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Short Communication

Ammonia exposure promotes algal biomass in an ombrotrophic peatland

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ABSTRACT

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

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

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

The Whim Moss experiment is a globally-unique pollution experiment on a *Calluna vulgaris*-*Eriophorum vaginatum* blanket bog in southern Scotland (3° 16'W, 55° 46'N: Leith et al., 2004). NH_3 is released from a pipe 1 m above the ground when air temperature exceeds 0°C, wind speed exceeds 2.5 m s^{-1} and wind direction is within the range 180-215°. Monthly average NH_3 concentrations are determined using passive ALPHA samplers (Sutton et al., 2001) positioned at 0.1 m above the vegetation along a downwind transect (Sheppard et al., 2011). After nine years of treatment (September 2011), the upper 50mm of ten stems of *Sphagnum capillifolium* (Ehrh.) Hedw. were extracted at seven locations along this transect and placed in 10% formaldehyde. Additional samples were taken off the main transect and from an area receiving only ambient deposition. Samples were prepared by multiple cycles of agitation and washing following Jassey et al. (2011) and examined under inverted microscopy. All larger micro-organisms were identified to group (cyanobacteria, microalgae, fungi, flagellates, ciliates and micrometazoa including rotifers and nematodes) and assigned to a sequence of broad morphotypes. More detailed morpho-species identification was carried out for testate amoebae (>100 individuals; Supplementary Table 1): an abundant group of protists which are sensitive to N deposition (Gilbert et al., 1998; Payne et al., 2012). Flow cytometry (BD FACSCalibur) was used for bacterial counts with samples stained with SYBR Green I (1/10,000 final concentration) for 15 minutes in the dark and run at medium speed (ca 40 µL min^{-1}). DAPI-treated sub-samples were examined by epifluorescence 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 µg 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 (>50000µm^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

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

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

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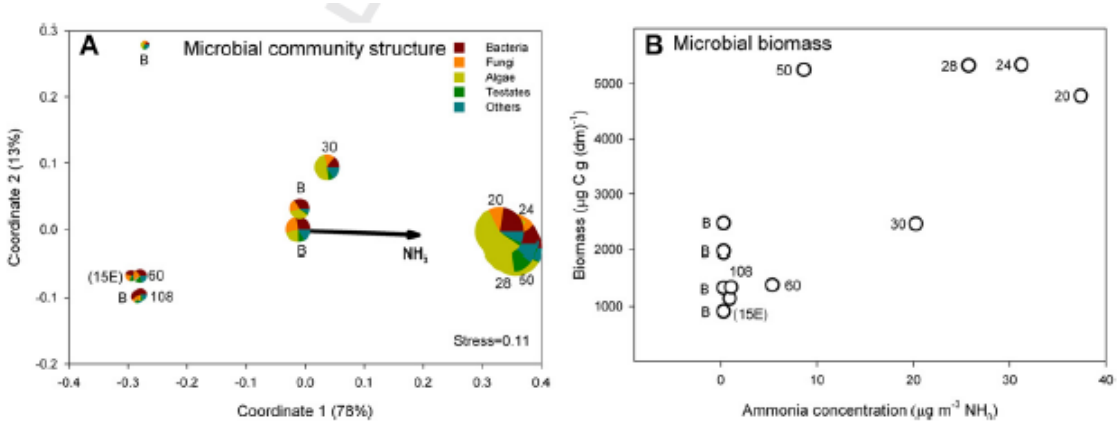
123 FIGURE CAPTIONS

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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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 µg C $g(dm)^{-1}$). 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.

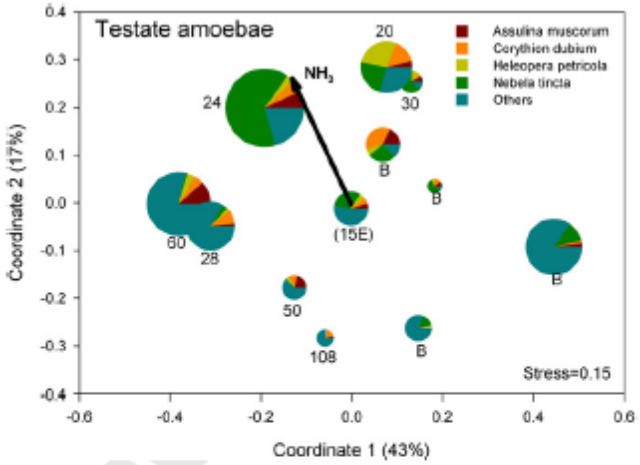

Fig. 2. Testate amoeba community. NMDS ordination based on Bray–Curtis dissimilarity of taxa biomass (calculated on the basis of both living and dead individuals, unlike Fig. 1 based on only living individuals for comparability with other microbial groups). Symbols sized in proportion to total biomass (range: 90–780 μg C g(dm)$^{-1}$). 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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