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REGULAR ARTICLE

Understanding drivers of peatland extracellular enzyme activity in the PEATcosm experiment: mixed evidence for enzymic latch hypothesis

Karl J. Romanowicz · Evan S. Kane · Lynette R. Potvin · Aleta L. Daniels · Randall K. Kolka · Erik A. Lilleskov

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Abstract
Aims Our objective was to assess the impacts of water table position and plant functional groups on peatland extracellular enzyme activity (EEA) framed within the context of the enzymic latch hypothesis.
Methods We utilized a full factorial experiment with 2 water table (WT) treatments (high and low) and 3 plant functional groups (PFG: Ericaceae, sedge, Ericaceae and sedge unmanipulated) in twenty-four 1 m^3 intact peatland mesocosms. We measured bulk peat and porewater phase oxidative and hydrolytic enzyme activities monthly from June - October 2012. We also measured physical and porewater chemical constituents in tandem to analyze environmental influences on seasonal enzyme activities.
Results No PFG effects on EEA with WT affecting only acid-phosphatase activity in porewater. Strong seasonal dynamics in EEAs overshadowed our manipulations. Analyses indicated phenolic concentrations were influenced by peat redox potential and negatively correlated with phenol oxidase activity as expected from enzymic latch hypothesis. However, no hydrolytic EEA was influenced by total phenolics, but driven largely by seasonal changes in soil temperature and increasing DOC concentrations in porewater.
Conclusions Our results suggest no support for final step in enzymic latch, in which phenolics are posited to regulate hydrolytic EEAs. Mechanisms regulating seasonal influences remain to be elucidated.

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K. J. Romanowicz (✉) · E. S. Kane · A. L. Daniels
School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA
e-mail: kjromano@umich.edu

E. S. Kane
e-mail: eskane@mtu.edu

A. L. Daniels
e-mail: aldaniel@mtu.edu

E. S. Kane · L. R. Potvin · E. A. Lilleskov
USDA Forest Service, Northern Research Station, Houghton, MI 49931, USA

L. R. Potvin
e-mail: lrpotvin@fs.fed.us

E. A. Lilleskov
e-mail: elilleskov@fs.fed.us

R. K. Kolka
USDA Forest Service, Northern Research Station, Grand Rapids, MN 55744, USA
e-mail: rkolka@fs.fed.us

Present Address:
K. J. Romanowicz
School of Natural Resources & Environment, University of Michigan, Ann Arbor, MI 48109, USA

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Introduction

Northern peatlands play a substantial role in global carbon (C) sequestration by storing over one third (~270–620 Pg) of soil C in the form of partially decayed organic peat (Gorham 1991; Turunen et al. 2002; Vasander and Kettunen 2006; Yu et al. 2010). Rates of organic matter decomposition and C mineralization in peatlands are controlled by abiotic factors such as soil temperature (Weltzin et al. 2000; Lafleur et al. 2005; White et al. 2008) and hydrology (Whiting and Chanton 1993; Blodau et al. 2004; Knorr and Blodau 2009; Deppe et al. 2010), and biotic factors, specifically plant functional groups (Verhoeven and Toth 1995; Joanisse et al. 2007) and the chemical composition of the peat itself (Belyea 1996; Yavitt et al. 2005). This combination of abiotic and biotic factors both directly and indirectly regulates synthesis and secretion of extracellular enzymes, and rates of extracellular enzyme activities (EEAs) that facilitate decomposition, mineralization, and nutrient cycling processes (Freeman et al. 1996, 1997; Shackle et al. 2000; Freeman et al. 2004; Fenner et al. 2005; Joanisse et al. 2007; Toberman et al. 2008; Jassey et al. 2011). Measurements of soil EEAs are used to evaluate the potential for microbially mediated decomposition and nutrient cycling (Sinsabaugh et al. 2008; Choi et al. 2009; Burns et al. 2013) as well as an early indicator of ecosystem responses to global change and other disturbances (Lipson et al. 2005; Finzi et al. 2006).

