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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  $\text{NH}_4\text{NO}_3$  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).  $\text{NH}_3$  is  
43 released from a pipe 1 m above the ground when air temperature exceeds 0°C, wind speed exceeds 2.5  
44  $\text{m s}^{-1}$  and wind direction is within the range 180-215°. Monthly average  $\text{NH}_3$  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  $\text{NH}_3$  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.

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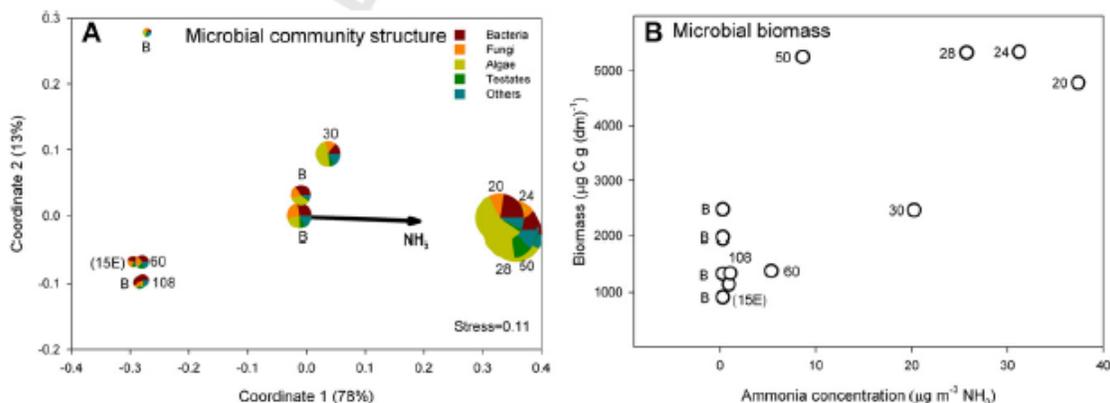
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122

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  $\text{NH}_3$  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  $r=0.99$ ,  $p<0.0001$ ).

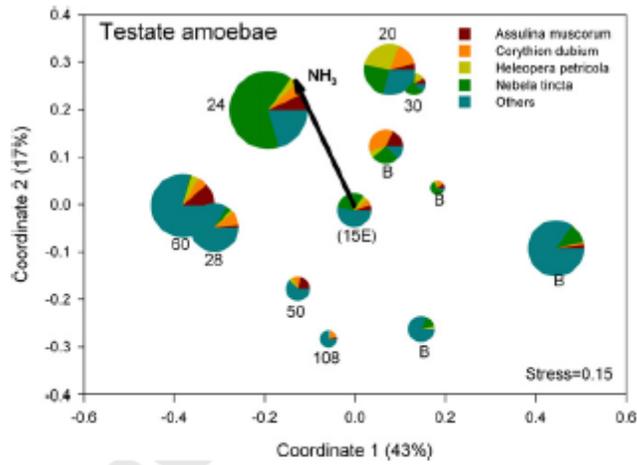


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136 Fig. 2. Testate amoeba community. NMDS ordination based on Bray-Curtis dissimilarity of taxa biomass  
 137 (calculated on the basis of both living and dead individuals, unlike Fig. 1 based on only living individuals  
 138 for comparability with 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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