taken off the main
49 transect and from an area receiving only ambient deposition. Samples were prepared by multiple cycles
50 of agitation and washing following Jassey et al. (2011) and examined under inverted microscopy. All
51 larger micro-organisms were identified to group (cyanobacteria, microalgae, fungi, flagellates, ciliates
52 and micrometazoa including rotifers and nematodes) and assigned to a sequence of broad
53 morphotypes. More detailed morpho-species identification was carried out for testate amoebae (>100
54 individuals; Supplementary Table 1): an abundant group of protists which are sensitive to N deposition
55 (Gilbert et al., 1998; Payne et al., 2012). Flow cytometry (BD FACSCalibur) was used for bacterial counts
56 with samples stained with SYBR Green I (1/10,000 final concentration) for 15 minutes in the dark and
57 run at medium speed (ca 40 $\mu\text{L min}^{-1}$). DAPI-treated sub-samples were examined by epifluorescence
58 microscopy to establish mean bacterial dimensions. All count data were converted to biomass by

59 calculating biovolumes based on geometric shapes (*cf.* Mitchell 2004) and applying established
60 conversion factors (Jassey et al., 2011).

61 Enhanced ammonia exposure was found to drive large changes in the microbial community.
62 Near to the ammonia source the biomass was dominated by algae, contributing over 50% of the total
63 microbial biomass (Fig. 1A), while in samples receiving ambient exposure the biomass was more evenly
64 distributed between algae, fungi, bacteria and protozoa (Fig. 1A). There was a positive trend in total
65 microbial biomass along the ammonia concentration gradient (Spearman's $r_s=0.71$, $p=0.009$; Fig. 1B);
66 mostly accounted for by difference between those samples receiving <8 and $>25 \mu\text{g m}^{-3}$ (t-test $t=-10.8$,
67 $p<0.001$). Biomass near the ammonia source was more than double that of samples receiving ambient
68 exposure. Considered separately, only algal ($r_s=0.63$, $p=0.03$) biomass was significantly correlated with
69 ammonia concentration. The algae increasing in abundance included euglenids (e.g. *Euglena cf.*
70 *mutabilis*), and to a lesser extent, desmids (e.g. *Cylindrocystis gracilis*). Cyanobacteria biomass (mostly
71 non-nitrogen fixing genera such as *Merismopedia* and *Chroococcus*) showed a non-significant positive
72 correlation with ammonia ($r_s=0.5$, $p=0.08$) but was a minor component of the total biomass ($<3\%$). Given
73 the many other influences on peatland microbial communities (Mitchell et al., 2000), the impact of
74 ammonia emerges strongly in our data (Fig. 1). The sample at 30 m did not have an elevated overall
75 biomass but does have a larger proportion of algae than untreated samples, perhaps reflecting local
76 micro-topographic sheltering (Fig. 1A).

77
78 Ammonia concentration was significantly ($p=0.02$) correlated with the second ordination axis in
79 an NMDS of testate amoeba data, showing ammonia-induced changes in testate amoebae community
80 structure (Fig. 2). Total (living + dead) testate amoeba biomass tends to increase with ammonia but this
81 is of marginal non-significance ($r_s=0.51$, $p=0.09$; Fig. 2). There was a significant positive correlation
82 between ammonia and the total biomass of testate amoeba taxa with larger tests ($>50000\mu\text{m}^3$, e.g.
83 *Nebela tincta*, *Heleopera rosea*], $r_s=0.78$, $p=0.003$) but not with smaller taxa ($p>0.05$). These larger taxa
84 with larger apertures are more likely to be algivorous (e.g. *N. tincta*: Jassey et al., 2012) suggesting that
85 greater biomass is driven by greater availability of algal prey. The lack of a similar biomass response in
86 other groups of predators (ciliates, rotifers) may relate to the palatability of the algal and cyanobacterial
87 species involved (Dokulil and Teubner, 2000).