Over the past several decades, northern regions have experienced more variability in precipitation patterns (Groisman et al. 2005) causing declines in peatland water table levels and increased frequency and intensity of mid-summer water table drawdown (Roulet et al. 1992; Hilbert et al. 2000). Climate change models predict that consistent lowering of the water table could cause peatlands to transition from C sinks to sources (Trettin et al. 2006) because of direct and indirect impacts on C cycling. One mechanism proposed for water table regulation of decomposition is the enzymic latch hypothesis (Freeman et al. 2001), which postulates that activity of oxidative enzymes (e.g., phenol oxidase) is suppressed under saturated water table conditions due to lack of oxygen (O_2) as a necessary reactant. These oxidative enzymes are deemed critical to C cycling because they contribute to breakdown of phenolic compounds that suppress hydrolytic enzymes responsible for carbon and nutrient cycling. Suppression of oxidative enzyme activity due to low oxygen availability in the peat soil leads directly to an increased abundance of polyphenols, which bind to hydrolytic enzymes and inhibit function (Freeman et al. 2001, 2004; Fenner and Freeman 2011).

In addition to the potential effects of water table drawdown on peat oxygen availability and extracellular enzyme activity related to the enzymic latch hypothesis, sustained changes in peatland water table have been shown to alter the relative abundance of plant functional groups (Weltzin et al. 2000; Breeuwer et al. 2009; Churchill et al. 2015). The dominant vascular plants such as sedges and ericaceous shrubs have the ability to regulate soil microbial processes by influencing litter quality (Joanisse et al. 2007), O_2 availability (Strack et al. 2006), root exudation (Inderjit 2002). root symbiosis (Read 1991), and nutrient competition (Bonfante and Genre 2010; Artz et al. 2007). Sedges utilize aerenchyma to promote diffusion of O_2 to deep roots in anoxic peat creating rhizosphere C oxidation and mineralization hotspots (Holzapfel-Pschorn et al. 1986) that promote changes in microbial populations and associated EEAs, potentially leading to peat subsidence and carbon loss (Flessa and Fischer 1992; Brix 1997; Potvin et al. 2015). In contrast, ericaceous shrubs are non-aerenchymatous and shallowly rooted, and form a symbiotic relationship with enzymatically active ericoid mycorrhizal fungi (Read 1991; Read et al. 2004). The mutualistic association between plant host and mycorrhizal fungi in the soil rhizosphere is hypothesized to suppress free-living heterotrophs and may promote decreased C mineralization by mediating changes in rhizosphere microbial communities (Gadgil and Gadgil 1971). Alternatively, the ability of the ericoid mycorrhizal fungi to mobilize a broad suite of oxidative and hydrolytic enzymes might enhance C mineralization in the shallow peat (Read and Perez-Moreno 2003). Shifts in the dominance of these plant functional groups due to sustained changes in water table levels could have significant effects on the growth of microbial populations

and subsequent EEAs. To our knowledge, no study to date has directly tested the anticipated combined effects of shifts in hydrology and plant functional group assemblages on extracellular enzymatic mediation of carbon and nutrient cycling in peatlands.

To explore the enzymatic mechanisms linked to peatland hydrology and plant functional groups, it was necessary to analyze multiple pools of belowground EEAs. Previous studies on peatland EEAs have focused on the bulk peat phase but neglected the potential EEAs in the porewater phase (Williams et al. 2000; Freeman et al. 2004; Kang et al. 2005a, 2005b; Fenner et al. 2005; Joanisse et al. 2007; Toberman et al. 2008; Sun et al. 2010; Jassey et al. 2011; Fenner et al. 2011; Jassey et al. 2012). There are some reasons why these two phases might differ. First, the bulk peat phase is more likely to be influenced by root and filamentous fungal EEAs, whereas the porewater phase, which naturally has larger particles and biota filtered out, is likely to have a larger impact from smaller-celled microbiota, including microfungal and prokaryote activity. Additionally, the primary source of labile substrates available for enzyme production by soil microorganisms is often contained in the bulk peat phase while porewater is composed mainly of dissolved organic matter highly enriched in humic substances, with a labile fraction often representing less than 10 % of the total dissolved fraction (Munster 1991, 1993; Kalbitz et al. 2003). However, recent studies have shown an increase in dissolved organic carbon in peatland porewater following climate-induced warming (Jassey et al. 2012; Kane et al. 2014), indicating greater potential for porewater EEAs in the future (Peacock et al. 2015).

