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PEATLANDS

Peatland Vegetation Patterns in a Long Term Global Change Experiment Find no Reflection in Belowground Extracellular Enzyme Activities

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Abstract

To assess the effects of global change on peatland vegetation and biogeochemistry we used a long term (21 years) in-situ plot scale manipulation experiment comprising nitrogen (N; ambient and 30 kg ha^{-1} yr^{-1}), temperature (T; ambient and + 3.6 °C during growing season) and sulfur (S; ambient and 20 kg ha^{-1} yr^{-1}) treatments in an oligotrophic boreal peatland. Vegetation was assessed by plant species cover estimates, while biogeochemical processes were characterized by measuring potential extracellular enzyme activity (EEA) of glucosidase, cellulase, aminopeptidase, phosphatase, and sulfatase in the peat matrix. We hypothesized that the plant communities would change in response to the N and T manipulations, and that belowground EEA would respond distinctively to the applied treatments as well as to changes in plant community. We found vascular plant cover to have strongly increased in the T treatment, whereas the *Sphagnum* cover collapsed in the high N treatment. Belowground we found enhanced enzymatic C and N acquisition activity in response to the N treatment, but EEA showed no response to the T treatment. No S effects were found, neither aboveground nor belowground. Contrary to our expectations, our data reveal a mismatch between above-ground vegetation patterns and belowground decomposition processes. In particular, the large increase in vascular plant cover in the warming treatment found no reflection in belowground EEA.

Keywords Plant–soil (below-ground) interactions · Global change ecology · Ecosystem function · Ecophysiology · Extracellular enzymes · Enzyme stoichiometry · Plant functional types · Peat

Introduction

Two global change factors with strong potential to alter peatland ecosystems are enhanced nitrogen (N) deposition and climate change (Limpens et al. 2008; Dise and Phoenix 2011). Over the last few decades, anthropogenic reactive nitrogen (Nr) production has been greater than production from all natural terrestrial systems combined (Galloway et al. 2008). The highest N deposition rates are measured in the northern hemisphere (Dentener et al. 2006; Reay et al. 2008; Bobbink et al. 2010), where peatlands form a dominant landscape element in boreal and sub-arctic areas. In these northern latitudes 95% of the global peat reserves are found, storing about 25% of the global soil carbon (C) pool (Gorham 1991; Smith et al. 2004; Loisel et al. 2014). Most peatlands are located at latitudes that have already seen substantial changes in climate (IPCC 2014) and are currently undergoing changes that could have severe implications for atmospheric C feedback processes. Thus, northern peatlands are under pressure from both N deposition and climate change, and may be impacted by either driver individually or in combination.

The reason why peat-forming, *Sphagnum*-dominated, oligotrophic wetlands are among the most sensitive ecosystems to enhanced N input, is that these strongly nutrient limited ecosystems rely heavily on atmospheric deposition for their N supply (Rydin and Jeglum 2006). Oligotrophic peatlands are usually dominated by *Sphagnum* moss building the bottom layer and by sparsely interspersed ericaceous shrubs and

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graminoid sedges (Rydin and Jeglum 2006). *Sphagnum* species are regarded as "ecosystem engineers" (Vanbreemen 1995), monopolizing sparse nutrients (Malmer et al. 2003; Turetsky 2003) and creating and maintaining an acidic, nutrient poor, wet, and anoxic environment (Rydin and Jeglum 2006) — environmental conditions unfavorable for many vascular plants. *Sphagnum* litter is by far the most recalcitrant plant component in peatlands (Turetsky 2003; Dorrepaal et al. 2005) and thus slows decomposition processes (Hájek et al. 2011) and fosters C sequestration. As long as N deposition does not exceed the uptake rate by *Sphagnum*, the genus will maintain its role as ecosystem engineer, and peatland vegetation will remain relatively stable (Lamers et al. 2000; Nordbakken et al. 2003). However, with elevated N deposition, peatland ecosystems typically respond with decreased *Sphagnum* cover and increased vascular plant abundance (Gunnarsson and Rydin 2000; Wiedermann et al. 2007; Limpens et al. 2011), with cascading effects on the entire peatland ecosystems (Limpens et al. 2008; Eriksson et al. 2010).

Despite the dominant role of Sphagna as ecosystem engineers, vascular plants substantially influence biogeochemistry, ecosystem function and response to global change in peatlands. Peatland plants differ in growth forms, root distribution and architecture, as well as leaf longevity and levels of resistance to decay – so the relative proportion and productivity of each plant functional type (PFT) has consequences for the rates of key ecosystem processes impacting C cycling (Belyea and Malmer 2004). For instance, the evergreen leaves of the ericaceous shrubs are relatively recalcitrant compared to the sedge leaf litter, which degrades easily (Dorrepaal et al. 2005). As a special adaptation to the anoxic conditions in peatlands, sedges have an aerenchymal tissue structure which provides the sedge roots with oxygen and allows for nutrient mining in the deep peat, altering biogeochemical cycles in the deep peat (Ström et al. 2003). Peatland sedge roots are typically non-mycorrhizal (Miller et al. 1999; Thormann et al. 1999). The co-occurring woody ericaceous shrubs lack aerenchymal tissue, resulting in superficial root distribution. Also, the ericaceous shrubs grow with associated mycorrhizal symbionts, which produce the most diverse assemblage of extracellular enzymes of any mycorrhizal fungi, and hence have the potential to assimilate nutrients from complex polymers via high extracellular activity of key enzymes, including proteases, phosphatases, phenol oxidases, chitinases, and cellulases (Cairney and Burke 1998; Martino et al. 2018). In contrast, peatland sedge roots are typically non-mycorrhizal (Miller et al. 1999; Thormann et al. 1999). Both the additional oxygen evading from the aerenchymal sedge roots as well as the diverse extracellular enzyme production by the ericaceous roots in conjunction with the ericaceous mycorrhizae alter biogeochemical processes in peat (Robroek et al. 2015; Ward et al. 2015; Wiedermann et al. 2017). The abundance and composition of vascular plants in peatlands are thus expected to influence belowground biogeochemical processes.

Only by linking aboveground and belowground patterns and processes (Wardle et al. 2004; Van der Putten et al. 2013) it is possible to draw a more comprehensive picture of ecosystem response to altered global change factors. Aboveground effects in peatlands are commonly assessed using vegetation surveys. Belowground activity is more difficult to assess, but measurements of potential extracellular enzyme activity (EEA) can provide information about important belowground processes (Sinsabaugh 1994). Extracellular enzymes are produced by plant roots, mycorrhizae and free living soil microorganisms, and catalyze reactions of N, phosphorus and C depolymerization in soil and peat (e.g., Allison and Vitousek 2005; Wallenstein and Weintraub 2008; Moorhead et al. 2012). Thus, environmental change-induced effects on vegetation communities and associated nutrient and C processing rates are expected to be reflected in changing EEA (Caldwell 2005; Bardgett et al. 2014) and enzyme stoichiometry (Moorhead et al. 2016).

