Enzymatic activities indicate mechanisms driving *Sphagnum* moss nitrogen and phosphorus co-limitation in a pristine bog and poor fen

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Introduction

- Peatlands are classicly defined as nutrient limited ecosystems, contributing to their sensitivity to anthropogenic inputs. While the boreal north is relatively pristine, ongoing Alberta oil sands development has elevated and is expected to increase nitrogen deposition in northern Alberta[4].
- **Our goal is to investigate nutrient limitations in pristine peatlands in order to assess how these pollutants will impact large soil carbon stores sequestered in peatlands and their potential feedbacks to climate change.**

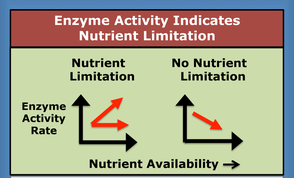


Figure 1. The production and activity enzymes are up-regulated when substrate supply is limited and down-regulated when substrate is freely available[4]. Nitrogen limitation increases the production of phosphatase activity while the absence of nitrogen limitation does not affect enzyme activity.

- Enzymes are the workhorses of biogeochemical processes, ultimately catalyzing the majority of reactions. Limitations of biogeochemical transformations are governed by the availability of nutrients **(Fig. 1)**. Rates of enzymatic activities can be used as a proximate representative of nutrient demand for biogeochemical transformations[1].
- **We hypothesized that phosphatase activity in *Sphagnum* moss would increase in both the bog and poor fen in response to nitrogen enrichment; but that chitinase (organic nitrogen mineralization) activity would decline in the bog but not the poor fen (Fig 2). This hypothesis is consistent with the idea that nitrogen is limiting in the poor fen, but not in the bog.**

Mariana Peatland Complex

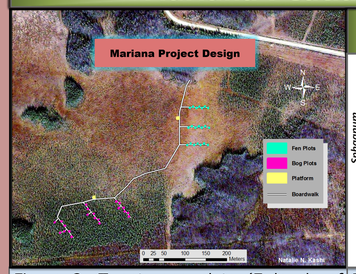


Figure 2. Twenty-one plots (7 levels of nitrogen, 3 replicates) were established in each the poor fen and the bog. Nitrogen, in the form of NH_4NO_3, was sprayed onto the plots at rates of 0, 5, 10, 15, 20 and 25 kg N ha^{-1} yr^{-1} from June-August 2011-2013. Additional control plots, receiving neither water nor nitrogen, served as a water control.

Net Primary Productivity

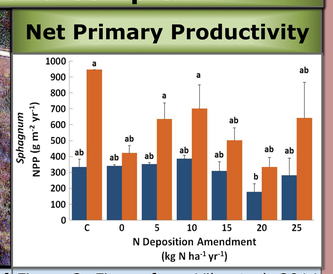
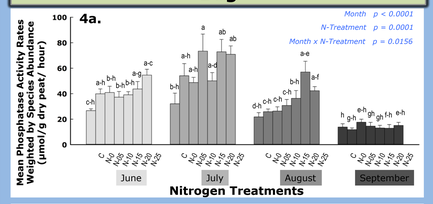
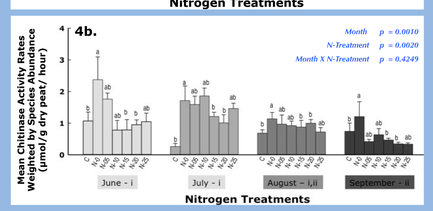


Figure 3. Figure from *Vile et al. 2014 illustrates* no significant NPP response to N-fertilization for either *Sphagnum* species (blue, n = 3, p = 0.246; orange, n=3, (except n=2 for C, 0, 5, and 15 kg ha^{-1} $year^{-1}$) p = 0.185) in the 2012 growing season, Mariana Lakes Peatland, Alberta Canada.

Enzyme Activities Weighted by Species % Cover of Each Plot

Bog

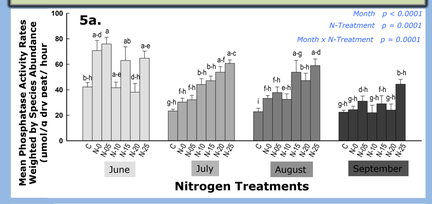




Phosphatase

Chitinase

Fen



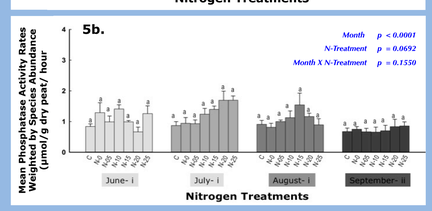
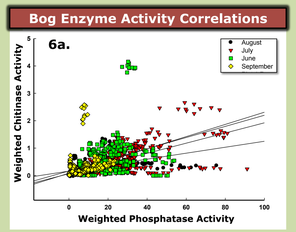


Figure 4. Enzyme activities as a function of month and nitrogen addition treatment of three **bog** *Sphagnum* species weighted by the percent cover within each plot. Bars represent the mean ± standard error (n=3), and letters above the bars indicate groups that are significantly different, based on a two-way nested randomized complete block design ANOVA for (**4a.**) phosphatase and (**4b.**) chitinase enzyme activities. Enzyme activity indicate co-nitrogen and phosphorus limitation and a water effect in the bog.