To this end, we utilized PEATcosm, a peatland mesocosm experiment where water table and plant functional group manipulations were implemented beginning June 2011 and EEAs were measured across the experimental design monthly from June through October 2012. Our study measured a broad suite of extracellular enzymes including two oxidative and four hydrolytic enzymes involved in carbon, nitrogen, or phosphorus cycling. The oxidative enzymes phenol oxidase and peroxidase contribute to the breakdown of lignin and other complex polymeric substances, while hydrolytic enzymes β-glucosidase and cellobiohydrolase degrade cellulose, *N*-acetyl-glucosaminidase degrades chitin, and acid-phosphatase liberates phosphate from organic molecules. Our primary hypotheses were framed within the context of the enzymic latch hypothesis (Freeman et al. 2001) and potential plant functional group effects such that 1) lowered water table levels would lead to higher EEAs in both phases due to increased peat oxidation, which would stimulate oxidative EEAs and subsequently increase hydrolytic extracellular enzyme expression; and 2) when oxygen was limiting (high water table conditions) sedges would be associated with elevated EEAs because of their transport of O_2 to the rhizosphere, whereas when oxygen was not limiting (low water table conditions) ericaceous shrubs would be associated with higher EEAs because they possess enzymatically competent mycorrhizae supported by host C subsidy. We also tested the validity of the enzymic latch hypothesis and potential plant functional group effects through hierarchical correlations of physical and porewater chemical constituents measured in tandem with oxidative and hydrolytic EEAs.

Materials and methods

Site description and experimental design

Mesocosm construction, peat harvesting, and environmental manipulations followed methods described in detail elsewhere (see Potvin et al. 2015). In brief, we collected twenty-four intact peat monoliths (~1m^3) in close proximity to one another in May 2010 from an acidic (pH ~4.0), oligotrophic bog in northeastern Minnesota (47°07′05″N, 92°47′59″W, Meadowlands, MN, USA), which were placed into individual mesocosm chambers and transported to the Houghton Mesocosm Facility, Houghton, Michigan. Vegetation included bryophytes (*Sphagnum fuscum* (Schimp.) Klinggr., *S. magellanicum* Brid., *S. rubellum* Wilson, *Polytrichum strictum* Brid., *P. commune* Hedw.), sedges (*Carex oligosperma* Michx., *Eriophorum vaginatum* L.), and ericaceous shrubs (*Chamaedaphne calyculata* (L.) Moench., *Kalmia polifolia* Wang., *Vaccinium oxycoccos* L., *Rhododendron groenlandicum* Oeder., and *Andromeda glaucophylla* Link.). We extracted peat monoliths from the treeless portion of the bog with a priori selection for relatively equal representation of both sedge and Ericaceae vascular plant cover (see Potvin et al. 2015 for details).

Beginning in June 2011, we randomly assigned individual mesocosm chambers one of the following plant functional group (PFG) treatments: *i.*) Unmanipulated

(U) – sedge and Ericaceae left intact; *ii.*) Sedge (S) – sedge left intact while Ericaceae removed; and *iii.*) Ericaceae (E) – Ericaceae left intact while sedge removed. Differences in plant cover biomass by treatment manipulations are described in greater detail in Potvin et al. (2015). Along with plant functional group treatments, we assigned each mesocosm chamber either a high (H) or low (L) water table (WT) treatment with seasonal hydrologic profiles based on data derived from the almost 50 year record of water tables at the Marcell Experimental Forest (Sebestyen et al. 2011), which is edaphically and climatically similar to the peatland our monoliths were extracted from. This effectively created a 3 × 2 factorial experimental design with four replicates arranged in four blocks where each block contained one replicate of each treatment combination, for a total of 24 experimental units.

Peat and porewater sampling

We collected bulk peat and porewater samples from each mesocosm chamber once per month from June through October 2012. For bulk peat samples, we collected ten replicates (~0.2 g replicate^{-1} wet weight for total wet weight ~ 2 g chamber^{-1}) at 15 cm below the peat surface using 5 mm laparoscopic spoon retrieval forceps (Teleflex Inc., USA) and homogenized to minimize micro-topography bias. We collected approximately 60 mL of porewater at 20 cm depth (± 5 cm) from a piezometer installed in each chamber. We constructed piezometers from ultra-high-density polyethylene (UHDPE), which had a 10 cm slotted region centered at the 20 cm, 40 cm, and 70 cm depths, each covered with Nitex nylon mesh (37 μm). Porewater was harvested from narrow Teflon tubes installed in each piezometer (at the three corresponding sampling depths) without introducing atmosphere by purging the lines through a 3-way stopcock prior to sampling with a syringe. Each depth was compartmentalized with a plug of inert glue and only the 20 cm depth porewater samples were utilized for extracellular enzyme assays for direct comparison with bulk peat EEA. To reflect realistic seasonal dynamics, water table levels between the high and low treatments were similar during the early season (June) but diverged greatly in July through October (Fig. 1). All bulk peat and porewater samples were immediately refrigerated at 4 °C and processed within 4 h from time of collection.