To assess the effects of global change on peatland biogeochemistry and vegetation, a long term (21 years) plot scale manipulation experiment in an oligotrophic boreal peatland was used. The factorial design of the experiment comprises enhanced reactive N, T and S treatments. Early results from the long-term experiment showed an increase in vascular plant cover both in the N and the T treatments along with a sharp decline of the *Sphagnum* carpet in response to the N treatment, at 8 and 12 years after establishment (Eriksson et al. 2010; Wiedermann et al. 2007). Sulfur effects on the peat vegetation were negligible (Wiedermann et al. 2007). However, long term vegetation responses are not known, nor have belowground processes as described by EEA been assessed in such an experiment.

In the current study, new measurements of vegetation cover from the long-term global change experiment were made to assess whether the vegetation responses after 8–12 years of experimental treatment represented a new stable state, or whether those patterns were transient. Additionally, the EEA were assayed to examine how belowground processes responded to the experimental treatments and concomitant vegetation responses. The specific enzyme assays were primarily chosen to gain insights into the acquisition of the main structural components of peatland biota (C, N, P). Our hypotheses focus on the T and N treatment effects since previous results from this experiment had shown no S effects on the vegetation (Wiedermann et al. 2007).

Central Questions & Hypotheses

Q1: Will projections of vegetation responses to increased T and N fertilization treatments based upon results from short term experiments hold true in the long term? Q2: How do

long-term enhanced T and N availability alone and in combination modify (A) the abundance and composition of peatland vegetation, and (B) belowground decomposition processes exemplified by extracellular enzymes responses?

Based on previous results from short term (8–12 years) experiments we expected that vascular plant species cover will increase in response to the T and N fertilization treatment, whereas *Sphagnum* cover was shown to decline in response to the N treatments (Limpens et al. 2008; Wiedermann et al. 2007). In terms of belowground responses we hypothesized that C (glucosidase (BG) and cellulase (CBH)) and phosphorus mining enzymes (phosphatase (PHOS)) would be up-regulated in response to the N treatment due to an anticipated shift of microbial demands from being N limited to C (Koyama et al. 2013; Cenini et al. 2016) and P limited (Olander and Vitousek 2000; Saiya-Cork et al. 2002). We hypothesized that the N mining enzymes (aminopeptidase (LAP) c.f. (Sinsabaugh 1994) and chitinase (NAG) c.f. (Kang et al. 2005)) would be down-regulated due to an excess of mineral N (Olander and Vitousek 2000; Saiya-Cork et al. 2002). In the T treatment plots we expected that the elevated T would directly increase microbial activity and enzyme process rates (Bell et al. 2013; Steinweg et al. 2013), and additionally that changes in the abundance and composition of vascular plants would strongly modify the responses of extracellular enzymes.

Materials and Methods

Site Description

The study was conducted in a *Sphagnum*-dominated boreal peatland, Degerö Stormyr, located within the Kulbäcksliden Research Park of the Vindeln Experimental Forests (64°11' N, 19°33′ E; 270 m a.s.l.), about 70 km from the Gulf of Bothnia in the province of Västerbotten, Sweden. The experimental site is a poor fen (pH 4.5) with a peat depth of about 5 m (Malmström 1923), and is part of a 6.5 km^2 mixotrophic peatland system. At the experimental site the bottom vegetation layer is a closed *Sphagnum* carpet dominated by *S. balticum* (Russ.). The vascular plant cover is sparse, and dominated by the graminoid *Eriophorum vaginatum* L. and the two ericoid dwarf-shrubs *Andromeda polifolia* L. and *Vaccinium oxycoccos* L.

Experimental Design

The experiment was set up in the central part of the peatland in 1994. Treatments were first applied in 1995. The experiment was set up according to a full factorial design with three experimental factors. The experimental plots used in this study encompassed two levels of temperature (T) (ambient and + 3.6 °C), two levels of nitrogen (N) (ambient (2 kg N ha^{-1} yr^{-1}), and (30 kg ha^{-1} yr^{-1})), and two levels of sulfur (S) (ambient (3 kg S ha^{-1} yr^{-1}), and (20 kg ha^{-1} yr^{-1})) respectively (Granberg et al. 2001). Each experimental combination was duplicated resulting in a total of 16 plots.

Plots measure 2 m × 2 m, and each plot is separated by 1 m buffer zones. To prevent horizontal movement of the added elements, all plots are surrounded by a polyvinyl chloride frame which extends to 0.4 m deep in the peat. Monthly additions of NH_4NO_3 and Na_2SO_4 during the growing season have been carried out regularly for 21 years to achieve the respective annual N and S loads. To simulate climate change, warming chambers were used to raise mean air temperature by +3.6 °C, as measured 0.25 m above the bottom layer. These warming chambers consist of 0.5 m high and 2 mm thick transparent polycarbonate side plates. During the snow-free period of the year the plots are covered with a perforated clear plastic film roof at 0.5 m height above the field layer. To allow precipitation to enter the plots and to reduce unintentional effects on humidity, the plastic film is perforated with holes (diameter 20 mm), spaced 100 mm apart. For a more detailed site description and for more information about the field experiment see Granberg et al. (2001).

Sampling of Peat and Plant Material for Chemical Analyses

In early July 2016, 21 years after the onset of the global change experiment, two surface peat cores were taken from each plot. One core was extracted from an *Eriophorum* dominated tussock area and the other from a mostly *Sphagnum* dominated inter-tussock. The cores measured 5 cm × 5 cm at the surface and were cut to 10 cm below the WT level using a sharp knife. For each core, the distance from the peat surface to the water table level was recorded individually. Water table levels were in a typical range for the time of year and ranged from 5 cm to 17 cm in the tussock areas and from -2 cm (submerged) to 12 cm in the inter-tussock areas. At the time of sampling the plots did not show any signs of drought. Following extraction from the peat matrix, each core was immediately transferred into individually labeled plastic bags and stored in a cooler in the dark until used for analyses within the next few days.