88

89 Our study only considers a relatively small number of samples from a single sampling occasion at
90 a single site, but results are sufficient to suggest that increased ammonia exposure can cause large
91 changes in the structure and functioning of the microbial food-web. Our results show a similar pattern
92 to those of Gilbert et al., (1998) with N enhancing overall microbial biomass, and particularly autotrophs.
93 The magnitude of change is greater in our data, which probably relates to the much longer duration of
94 the experiment but may also reflect differences in sensitivity to N forms and peatland type. Our results
95 are consistent with a direct eutrophying influence of ammonia, suggesting that peatland algae and
96 cyanobacteria are N-limited (or N and P co-limited). However we cannot exclude indirect impacts
97 through changes in pH, soil chemistry and biotic interactions. The positive impact of ammonia on algae
98 contrasts with the deleterious impacts on bryophytes and vascular plants (Sheppard et al., 2011),
99 probably because the microbial community was primarily exposed to ammonium in the moist
100 bryosphere rather than to dry ammonia which can produce direct physiological impacts on plants.
101 Microbial C biomass with high ammonia exposure is only approximately 1% of *Sphagnum* C but may be
102 an important C pool given its greater lability.

103 Increased autotroph biomass is apparent with ammonia concentrations above approximately 6
104 $\mu\text{g m}^{-3}$, which corresponds to an N deposition of approximately $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Sheppard et al., 2011).
105 N deposition in Europe is regulated using a critical load, currently set at $5\text{-}10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and NH_3 using
106 a critical level currently set at $1 \mu\text{g m}^{-3}$ (Cape et al., 2009). Such values are primarily assigned on the basis
107 of impacts on plant communities with a (usually unstated) assumption that if plants are protected, other
108 ecosystem components will also be conserved. Our results do not contradict this assumption: after 9
109 years of treatment we find no indication of impacts on microbial communities below either the critical
110 load or level, our results suggest either should be sufficient to avoid major microbial change. Changes in
111 microbial community composition might be a useful addition to the suite of techniques used for the
112 bioindication of N pollution (Sutton et al., 2004), perhaps by considering a ratio of algae to other
113 microbial groups.

114

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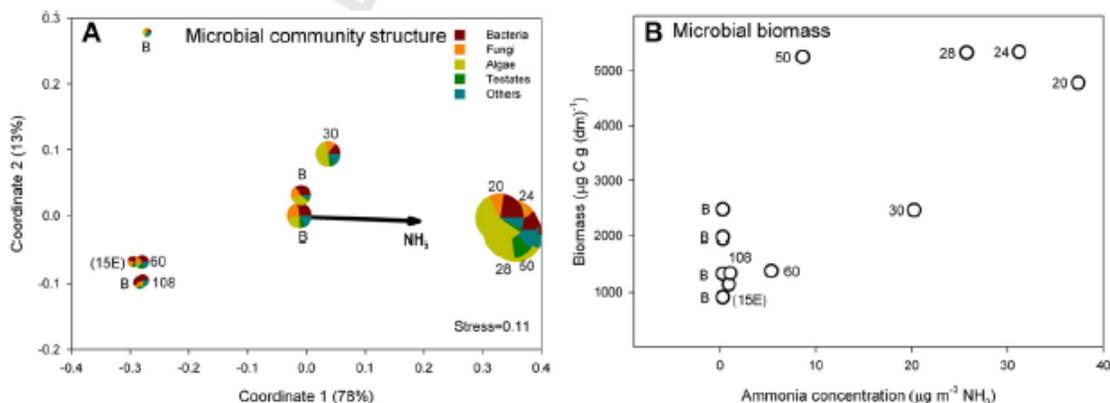
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122

123 FIGURE CAPTIONS

124 Fig. 1. Microbial response to ammonia. A) NMDS ordination based on Bray-Curtis dissimilarity of
 125 microbial group biomass, pies show relative abundance of groups with symbols sized in proportion to
 126 total biomass. Ammonia concentration is significantly correlated with NMDS axis one ($r=0.78$, $p=0.002$).
 127 B) Microbial total biomass against NH_3 concentration measured 0.1m above the vegetation. Symbols on
 128 both plots labelled with distance along transect (20-108 m, B=background sampling area, (15E)=15 m off
 129 main transect). Biomass calculations are likely to under-estimate biomass of some groups not removed
 130 in preparation (notably fungi) but provide internally consistent estimates. Ammonia concentrations are
 131 means for January 2007-September 2011; these long-term values are very strongly correlated with
 132 those for the month of sampling and the preceding 3 month period (both $r=0.99$, $p<0.0001$).



