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Effects of airborne ammonium and nitrate pollution strongly differ in peat bogs, but symbiotic nitrogen fixation remains unaffected

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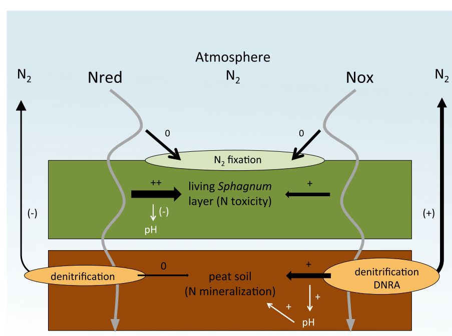
HIGHLIGHTS

- N_2 fixation of moss symbionts is not down-regulated by increased N deposition.
- Ammonium N deposition leads to N stress response in keystone spp. of bogs.
- Nitrate N deposition, in contrast, leads to increased peat N mineralization.
- Differential N effects on bog ecosystem functioning should be taken into account.

GRAPHICAL ABSTRACT



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ABSTRACT

Pristine bogs, peatlands in which vegetation is exclusively fed by rainwater (ombrotrophic), typically have a low atmospheric deposition of reactive nitrogen (N) (<0.5 kg ha^{-1} y^{-1}). An important additional N source is N_2 fixation by symbiotic microorganisms (diazotrophs) in peat and mosses. Although the effects of increased total airborne N by anthropogenic emissions on bog vegetation are well documented, the important question remains how different N forms (ammonium, NH_4^+, versus nitrate, NO_3^-) affect N cycling, as their relative contribution to the total load strongly varies among regions globally.
Here, we studied the effects of 11 years of experimentally increased deposition (32 versus 8 kg N ha^{-1} y^{-1}) of either NH_4^+ or NO_3^- on N accumulation in three moss and one lichen species (*Sphagnum capillifolium*, *S. papillosum*, *Pleurozium schreberi* and *Cladonia portentosa*), N_2 fixation rates of their symbionts, and potential N losses to peat soil and atmosphere, in a bog in Scotland.
Increased input of both N forms led to 15–90% increase in N content for all moss species, without affecting their cover. The keystone species *S. capillifolium* showed 4 times higher N allocation into free amino acids, indicating N stress, but only in response to increased NH_4^+. In contrast, NO_3^- addition resulted in enhanced peat N mineralization linked to microbial NO_3^- reduction, increasing soil pH, N concentrations and N losses via denitrification. Unexpectedly, increased deposition from 8 to 32 kg ha^{-1} y^{-1} in both N forms did not affect N_2 fixation rates for any of the moss species and corresponded to an additional input of 5 kg N ha^{-1} y^{-1} with a 100% *S. capillifolium* cover. Since both N forms clearly show differential effects on living *Sphagnum* and biogeochemical processes in the underlying peat, N form should be included in the assessment of the effects of N pollution on peatlands.

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1. Introduction

Nitrogen (N) is a key limiting nutrient for primary production in terrestrial ecosystems (LeBauer and Treseder, 2008), and the availability of this nutrient affects plant competition and biodiversity (Bobbink et al., 1998; Porter et al., 2013). Ombrotrophic bogs typically develop under very nutrient poor conditions, and their keystone genus, *Sphagnum* (peat moss), is highly adapted, showing a high N uptake and N use efficiency (Aerts et al., 1999; Fritz et al., 2014). By covering the peat soil, peat mosses function as a filter that efficiently absorbs N from rainwater, preventing it from leaching to the rhizosphere of vascular plants, which makes *Sphagnum* an effective competitor for nutrients (Bragazza et al., 2004; Fritz et al., 2014; Lamers et al., 2000). However, increasing anthropogenic N emissions of the last century have led to much higher N deposition loads (Dentener et al., 2006; Vitousek et al., 1997), presenting a severe threat to bogs (Bragazza et al., 2006; Tomassen et al., 2003). Reactive N in atmospheric deposition consists of two major forms: reduced N as ammonia (NH_3) and ammonium (NH_4^+), and oxidized N as nitrate (NO_3^-). Highly detrimental effects of NH_3 deposition on vegetation have been demonstrated for various ecosystems including peatlands (Fangmeier et al., 1994; Krupa, 2003; Sheppard et al., 2011), but potential differences between effects of NH_4^+ deposition (here referred to as Nred) and NO_3^- deposition (referred to as Nox) remain largely obscure. The ratio between these two forms of N deposition varies worldwide and is important in explaining changes in species composition and ecosystem functioning (Stevens et al., 2011).

Both N forms can be rapidly taken up by *Sphagnum* (Fritz et al., 2014; Rudolph et al., 1993) and other mosses (Li and Vitt, 1997) and assimilated in their tissue. In particular NH_4^+ can enter *Sphagnum* by binding to cation-exchange sites, leading to subsequent translocation to the cytoplasm (Clymo and Hayward, 1982) and can in this way be taken up 10 times faster than NO_3^- (Fritz et al., 2014; Liu et al., 2013), which must be reduced to NH_4^+ before it can be assimilated. At high loads, N deposition can have a direct detrimental effect on *Sphagnum*, as internal accumulation of NH_4^+ can become toxic (Baxter et al., 1992; Limpens and Berendse, 2003; Nordin and Gunnarsson, 2000; Tomassen et al., 2003; Wiedermann et al., 2009). Excess N is stored in N rich free amino acids in *Sphagnum*, functioning as an internal N detoxification mechanism. However, the imbalance between N uptake and N assimilation leads to N stress in *Sphagnum* that can lead to *Sphagnum* decline and gradual ecosystem change (Tomassen et al., 2003; van der Heijden et al., 2000). The amino acid content of *Sphagnum* therefore represents a sensitive indicator of *Sphagnum* N stress (Tomassen et al., 2003).