The depth of the water Table (WT) relative to the peat surface varies between and within plots, with N treatment plots having particularly shallow, and heterogeneous, depth to WT (Eriksson et al. 2010), see Plate 1). We decided to use the average growing season WT (mean growing season water table level is 13.1 cm; Eriksson et al. (2010) as a reference, and utilized the peat material from the cores above this WT level for subsequent analyses. This approach resulted in an average 11 cm tall core for the tussock samples, and an average 5 cm tall core for the inter-tussock samples. The

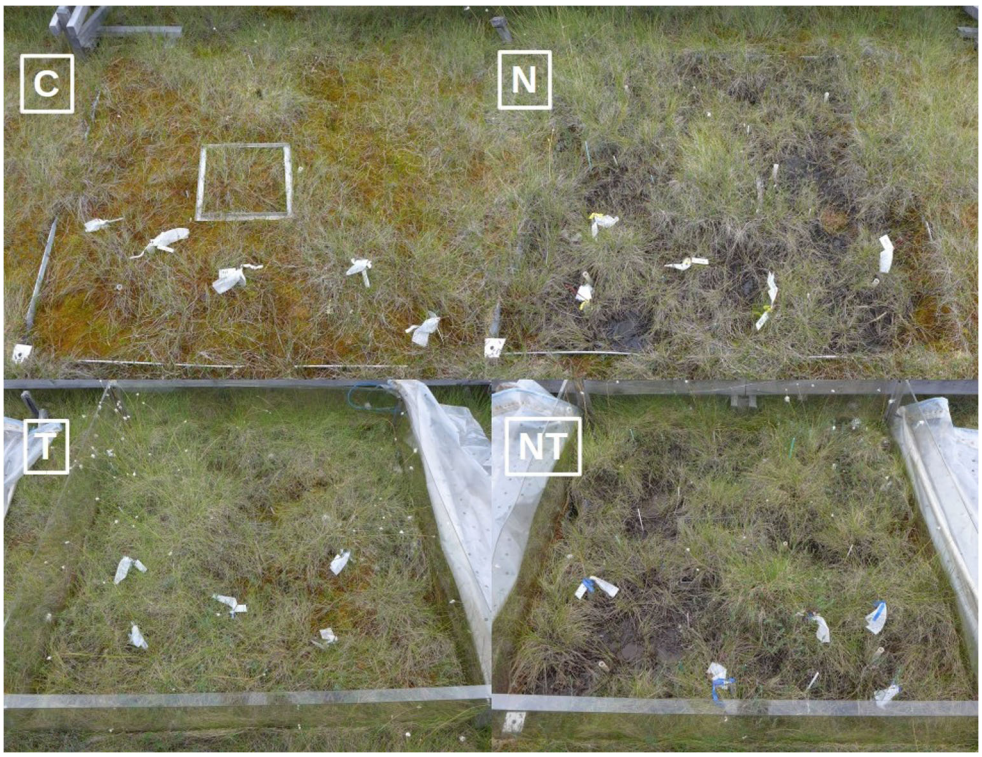

PLATE 1 Photographs of the experimental plots (2 × 2 m) (clockwise from upper left): control (C), nitrogen treatment (N), nitrogen and temperature treatment (NT), temperature treatment (T). Photo credits: M. M. Wiedermann

rationale for this sampling approach was to compare peat samples that are similar in age and WT regime. All aboveground green plant material was removed, then each core was homogenized by first cutting the core into ~1 cm depth increments and then mixing these together by hand to create one homogeneous sample of the entire core. Thereafter the homogenized peat cores were subsampled (15–20 pinches per core), and about 6 g of peat was weighed out for the enzyme slurry, and 20 g for dry weight and elemental analyses.

In addition to the peat cores, plant material from the three dominant vascular plants and from *S. balticum,* the dominant *Sphagnum* species, was sampled in mid July 2016 from the same experiment. At ten locations within each plot the green parts (including the capitula) of *S. balticum* were sampled together with ten current annual shoots of *V. oxycoccos* and *A. polifolia*, as well as green leaves from ten *E. vaginatum* individuals. Samples were placed in labeled paper bags separated by species and transported to the lab. The same day all dead and foreign plant material was removed from the samples, which were then dried at 50 °C for 48 h for later chemical characterization.

Vegetation Cover Analyses

In order to be consistent with the annual vegetation recordings throughout the 21-year experimental period, cover estimates by eye in percent cover of the entire plots were recorded for each plant species in October 2016. The autumn colors make it easier to distinguish the vascular and *Sphagnum* species and thus provide for more accurate cover estimates.

Chemical Analyses

The dried leaf material from the three vascular plants and *S. balticum* as well as the two dried peat samples (tussock, inter-tussock) from each plot were ground using a ball mill. Thereafter, all samples were analyzed for mass fraction of C and N using an elemental analyzer (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany).

Rationale for Selection of Specific Extracellular Enzyme Assays

The measured enzymes in this study except sulfatase (SULF) are assumed to be representative indicators of overall C, N, and P acquisition (Sinsabaugh 1994). Glucosidase (BG) and cellulase (CBH) are the primary enzymes that catalyze cellulose and hemicellulose degradation (Sinsabaugh 1994). Aminopeptidase (LAP) represents a key protease/peptidase enzyme that catalyze the cleavage of amino acids from proteins or other peptide substrates (Sinsabaugh 1994), and chitinase (NAG) - frequently used to represent N cleaving enzymes (e.g.: Kang et al. 2005; Bell et al. 2013; Li et al. 2019) - hydrolyzes oligomers of N-acetyl glucosamine in chitin, found in fungal cell walls and invertebrate exoskeletons, and peptidoglycan, the principal component of bacterial cell walls. Phosphatase (PHOS) hydrolyzes phosphomonoesters,

liberating phosphate from common organic molecules such as phospholipids and nucleic acids (Sinsabaugh 1994). Sulfatase activity is known to increase in anoxic conditions (Wiedermann et al. 2017), which is a crucial driver of biochemical processes in peatlands (Bergman et al. 1999; Klüpfel et al. 2014).

Assay for Measuring Potential Extracellular Enzyme Activity (EEA) and Enzyme Stoichiometry

To quantify potential extracellular hydrolytic enzyme activity in the peat matrix we closely followed the protocol by Bell et al. (2013) using deep well incubations. To create the peat slurry we used a 0.05 M sodium acetate buffer at pH 4.2 (ambient pH), added 6 g wet peat and used a hand blender for 30 s to create a homogeneous suspension. Separate enzyme assays were run with each of the following six substrates added at a concentration of 200 μM, which is enough to avoid substrate limitation: 4-Methylumbelliferyl β-D-cellobioside (Sigma M6018) for potential cellulase activity (CBH), 4-Methylumbelliferyl β-D-glucopyranoside (Sigma M3633) for potential glucosidase activity (BG), 4-Methylumbelliferyl N-acetyl-β-D-glucosaminide (Sigma M2133) for potential chitinase activity (NAG), 4-Methylumbelliferyl phosphate (Sigma M888) for potential phosphatase activity (PHOS) and 4-Methylumbelliferyl-sulfate (Sigma M7133) for potential sulfatase activity (SULF), and L-Leucine-7-amido-4-methylcoumarin hydrochloride (Sigma L2145) for potential aminopeptidase activity (LAP).