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134

135

136 Fig. 2. Testate amoeba community. NMDS ordination based on Bray-Curtis dissimilarity of taxa biomass
 137 (calculated on the basis of both living and dead individuals, unlike Fig. 1 based on only living individuals
 138 for comparability with other microbial groups). Symbols sized in proportion to total biomass (range: 90-
 139 780 $\mu\text{g C g}(\text{dm})^{-1}$). Ammonia concentration significantly correlated with axis two ($r=0.66$, $p=0.02$). Pies
 140 show relative abundance of selected taxa, labelled as for Fig. 1.

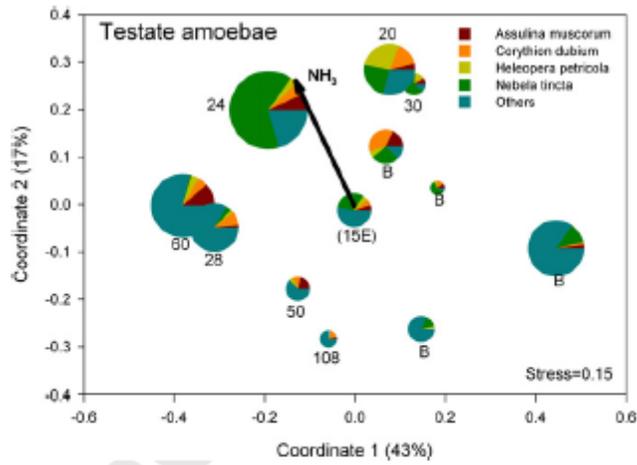


Fig. 2. Testate amoeba community. NMS ordination based on Bray-Curtis dissimilarity of taxa biomass (calculated on the basis of both living and dead individuals, unlike Fig. 1 based on only living individuals for comparability with other microbial groups). Symbols sized in proportion to total biomass (range: 90–780 $\mu\text{g C}(\text{dm}^{-3})$). Ammonia concentration significantly correlated with axis two ($r = 0.66, p = 0.02$). Pies show relative abundance of selected taxa, labelled as for Fig. 1.

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143 Supplementary Table One. Full list of testate amoeba taxa identified in Whim Moss samples.

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146 REFERENCES

147 Aerts, R., Wallén, B., Malmer, N., 1992. Growth-limiting nutrients in *Sphagnum*-dominated bogs subject
148 to low and high atmospheric nitrogen supply. *Journal of Ecology* 80, 131-140.

149 Bragazza, L., Freeman, C., Jones, T., Rydin, H., Limpens, J., Fenner, N., Ellis, T., Gerdol, R., Hájek, M.,
150 Hájek, T., Iacumin, P., Kutnar, L., Tahvanainen, T., Toberman, H., 2006. Atmospheric nitrogen deposition
151 promotes carbon loss from peat bogs. *Proceedings of the National Academy of Sciences of the USA* 103,
152 19386-19389.

153 Cape, J.N., van der Eerden, L.J., Sheppard, L.J., Leith, I.D., Sutton, M.A., 2009. Evidence for changing the
154 critical level for ammonia. *Environmental Pollution* 157, 1033-1037.

155 Dokulil, M.T., Teubner, K., 2000. Cyanobacterial dominance in lakes. *Hydrobiologia* 438, 1-12.

156 Gilbert, D., Mitchell, E.A.D., 2006. Microbial diversity in *Sphagnum* peatlands. In: Martini, I.P., Martinez-
157 Cortizas, A., Chesworth, W. (Eds.), *Peatlands: Evolution and Records of Environmental and Climate*
158 *Changes*. Elsevier, Amsterdam, pp. 287-318.

159 Gilbert, D., Amblard, C., Bourdier, G., Francez, A.-J., 1998. Short-term effect of nitrogen enrichment on
160 the microbial communities of a peatland. *Hydrobiologia* 373/374, 111-119.