Extracellular enzyme assays

We conducted extracellular enzyme assays using methods described by Saiya-Cork et al. (2002), with modifications for organic soil. We assayed bulk peat and porewater samples for two oxidative EEAs: phenol oxidase (POX), peroxidase (PER) using L-3,4-dihydroxyphenylalanine (L-DOPA) substrate; and four hydrolytic EEAs: β-1,4-glucosidase (BG), β-1,4-cellobiohydrolase (CBH), β-1,4-*N*-acetyl-glucosaminidase (NAG), and acid-phosphatase (AP) using methylumbelliferyl-based (MUB) substrates (see Table S1 in Supplementary Materials for more details). In brief, we prepared bulk peat samples in suspension by adding 1.0 g (wet weight) peat to 125 ml of 50 mM, pH 5.0 sodium acetate buffer that was then shaken vigorously for 2 min to homogenize thoroughly. Peat suspensions were stirred continuously on a magnetic stir plate while 200 μl aliquots were dispensed into 96-well microplates with 4 replicate wells per sample per assay. For porewater samples, 200 μl aliquots were dispensed directly into 96-well microplates with 4 replicate wells per sample per assay. For oxidative assays, we added 50 μL of 5 mM L-DOPA to each sample well for measuring EEA as modified from 25 mM L-DOPA for ease of dissolving substrate in 50 mM sodium acetate buffer, pH 5.0. Comparison of measured EEA between 5 mM and 25 mM L-DOPA showed no significant differences (*results not shown*). For hydrolytic assays, we added 50 μL of 200 μM MUB-based substrate concentrations to each sample well for measuring potential EEA. All subsequent plating conditions were followed according to Saiya-Cork et al. (2002) with the following modifications: four replicate wells for each blank, negative control, and quench standard. For oxidative enzyme assays, we incubated microplates in the dark at 25 °C for 24 h prior to quantifying absorbance spectrophotometrically at λ = 450 nm. For hydrolytic enzyme assays, we incubated microplates in the dark at 25 °C for 3 h then terminated all reactions by adding a 10 μL aliquot of 1.0 M NaOH to each well. Following reaction termination, we analyzed all hydrolytic assays fluorimetrically with 365 nm excitation and 450 nm emission filters. All spectrophotometric and fluorimetric measurements were read on a SpectraMax M2 plate reader (Molecular Devices, Sunnyvale, California). After we corrected for negative controls and quenching, potential EEA for bulk peat and porewater assays were expressed as 1 nmol of substrate consumed h^{-1} g^{-1} dry mass or h^{-1} ml^{-1}, respectively.