We used eight analytical replicates for each enzyme assay and incubated 1000 μl of the peat-substrate slurry in the 2 ml deep well trays at 25 °C in the dark for three hours. The peat slurries were then centrifuged at 41 RCF for 3 min, and 250 μl of the supernatant was transferred into opaque microplates and read for fluorescence with excitation and emission wavelengths of 365 and 460 nm respectively (BioTek Synergy H4 plate reader). To convert the fluorescence readings (output from the software program Gen 5 2.01) into EEA we followed the protocol by Bell et al. (2013), which utilizes a 4-Methylumbelliferone Sigma (M1381) standard curve for each methylumbelliferone sample and a 7-Amino-4-methylcoumarin Sigma (A9891) standard curve for the methylcoumarin substrate (LAP).

Following the acquisition of the EEA, we used stoichiometric equations (c.f., Moorhead et al. 2016) with the measured EEA to assess how changes in N availability and T translate into altered resource limitations.

Statistical Analyses and Data Visualization

Mixed effects models (Bolker et al. 2009) were used on the peat core data to account for the nesting of the two microsites (tussock, inter-tussock = "topography") within each plot. For data visualization and statistical analysis, R version 3.6.1 for linux-gnu (R-Core Team 2019) and the following packages: "lme4" (Bates et al. 2015), "car" (Fox and Weisberg 2011), "MASS" (Venables and Ripley 2002), and "ggplot2" (Wickham 2009), were used. The applied treatments (N- and S addition and T treatment) were analyzed as fixed factors, while the plot identity was analyzed as a random term.

For data from the peat cores with a normal distribution (tested for with Shapiro test), linear mixed model fits using REML (restricted maximum likelihood – preferred for small sample sizes) were applied. The statistical results were extracted from ANOVA tables using the "car" package in "R": Analysis of Deviance Table (Type III Wald F tests with Kenward-Roger df). In the case of non-significant interaction terms, the models were reduced to the main factors (N, S, T or topography) only and the statistical results were extracted from ANOVA tables using the "car" package: Analysis of Deviance Table (Type II Wald F tests with Kenward-Roger df). Following Bolker et al. (2009), results were extracted from linear mixed-effects model fits by maximum likelihood using Wald t-tests.

Vegetation cover data, which consisted of a single value per plot rather than having a nested data structure, were analyzed via multiple regression models using the "lm" function in R (p-values are based on Type III t-tests). In case of non-significant interaction terms the models were reduced to the main factors only. Model assumptions were tested with qq-plots to assess model residuals.

Results

Vegetation Response

The most unexpected result was that, unlike after eight years of experimental treatment (Wiedermann et al. 2007), the total vascular plant cover did not show a significant positive response to the applied N treatment after twenty-one years (Table 1). Twenty-one years of experimental warming (T) have though led to a large increase in total vascular plant cover, from an average of 20% in the control plots to an average of 62% in the warmed plots (Fig. 1). Concurrently, vascular plant litter inputs have increased in the warmed plots (Table 1, Fig. 1). No effects on the *Sphagnum* cover were detected as a function of T (Table 1, Fig. 1), while *Sphagnum* cover declined drastically in response to the N treatment (Table 1, Fig. 1). The two ericaceous dwarf shrubs responded differently to the applied experimental treatments. *V. oxycoccos* cover increased in response to the T treatment and declined in response to the TxN treatment (Table 1), whereas *A. polifolia* did not show any significant responses to the applied N treatments (Table 1). *E. vaginatum* cover increased in response to the N treatment and the T treatment and declined with the SxN interaction (Table 1). All three

Table 1 Statistical results from multiple regression analyses on percent areal cover: total vascular plants, plant litter, individual plant species; as well as foliar tissue nitrogen (N) mass percent, using all 16 experimental plots; Variables: N (nitrogen addition), T (temperature increase), S (sulfur addition), plus interactions between treatments: TxN, SxN, and SxT. Plus signs (+) to the right of the *p*-values indicate an increase, minus signs (−) a decrease in response to the respective treatment

response variables			experimental factors					
	model R2	model	N	T	S	TxN	SxN	SxT
Total vascular plant cover	0.73	F(3,12) ***p* < 0.001**		***p* < 0.001**(+)				
Total vascular plant litter	0.53	F(3,12) $p = 0.024$		$p = 0.006$(+)				
Andromeda cover	0.58	F(3,12) $p = 0.013$		$p = 0.003$(+)				
Vaccinium cover	0.94	F(6, 9) ***p* < 0.001**		***p* < 0.001**(+)		***p* < 0.001**(−)		
Eriophorum cover	0.86	F(6, 9) $p = 0.002$	$p = 0.020$(+)	$p = 0.003$(+)			$p = 0.005$(−)	
Sphagnum cover	0.98	F(3,12) ***p* < 0.001**	***p* < 0.001**(−)					
Andromeda %N	0.63	F(3,12) $p = 0.006$		$p = 0.002$(−)				
Vaccinium %N	0.81	F(6, 9) $p = 0.007$						$p = 0.014$(−)
Eriophorum %N	0.68	F(3,12) $p = 0.003$	$p = 0.018$(+)	$p = 0.002$(−)				
Sphagnum %N	0.97	F(3,12) ***p* < 0.001**	***p* < 0.001**(+)					

vascular plants individually responded with increased cover to the T treatment (Table 1, Fig. 1), while the sulfur (S) treatment alone had no effect on the vegetation cover (Table 1, Fig. 1).

Plant Chemistry

Mass percent N content increased in the annual leaves of *E. vaginatum* and *Sphagnum* in the N addition plots (Table 1, and supplemental material). Most drastically, for *Sphagnum* the tissue N content tripled from an average of 0.53 (± 0.03) mg g^{-1} DM across the ambient N treatments to an average of 1.54 (± 0.05) mg g^{-1} DM across the high N treatments (for individual plot data see supplemental material). The T treatment led to lower foliar N concentrations for *E. vaginatum* and *A. polifolia.* The T treatment also had a negative effect on the foliar N concentrations of *V. oxycoccos*, but only in combination with the S treatment (SxT) (Table 1, supplemental material). There were no significant changes in mass percent C content in the leaf tissue for any of the study species.

Microtopography and Peat Chemistry

Mass percent C content of the peat was higher in the tussock area than in the inter-tussock area (Table 2, supplemental material). Regardless of microtopographic position, both mass percent C content and mass percent N content in the peat increased in response to the N treatments (Table 1, supplemental material).