161

162 Jasey, V.E.J., Gilbert, D., Binet, P., Toussaint, M.-L., Chiapusio, G., 2011. Effect of a temperature gradient
163 on *Sphagnumfallax* and its associated living microbial communities: a study under controlled conditions.
164 *Canadian Journal of Microbiology* 57, 226–235.

165

166 Jasey, V.E.J., Shimano, S., Dupuy, C., Toussaint, M.-L., Gilbert, D., 2012. Characterizing the feeding habits
167 of the testate amoebae *Hyalosphenia papilio* and *Nebela tinctoria* along a narrow “Fen-Bog” gradient using
168 gigestive vacuole content and ¹³C and ¹⁵N isotopic analyses. *Protist* 163, 451-464.

169 Leith, I.D., Sheppard, L.J., Fowler, D., Cape, J.N., Jones, M., Crossley, A., Hargreaves, K.J., Tang, Y.S.,
170 Theobald, M., Sutton, M.R., 2004. Quantifying dry NH₃ deposition to an ombrotrophic bog from an
171 automated NH₃ field release system. *Water Air and Soil Pollution: Focus* 4, 207-218.

172 Liu, L., Greaver, T.L., 2009. A review of nitrogen enrichment effects on three biogenic GHG's: the CO₂
173 sink may be largely offset by stimulated N₂O and CH₄ emission. Ecology Letters 12, 1103-1117.
174

175 Mitchell, E.A.D., 2004. Response of testate amoebae (Protozoa) to N and P fertilization in an Arctic wet
176 sedge tundra. Arctic Antarctic and Alpine Research 36, 77-82.
177

178 Mitchell, E.A.D., Borcard, D., Buttler, A.J., Grosvernier, P., Gilbert, D., Gobat, J-M., 2000. Horizontal
179 distribution patterns of testate amoebae (Protozoa) in a *Sphagnum magellanicum* Carpet. Microbial
180 Ecology 39, 290–300.
181

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

184 Sheppard, L.J., Leith, I.D., Mizunuma, T., Cape, J.N., Crossley, A., Leeson, S., Sutton, M.A., van Dijk, N.,
185 Fowler, D., 2011. Dry deposition of ammonia gas drives species change faster than wet deposition of
186 ammonium ions: evidence from a long-term field manipulation. Global Change Biology 17, 3589-3607.

187 Stevens, C.J., Manning, P., van den Berg, L.J.L., de Graaf, M.C.C., Wamelink, G.W.W., Boxman, A.W.,
188 Bleeker, A., Vergeer, P., Arroniz-Crespo, M., Limpens, J., Lamers, L.P.M., Bobbink, R., Dorland, E., 2011.
189 Ecosystem responses to reduced and oxidised nitrogen inputs in European terrestrial habitats.
190 Environmental Pollution 159, 665-676.
191

192 Sutton, M.A., Tang, Y.S., Dragosits, U., Fournier, N., Dore, A.J., Smith, R.I., Weston, K.J., Fowler, D., 2001.
193 A spatial analysis of atmospheric ammonia and ammonium in the U.K. The Scientific World Journal 1,
194 275-286.
195

196 Sutton, M.A., Pitcairn, C.E.R., Whitfield, C.P., 2004. Bioindicator and biomonitoring methods for
197 assessing the effects of atmospheric nitrogen on statutory nature conservation sites, JNCC Report 356.
198 JNCC, Peterborough. Available at: <http://jncc.defra.gov.uk/pdf/jncc356.pdf>. Last accessed 16/08/2012.

199 Sutton, M.A., Leith, I.D., Bealey, W.J., van Dijk, N., Tang, Y.S., 2011. Moninea Bog-Case study of
200 atmospheric ammonia impacts on a Special Area of Conservation, In: Hicks, W.K., Whitfield, C.P., Bealey,
201 W.J., Sutton, M.A. (Eds.), Nitrogen Deposition and Natura 2000: Science and Practice in Determining
202 Environmental Impacts. COST, Brussels pp. 59-71.

203

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206