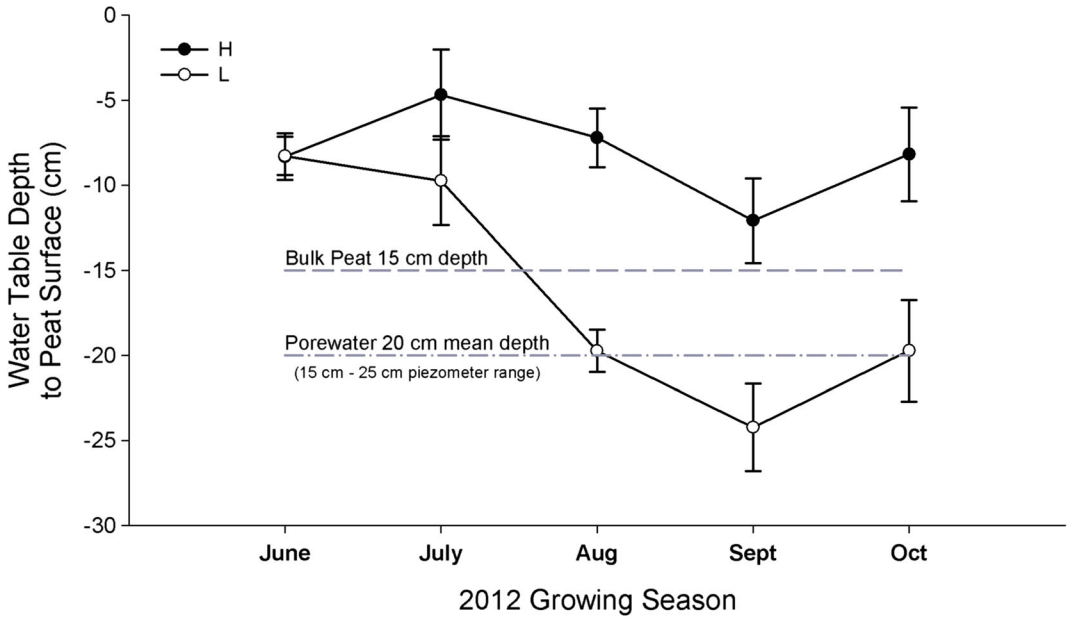

Fig. 1 Seasonal water table levels for high water table (H) and low water table (L) treatments with bulk peat and porewater phase sampling depths denoted. Water table depths based on mean monthly measurements by treatment (n = 12/month)

Seasonal measurements of physical, biogeochemical, and porewater chemical characteristics

To further elucidate the effects of environmental drivers on seasonal EEAs in the context of the enzymic latch hypothesis and plant functional group effects, we quantified multiple physical and porewater chemical constituents in tandem with EEA assays for the months of July, August, and September 2012 (see Table S2 in Supplementary Materials). In brief, we measured the vertical and horizontal temperature profiles in the peat soil using an NI Controller (CompactRIO 9074) linked to monitoring software (LabVIEW, Austin, TX) using two probes in each mesocosm bin, one at the center and another 10 cm from an edge. Each temperature probe has thermistors at five depths (5, 10, 20, 40 and 80 cm below peat surface). In this study we calculated the mean temperature between the 10 cm and 20 cm depths from the date of sampling for EEAs. We derived bulk peat water content (% moisture) from samples collected for EEA assays by calculating the difference between wet weight and dry weight and then dividing from the wet weight. Oxidation-reduction potential (ORP) was measured by harvesting purged porewater from the piezometers as previously described; in effect the sealed piezometers act as equilibration chambers (cf., Megonigal and Rabenhorst 2013). This water was then injected into a sealed flow-through cell containing a Hach ORP probe (Hach Co., Loveland, Co., USA, IntelliCAL MTC301). The cell was purged with sample, sealed, and then approximately 10 mL was flushed across the probe with two syringes while the probe equilibrated. The probe was calibrated daily with an ampoule of Light's solution (Hach Co., no. 2,612,520) and conditioned with Reducing solution (Hach Co., no. 2,965,349). All E_h values were normalized to a pH of 7 (E_h7), based on pH – E_h relationships for Quinhydrone (Bier 2009).

From the original porewater samples used in EEA assays we initially isolated a 20 mL split and acidified with hydrochloric acid to be analyzed for dissolved organic carbon (DOC). We measured DOC using a Shimadzu TOC-V Combustion Analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). In addition, we analyzed total phenolics (tannin and lignin) using Hach (Loveland, CO, USA) reagents scaled to a microplate technique. In brief, we quantified total phenolics at a 1:3 dilution by adding 83 μL sample to 166 μL RO water to each microplate well. We then added 10 μL of TanniVer reagent. Next, we dissolved sodium pyrophosphate (NaP_2O_7) in RO water (0.3 g per 3 mL), 20 μL of which was added to each well to eliminate the possibility of ferrous iron interference. We followed with the addition of 50 μL of sodium

carbonate solution. Plates were shaken for 30 s to allow all products to mix, incubated for 25 min, and read at 700 nm absorbance on SpectraMax M2 plate reader (Molecular Devices, Sunnyvale, California). A total phenolic standard curve was produced from tannic acid diluted to 1.5, 3, 6, and 9 ppm.