Figure 5. Enzyme activities as a function of month and nitrogen addition treatment of three **poor fen** *Sphagnum* species weighted by the percent cover within each plot. Bars represent the mean ± standard error (n=3), and letters above the bars indicate groups that are significantly different, based on a two-way nested randomized complete block design ANOVA for (**5A.**) phosphatase and (**5B.**) chitinase enzyme activities. Enzyme activity indicate co-nitrogen and phosphorus limitation in the poor fen.

Bog Enzyme Activity Correlations

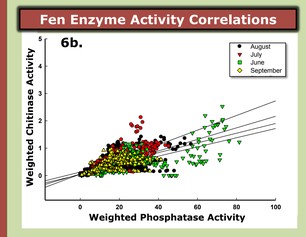


Weighted Phosphatase and Chitinase Correlations

	June	July	August	September
Bog				
P	<.0001	<.0001	<.0001	<.0001
R	0.4358	0.7356	0.5124	0.3776
Fen				
P	<.0001	<.0001	<.0001	<.0001
R	0.753	0.8025	0.6624	0.7855

Figure 6. Positive phosphatase and chitinase enzyme activity correlations during all four months in both the **6a**. bog and **6b.** poor fen.

Fen Enzyme Activity Correlations



Enzyme Activity Materials and Methods

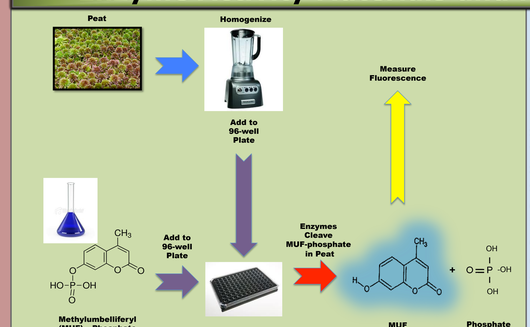


Figure 7. Enzyme assay schematic. Methylumbelliferyl (MUF) synthetic substrates fluoresce when enzymes cleave substrates in peat samples. Potential enzyme activity is then calculated based on fluorescent detection units and sample dry weights.

Chitinase by *Sphagnum* species-Poor Fen

Sphagnum magellanicum

Sphagnum fuscum

Sphagnum angustifolium

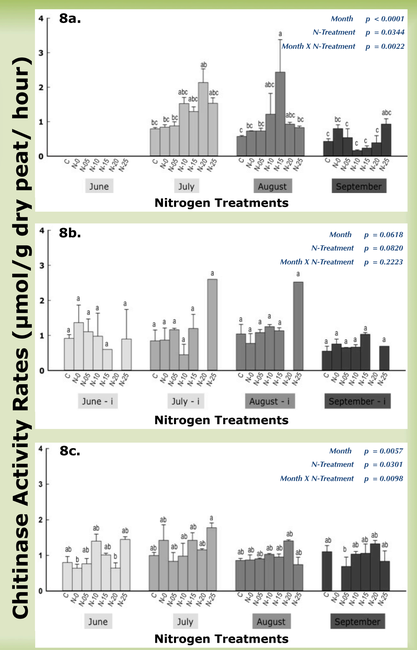


Figure 8**.** Chitinase enzyme activities of each *Sphagnum* species in the poor fen as a function of month and nitrogen addition treatment. Bars represent the mean ± standard error (*n*=3), and letters above the bars indicate groups that are significantly different, based on a two-way nested randomized complete block design ANOVA. No significant differences for chitinase by species in the bog. **8a.** *Sphagnum magellanicum* chitinase activity significantly increased in response to nitrogen in July and August indicating a stronger nitrogen limitation than the other species.

Discussion

- Despite no NPP response to nitrogen fertilization at the Mariana peatland (Fig. 3), enzyme activities indicate nitrogen effect by plant and / or microbial communities.
- Phosphatase enzyme activity increased incrementally from the control to 25 kg N ha^{-1} yr^{-1} up to **five-folds** in **all three species and in both peatlands** in response to three years of nitrogen addition (Fig. 4a & 5a).
- Weighted by species % abundance, a water effect on chitinase activity in the **bog illustrates water and/ or micronutrient limitation** (Fig. 4b).
- Both weighted and species specific chitinase activity suggest an incremental increasing need for organic nitrogen with inorganic nitrogen fertilization in the poor fen (Fig. 5b & 8). In August, *Sphagnum magellanicum* chitinase activity was 0.83 ± 0.05 µmol/g/dry peat/hour in the 25 kg N ha-1 yr-1 treatments, 1.5 times higher than the control treatment with rates of 0.57 ± 0.02 µmol/g/ dry peat/hour (Fig. 8a).
- Increased *Sphagnum magellanicum* chitinase activity indicate a stronger nitrogen limitation as compared to the other poor fen *Sphagnum* species. This is consistent with N_2-fixation rate differences between *Sphagnum* species[3].
- Enzyme activity in response to nitrogen addition supports our hypothesis that nitrogen is limiting in the poor fen; however, contrary to our hypothesis, both the bog and poor fen illustrated a co-nitrogen and phosphorus limitation, with a stronger nitrogen limitation in the poor fen.
- Seasonal variations of phosphatase enzymes of these three *Sphagnum* moss species suggests specific nutrient acquisition strategies unique to each species microhabitat in the peatland.

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