Potential Extracellular Enzyme Activity (EEA)

The T and S treatments had no effect on the EEA of any of the six measured enzymes (Table 2, Fig. 2). Nitrogen addition was the only experimental factor leading to significantly altered extracellular enzyme activity (Table 2, Fig. 2). In response to the N treatments, potential cellulase activity (CBH), potential glucosidase activity (BG), potential chitinase activity (NAG), and potential sulfatase activity (SULF) all increased (Table 2, Fig. 2). We also found different EEA activity depending on the microtopographic position within the plots (Table 2, Fig. 2). Potential chitinase activity (NAG) was significantly higher in elevated tussock areas (Table 2, Fig. 2), whereas potential phosphatase activity (PHOS) was significantly lower in the elevated tussock areas (Table 2, Fig. 2). Overall, potential aminopeptidase activity (LAP) was very low compared to the rest of the measured enzyme assays (Fig. 2). There was significantly lower potential LAP activity in elevated tussock areas (Table 2, Fig. 2). The statistical results of the measured LAP are highly driven by two inter-tussock samples with particularly high activity in response to the high N treatment. The LAP data thus needs to be interpreted with caution.

Enzyme Stoichiometry

The total enzyme N acquisition activity (NAG+LAP) rose in response to the N treatment, and also had

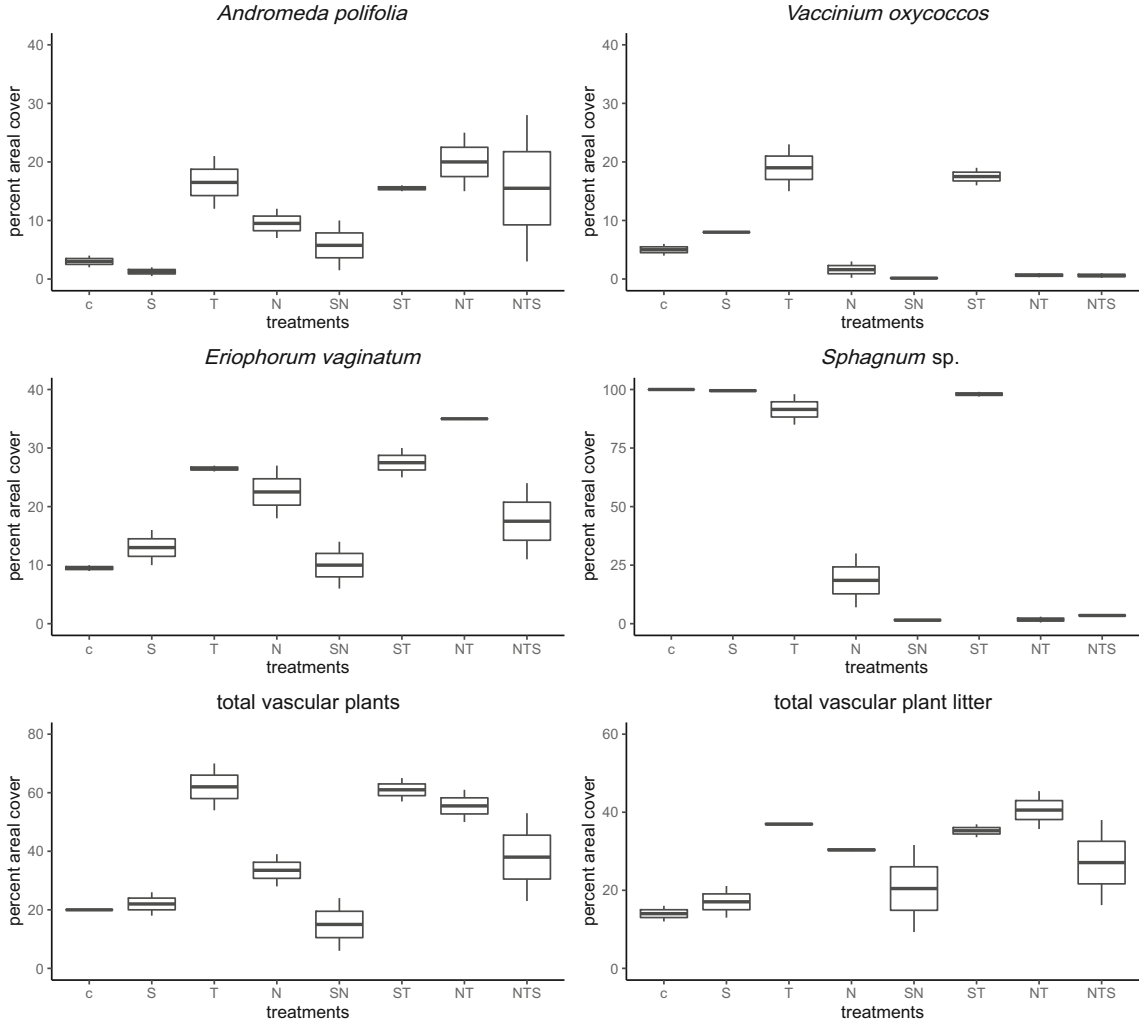

Fig. 1 Cover estimates (% areal cover) of vascular plant- and *Sphagnum* vegetation as well as vascular plant litter at the long term fertilization experiment at Degerö Stormyr in July 2016; y-axes: cover estimate; x-axes: experimental treatments: c = control, T = temperature (+3.6 °C), S = sulfur (20 kg ha^{-1} yr^{-1}), N = nitrogen (30 kg ha^{-1} yr^{-1}), ST = sulfur + temperature, SN = sulfur + nitrogen, NT = nitrogen + temperature, NTS = nitrogen + temperature + sulfur; total number of plots = 16

significantly higher values in the elevated tussock areas. In contrast, the per unit N acquisition ratio (NAG+LAP normalized to peat N content) declined in response to the N treatment, again differing depending on microtopography with overall higher values in the tussock areas (Table 2, Fig. 3). Both the total enzyme C acquisition activity (CBH + BG) and the per unit C acquisition ratio (CBH + BG normalized to peat C content) increased in response to the N treatment (Table 2, Fig. 3). The enzyme N:P acquisition ratio (NAG+LAP)/PHOS and the enzyme C:P acquisition ratio (CBH + BG)/PHOS both responded positively to N addition in the tussock areas (Table 2, Fig. 3). The enzyme C:N acquisition ratio (CBH + BG)/(NAG+LAP) was unchanged in response to the N treatment but differed between the two microtopographic areas, with higher values in the inter-tussock areas (Table 2, Fig. 3).

Discussion

Short term (8–12 years) experiments in peatlands often show an increase in vascular plant abundance in response to enhanced N conditions (Wiedermann et al. 2007; Kool and Heijmans 2009). However, in this experiment after 21 years of treatment the net effect of N addition on vascular plant cover was relatively minor or neutral. Hence, the observed complete crash of the *Sphagnum* layer due to N addition left substantial patches of bare peat uncovered by vegetation (see Plate 1). Further, we observed an intriguing mismatch between effects on aboveground vegetation patterns and belowground processes. Whereas the total vascular plant cover and the total vascular plant leaf litter production responded with a pronounced increase in abundance to the T treatment, EEA in the peat matrix responded solely to the N treatments and not to T. The effects of S addition were negligible both aboveground and belowground.