In addition, increased availability of N can lead to indirect negative effects on *Sphagnum*, since excess N is not assimilated or immobilized and becomes available in the rhizosphere of fast growing vascular plants that may subsequently outcompete *Sphagnum* for light. It is assumed that N leaches through the *Sphagnum* filter at deposition rates above 20 kg ha^{-1} y^{-1} (Harmens et al., 2014; Lamers et al., 2000). Deposition above this load may lead to ecosystem changes, from *Sphagnum* covered bogs to bogs that are more vascular plant dominated (Bubier et al., 2007; Heijmans et al., 2002; Lamers et al., 2000; Tomassen et al., 2003). Besides, N leaching to deeper anoxic peat layers may become available to the denitrifying microbial community that can quickly convert it to N_2O and subsequently to N_2. This loss of N to the atmosphere potentially represents an important pathway of N removal from peatlands (Silvan et al., 2002). However, denitrification rates reported are low, attributed to the low pH and N availability in peatlands (Aerts, 1997; Hayden and Ross, 2005).

Next to atmospheric deposition, N input to pristine ecosystems results to a large extend from N_2 fixation by microorganisms associated with peat soil and vegetation (Vitousek et al., 2013). In peatlands, the symbiosis between *Sphagnum* spp. and associated N_2 fixing microorganisms (diazotrophs) is considered a very effective mechanism to obtain sufficient N for growth (Santi et al., 2013). *Sphagnum* spp. have hyaline cells that are colonized by a diverse microbial community (Bragina et al., 2012; Opelt et al., 2007) containing several species of diazotrophs (Bragina et al., 2013). In a pristine boreal bog, this community was even found to fix 85–96% of the total bog N-input (Vile et al., 2014). In boreal forests, the bryophyte *Pleurozium* sp. (a feather moss) also grows in symbiosis with N_2 fixing cyanobacteria, supplying up to 50% of its total N input (Rousk et al., 2013). In pristine peatlands, associations between mosses and diazotrophs therefore represent an important contribution to the total N pool (Rousk et al., 2013), boosting peat accumulation through their stimulation of primary production (Vile et al., 2014). In addition to mosses, lichens such as *Cladonia portentosa* are also known to have similar symbioses (Grube et al., 2009). Although increased N availability can be expected to lower N_2 fixation rates, given the energy consuming nature of the reaction, inconsistent results have been reported on the effect of increased N deposition (0.1–2 kg N ha^{-1} y^{-1} background deposition compared to 12.5, 40 or 50 kg N ha^{-1} y^{-1}) on N_2 fixation for both feather mosses and *Sphagnum* spp. (Ackermann et al., 2012; Gundale et al., 2013; Kox et al., 2016; Leppänen et al., 2013). In addition, little is known about the relative contributions of the diazotrophic microbiomes of different species to the total N input in bogs where both bryophytes and lichens are present.

In this paper, we report on the effects of long-term (11 years) experimental addition of N deposition of 24 kg ha^{-1} y^{-1} as NO_3 versus NH_4 on the biogeochemical cycling of N in *Sphagnum* and peat soil with respect to N_2 fixation, denitrification and N loss to deeper peat. In the real-time watering experiment in Whim bog in Scotland we tested our hypotheses that: 1) increased N deposition reduces N_2 fixation of moss and lichen symbionts; 2) *Sphagnum* accumulates N rich amino acids, especially with NH_4^+; 3) N deposition, especially NO_3^-, leaches through the *Sphagnum* N filter to deeper peat layers, affecting biogeochemical processes in the soil including denitrification.

2. Methods

2.1. Study site

Whim bog is situated in the Scottish Borders, close to Edinburgh (3° 16′ W, 55° 46′ N) and represents a transition between a lowland raised bog and a blanket bog. It has a peat soil of 3–6 m deep that is relatively wet and acidic, with a pH of around 4.2. The mean annual air temperature and annual precipitation between 2003 and 2013 were 7.9 °C and 1124 mm respectively, and the ambient N deposition rate was around 8 kg N ha^{-1} y^{-1}, with similar contributions of ~3 kg of each wet N deposition form, i.e. NO_3^-, NH_4^+ and ~2 kg of dry deposition (NH_3) (Leith et al., 2004; Sheppard et al., 2004; Sheppard et al., 2014). The vegetation is classified as a *Calluna vulgaris-Eriophorum vaginatum* community (UK NVC M19) (Rodwell, 1991) with hummocks of *Sphagnum capillifolium* and hollows containing mostly *S. papillosum*. Other common species are *Calluna vulgaris*, *Eriophorum vaginatum*, *Erica tetralix*, the mosses *Pleurozium schreberi*, *Hypnum jutlandicum* and the lichen *Cladonia portentosa*.

In June 2002 a long-term deposition experiment was set up in this bog, where N treatments were continuously being added by real time watering in different doses and different forms to circular plots of 12.8 m^2. The treatments, replicated in four plots, were supplied to each plot from a central spinning disc generating fine rain droplets when activated by rainfall. In this study, we focus on the N treatments of annual addition of 24 kg N ha^{-1} y^{-1} of 2 different forms of wet deposition: oxidized N or NO_3^- (Nox) applied as $NaNO_3$ and reduced N or NH_4^+ (Nred) applied as NH_4Cl. No increased concentration of Na^+ was found in the pore water or bound to soil with Nox, nor an increase in concentration of Cl^- in pore water with Nred (results not shown). Including the background N deposition of 8 kg ha^{-1} y^{-1} this translates to total N loads of 4 times ambient deposition: 32 kg N ha^{-1} y^{-1}. The