Table 2 Estimates of fixed effects produced by mixed effects models with "plot" as random effect. For normal distributed data Wald F tests with Kenward-Roger df are presented. Response variables: 1–6: potential extracellular enzyme activity (EEA) (in nmol activity g^{-1} dry peat hr^{-1}); 7–13: enzyme stoichiometry; and 14 + 15: peat mass percent C and N content. Modeled factors (fixed effects): N (nitrogen addition), topography (tussock vs. inter-tussock); $n = 16$ plots. No temperature, sulfur or interaction effects with T and S were significant . Only significant ($p < 0.05$) p-values are presented in the table. Plus signs (+) to the right of the p-values indicate an increase, minus signs (−) a decrease in response to the respective treatment/variable

response variables	experimental factors		
	N	**tussock**	**N x topography**
1) CBH	F(1,12) ***p* < 0.001** (+)		
2) BG	F(1,12) ***p* < 0.001** (+)		
3) NAG	F(1,12) $p = 0.040$ (+)	F(1,15) $p = 0.002$ (+)	
4) PHOS		F(1,15) ***p* < 0.001** (−)	
5) SULF	F(1,9) $p = 0.004$ (+)		F(1,12) $p = 0.010$
6) LAP		F(1,12) $p = 0.049$ (−)	
7) NAG+LAP	F(1,12) $p = 0.026$ (+)	F(1,15) $p = 0.004$ (+)	
8) BG + CBH	F(1,12) ***p* < 0.001** (+)		
9) BG + CBH/unit C	F(1,12) ***p* < 0.001** (+)		
10) NAG+LAP/unit N	F(1,12) p = 0.004 (−)	F(1,15) $p = 0.028$ (+)	
11) N:P EEA	F(1,9) p = 0.007 (+)	F(1,12) ***p* < 0.001** (+)	F(1,12) $p = 0.017$
12) C:P EEA	F(1,9) ***p* < 0.001** (+)	F(1,12) ***p* < 0.001** (+)	F(1,12) $p = 0.016$
13) C:N EEA		F(1,15) $p = 0.009$ (−)	
14) peat % N	F(1,12) ***p* < 0.001** (+)		
15) peat % C	F(1,12) ***p* < 0.001** (+)	F(1,15) ***p* < 0.001** (+)	

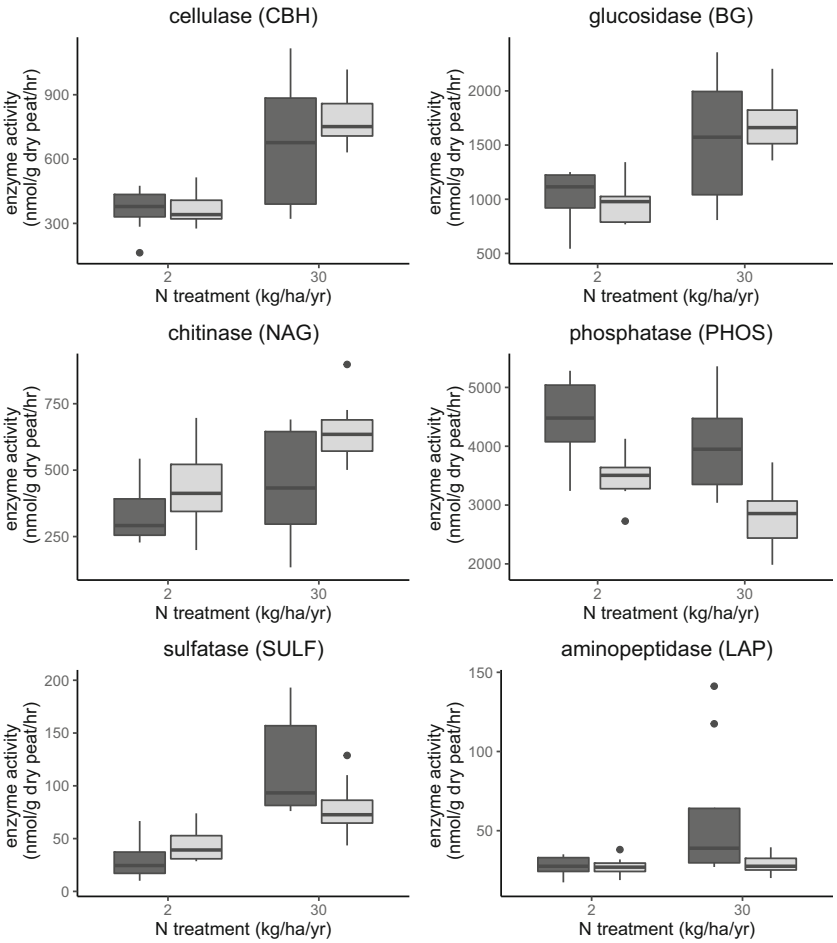

Fig. 2 EEA, measured as nmol activity g^{-1} dry peat hr^{-1} of C, N and P cycling enzymes in the long term fertilization experiment at Degerö Stormyr. Since no other factor (T and S) showed significant responses we plot only the responses to the N treatments (2 and 30 kg ha^{-1} yr^{-1} for the control and N-addition, respectively) and present them separately for the **inter-tussock (dark gray)** and **tussock (light gray)** areas. Plotted quantities are the median, and the 25th and 75th percentiles. Whiskers show either the maximum values or 1.5 times the interquartile range of the data. Points more than 1.5 times the interquartile range at both ends are individually plotted outliers

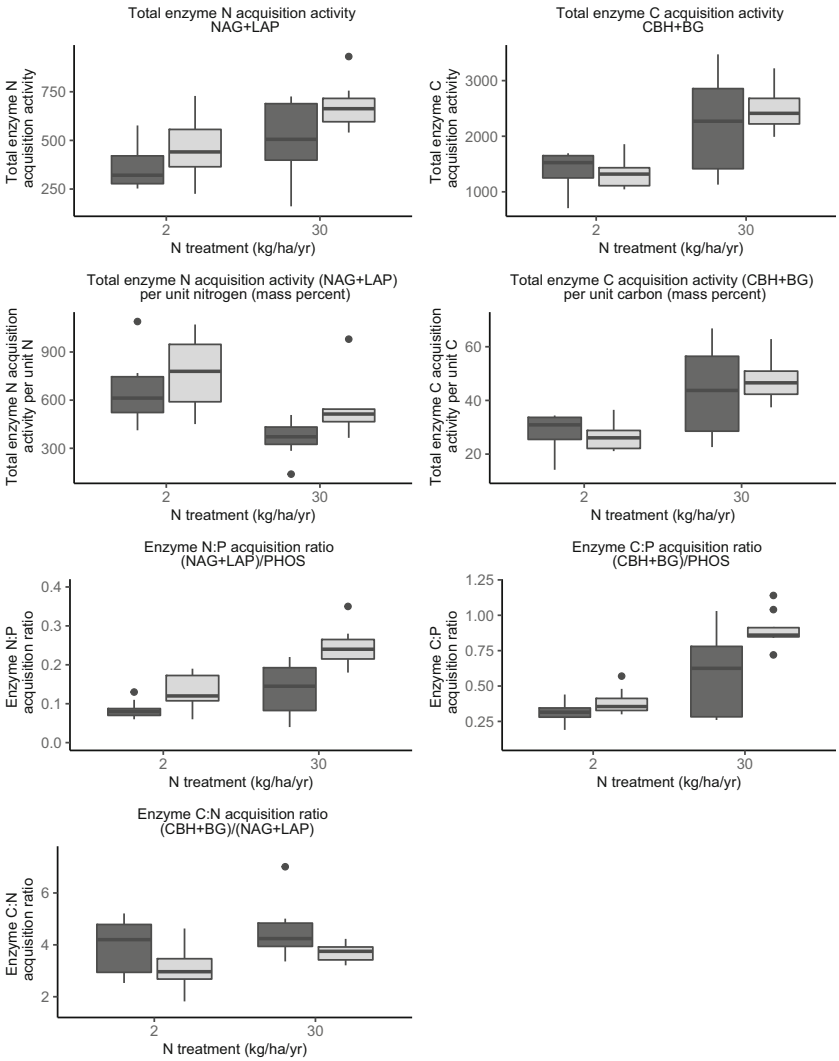

Fig. 3 Stoichiometric ratios of EEA, measured as nmol activity g^{-1} dry peat hr^{-1} of C, N and P cycling enzyme in the long term fertilization experiment at Degerö Stormyr. Since no other factor (T and S) showed significant responses we plot only the response to the N treatments (2 and 30 kg ha^{-1} yr^{-1} for the control and N-addition, respectively) and present them separately for the **inter-tussock (dark gray) and tussock (light gray)** areas. Plotted quantities are the median, and the 25th and 75th percentiles. Whiskers show either the maximum value or 1.5 times the interquartile range of the data. Points more than 1.5 times the interquartile range at both ends are individually plotted outliers

Vegetation Response

Sphagnum

The sharp decline in *Sphagnum* cover from the N treatment coincided with a substantial increase in N tissue concentration of the *Sphagnum* species as a function of the added N, which has also been reported by similar studies (Berendse et al. 2001; Wiedermann et al. 2009b) and hints toward a direct negative effect of high N availability on Sphagna (Table 1, Fig. 1, and supplemental material). The unicellular leaf structure of the mosses and the absence of a cuticle permits Sphagna to very effectively intercept and absorb airborne nutrients, in particular N sources, which leads to a competitive advantage of the mosses over vascular plants in oligotrophic conditions (Malmer et al. 2003; Vanbreemen 1995). However, in eutrophic conditions the apparent lack of a mechanism to regulate N uptake, especially under high NH_4^+ conditions (Wiedermann et al. 2009a; Chiwa et al. 2016) potentially leads to toxic N levels accumulating in the *Sphagnum* tissue (Van Der Heijden et al. 2000).

Surprisingly, *Sphagnum* abundance remained unchanged in the T treatments (Table 1, Fig. 1) despite the dense vascular plant cover, on average 62%, which suggests that light levels were not limiting to the *Sphagnum* species. In support of our results, it has previously been reported by for instance Hayward and Clymo (1983) and Malmer et al. (2003) that *Sphagnum* can tolerate shading up to a vascular plant cover of about 60%. Another possible indirect negative effect of the T treatment on *Sphagnum* cover could be through drought effects. In our study negative drought effects can likely be ruled out due to the year-round comparatively high water table at the experimental site (c.f. Peichl et al. 2014; Nijp et al. 2015).

Vascular Plants

For the cover of ericaceous dwarf shrubs, the earlier observed positive responses after 8 years of experimental N

addition (Wiedermann et al. 2007) had mostly disappeared after 21 years, which highlights the value of long term experiments. Numerous studies report positive vascular plant species responses to elevated N conditions (e.g., Kool and Heijmans 2009; Limpens et al. 2011). The absence of a positive vascular plant response to the applied N treatment in this study might in part be explained by the collapse of the matrix species (*Sphagnum*), thereby reducing structural support for some of the species and increasing flooding stress. The collapse of the decaying moss brings the water table closer to the peat surface (Eriksson et al. 2010) thus reduces the aerated rooting space for the ericaceous dwarf shrubs, whereas at the same time providing aerenchymal graminoids with a competitive advantage, which is supported by our data in this study (Table 1, Fig. 1). These hydrological impacts of N addition in peatlands might not only result in a vegetation shift towards eutrophic, aerenchymal graminoids but also shift biogeochemistry toward predominantly anaerobic processes. In this study, we found support for this trend toward a dominance of anaerobic processes from the measured potential sulfatase activity (SULF) where we find highest SULF in response to the N treatment particularly in the inter-tussock areas (Table 2, Fig. 2).

Our findings showed dissimilar responses of the two ericaceous dwarf shrubs (Table 1, Fig. 1), challenging the utility of the concept of trait based plant functional types in this context (Cornelissen et al. 2003; Pérez-Harguindeguy et al. 2013). For practical reasons, ecosystem modelers tend to operate with species groups based on the concept of plant functional types (Lavorel et al. 1997). However, our study demonstrates that caution must be taken when generalizing individual species responses to global change. The central role of *Sphagnum* spp. (Turetsky 2003; Vanbreemen 1995) might have contributed to the dissimilar response of the two ericaceous species, which present different growth forms. Whereas *A. polifolia* is an erect woody dwarf shrub, which at the experimental site typically grows up to a height of ~20 cm, *V. oxycoccos* with its prostrate growth creeps on the *Sphagnum* surface. We attribute the significant reduction of *V. oxycoccos* abundance in response to the NT treatment in part to the complete collapse of the *Sphagnum* matrix, which when intact provides structural support for *V. oxycoccos* growth. Greater tolerance toward flooding stress shown by *Andromeda* spp. as compared to *Vaccinium* spp. was also reported from a mesocosm experiment where water table level manipulations were part of the experimental setup (Potvin et al. 2015). Our findings are in line with previous results (e.g., Reich et al. 2001; Bret-Harte et al. 2008) who also demonstrated that individual species responses to global change can deviate greatly from expected trait based plant functional type responses.

Potential Extracellular Enzyme Activity (EEA)

Despite the many factors that were anticipated to result in altered EEA in response to the T treatment, our results showed no responses of EEA to the T treatment. Instead the only experimental factor that strongly influenced EEA was N addition (Table 2, Fig. 2). Unlike in other ecosystems where enhanced temperature itself coupled with sufficient soil moisture often leads to enhanced extracellular enzyme activity (EEA) (Bell et al. 2013; Steinweg et al. 2013), in oligotrophic peatlands the anticipated positive T effect on EEA seems to be missing. In line with our results a similar lack of T response of EEA was also reported from nutrient poor arctic peatlands (Weedon et al. 2013, 2014). Enzymes are protein structures, thus requiring N for their syntheses. The sparse N resources in oligotrophic peatlands, although creating a high demand for decomposition processes to provide the much-needed nutrient, may also limit extracellular enzyme production. Nitrogen limitation for enzyme production has also been found in arctic tussock soils (Sistla et al. 2012) and has been discussed earlier by Allison and Vitousek (2005).

Contrary to our expectation, elevated N conditions did not induce higher potential phosphatase activity (Table 2, Fig. 2). These results find support in another peatland study by Li et al. (2019), who found a decline in phosphatase activity in response to N addition. Phosphatases are produced by plants and microbes alike and despite higher demand for labile C in response to N addition, no such limitation was seen for P in our study. The lack of apparent P limitation in this minerotrophic portion of the peatland is in line with results from Olid et al. (2017) and from previous results from the experiment in 2003, where no differences in P concentrations of *Sphagnum* tissue were found in response to the applied treatments (Wiedermann, unpublished data). Alternatively, there might be less of a demand for P and consequently for phosphatases since the ericaceous dwarf shrub density ultimately declined in response to the N treatment, and the rooting depth of the sedges is primarily in the deeper peat zones below our peat core sampling depth.

Enzyme Stoichiometry

The long term experimental N addition might have resulted in a shift in microbial demands from being N limited to increased belowground C limitation as indicated by the applied stoichiometric equations on the measured EEA (Moorhead et al. 2016). In accordance with our expectations, the total enzyme C acquisition activity (glucosidase (BG) + cellulase (CBH)) per unit C (mass percent)

increased with N addition, whereas the total enzyme N acquisition activity (chitinase (NAG) + aminopeptidase (LAP)) per unit N (mass percent) decreased with N addition (Table 2, Fig. 3). This suggests that in the N treatments N availability now exceeds demand, thus N may no longer limit decomposition processes, and resources are exceedingly placed toward provision of C, which might promote C loss from peatlands as described by for instance Bragazza et al. (2006).

Discrepancy between the Aboveground and Belowground Responses

The most striking outcome of this long term global change experiment was the apparent discrepancy between the aboveground vegetation response and the belowground decomposition processes, which seem at first glance largely decoupled. We had anticipated that in the T plots the substantial increase in vascular plant species abundance of both the mycorrhizal ericaceous shrubs as well as *E. vaginatum,* an aerenchymal graminoid, would be reflected in increases in EEA (Bragazza et al. 2013; Wiedermann et al. 2017). In terms of specific effects of the increased vascular plant cover following the T treatment, we expected additional root exudates from the vascular plants to provide for additional labile C (Ström et al. 2003; Crow and Wieder 2005; Drake et al. 2013) which might enhance glucosidase (BG) and cellulase (CBH) activity. With higher ericoid species abundance in the T treatment (thus increased ericoid mycorrhizal abundance), we expected to measure higher potential phosphatase activity (PHOS) due to a strong enzymatic capacity for P mining by the ericoid mycorrhiza (Leake and Miles 1996). In addition, we anticipated the higher mycorrhizal fungal biomass accompanied by higher chitin inputs to lead to elevated chitinase (NAG) activity. Given the increased vascular plant cover in the long term T treatment, we had also anticipated that the higher leaf litter inputs would provide for enhanced resource availability to belowground decomposers and thus overall higher EEA.

The observed responses to the N addition treatment and experimental temperature enhancement differing between aboveground and belowground patterns invites several plausible explanations: 1) The hydrological effects from N addition overrides any positive N effects on the vascular plants. The N treatment led to a collapse of the peat matrix which resulted in relatively higher water table levels that could induce flooding stress on the ericaceous dwarf shrubs. 2) Contrary to the belowground microbial communities, the vascular plants are possibly less N limited. In response to the T treatment, both *Eriophorum* and *Andromeda* increased in cover at the expense of tissue N concentration, which dropped. The lower tissue N concentrations in the T treatments indicate that despite the universal low N availability in oligotrophic peatlands and the already high C/N ratios of 47 for *Andromeda* and 35 for *Eriophorum,* both species seem to be more temperature limited. 3) N might have negative effects on mycorrhizal colonization as shown for Ecto-mycorrhizal mutualists (Yesmin et al. 1996; Lilleskov et al. 2002, 2011). Negative N effects on belowground processes can result from a complex web of interactions, about which relatively little is known. It is however known that N addition leads to shifts in microbial community composition (e.g., (Wallenstein et al. 2006; Dean et al. 2014) and has particularly negative effects on mycorrhizal fungi (Lilleskov et al. 2011). Mycorrhizal fungi provide many benefits to the host plant, such as enhanced nutrient uptake, additional water supply, and protection from root pathogens (Allen et al. 2003; Albornoz et al. 2016). It is thus possible that altered plant mycorrhizal interactions have led to additional negative effects on the dwarf shrub abundance.

Conclusions

Our long term study in an oligotrophic boreal peatland found an interesting contrast with aboveground vascular plants cover strongly increasing in the T treatments, and belowground enzymatic processes only responding to the N treatment. The large increase in vascular plant cover in the warming treatment finding no reflection in belowground extracellular activity remains a mystery awaiting to be explored in follow-up studies. The initial response of the ericaceous dwarf shrubs to the experimental N addition were found to be positive after 8 years of treatment (Wiedermann et al. 2007), but after 21 years of experimental treatment the initial effect was lost. An important mechanism explaining the lack of positive responses of the ericaceous draft shrubs to the applied N treatment in the long term might be that the high N treatment led to the collapse of the peat matrix, which resulted in relatively higher water table levels that could induce flooding stress on the ericaceous dwarf shrubs. We thus emphasize that such indirect effects of chronic high N levels far exceeding the demands of the *Sphagnum* species can only be elucidated by long term experiments. Another interesting facet of our study are the negligible effects of S addition on both aboveground vegetation as well as belowground enzymatic processes. Our study, by integrating effects of long term exposure to both elevated N, S and T on peatland vegetation and biogeochemistry, provides critical new insights contributing to a more coherent understanding of peatland ecosystem responses to global change.

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Author Contribution MMW and MBN conceived the study; MBN provided the long-term field experiment; MMW carried out the field sampling, laboratory and statistical analyses and wrote the manuscript; MBN contributed critically to the evaluation and interpretation of the data and to the manuscript writing.

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