Statistical analysis

We analyzed the effects of experimental treatments on the potential activity of each extracellular enzyme assayed using a generalized linear mixed (GLM) model analysis (type II Wald test with restricted maximum likelihood) for repeated measures (lme4 package; R, Version 3.0.2) with plant functional group, water table regime, and month as main effects, block as fixed effect, and mesocosm bin as a random effect to account for repeated measures, assuming a multivariate normal distribution. We also explored seasonal dynamics using a two-way ANOVA followed by Tukey's HSD post hoc test performed in R (version 3.0.2).

Furthermore, we established a 4-level hierarchical conceptual model to empirically test the hypothetical effects of the enzymic latch hypothesis, potential plant functional group effects, as well as alternative environmental effects on peatland extracellular enzyme activity over the growing season in our PEATcosm experimental system. The continuous variables in each level of the hierarchical model were evaluated via GLM model analysis where main effects and dependent variables varied between levels but block was held as a fixed effect and mesocosm bin as a random effect to account for repeated measures. All continuous variables for hierarchical GLM models were derived from sampling efforts made in tandem with EEA assays in July, August, and September 2012. Within Level 1, we investigated the environmental controls influencing oxidation-reduction potential (ORP); whether it was water content (WC) as predicted from the enzymic latch hypothesis; Ericaceae (Er) or sedge (Sed) biomass as PFG effects; or temperature (temp) as an alternative influence. Within Level 2, we investigated the environmental controls influencing total phenolic concentrations by building from Level 1 to test whether ORP influenced phenolics as predicted from the enzymic latch hypothesis. We also tested the effects of Ericaceae (Er) or sedge (Sed) biomass as PFG effects and temperature (temp) and dissolved organic carbon (DOC) as alternative influences. The predictor variables in Level 3 for the enzymic latch hypothesis built from Levels 1 and 2 to test if phenolics or ORP influence phenol oxidase (POX) and peroxidase (PER) activities in the bulk peat and porewater phases. The same predictor variables for PFG effects and alternative influences found in Level 2 were also simultaneously tested. Within Level 4, we investigated the environmental controls influencing hydrolytic EEAs in the bulk peat and porewater phases. In this case, we tested the influence of total phenolics on hydrolytic EEAs from both phases as predicted from the enzymic latch hypothesis as well as all previous predictor variables for PFG effects and alternative influences. All statistical analyses were considered significant at $P \leq 0.05$.

Results

Treatment effects and their interactions on seasonal EEAs within phases

No plant functional group (PFG) treatment had a significant main effect on any EEA assayed (Table 1). In the bulk peat phase, WT had a significant effect on PER activity (P-value =0.004; Table 1; see also Table S3 in Supplementary Materials) where activity increased under HWT conditions, specifically during the month of August (Fig. 2). In the porewater phase, WT only had a significant effect on AP activity (P-value =0.030; Table 1; see also Table S3 in Supplementary Materials) where activity increased under LWT conditions. Within both phases, all oxidative and hydrolytic enzymes had significant variations among sampling months with the exception of POX activity in the bulk peat phase (Table 1). There were no significant interactions between WT, PFG, or month for any EEA in either phase (Table 1).

Seasonal variations in EEAs

We found that mean oxidative EEA rates between phases differed by up to four orders of magnitude and hydrolytic EEA rates varied by two orders of magnitude, yet we observed similar seasonal trends for most enzymes (Table 2; see also Figure S1 in Supplementary Materials). POX activity in the bulk peat phase was the only EEA assayed which lacked any significant monthly response over the 2012 growing season. However, in the porewater phase, POX activity in June and September

Table 1 Generalized linear mixed effects (GLM) model (type II Wald test with restricted maximum likelihood) for categorical treatment effects and their interactions on potential EEAs. GLM model factors each accounted for 120 observations

	df	POX Chi^2 (*P*-value)	PER Chi^2 (*P*-value)	BG Chi^2 (*P*-value)	CBH Chi^2 (*P*-value)	NAG Chi^2 (*P*-value)	AP Chi^2 (*P*-value)
Bulk Peat							
PFG	2	0.7 (0.692)	4.6 (0.101)	1.4 (0.506)	1.8 (0.399)	1.4 (0.498)	1.6 (0.458)
WT	1	0.8 (0.377)	**8.5 (0.004)**	0.7 (0.413)	0.3 (0.562)	0.7 (0.401)	0.2 (0.672)
Month	4	1.5 (0.834)	**66.3 (<0.001)**	**75.2 (<0.001)**	**23.9 (<0.001)**	**30.5 (<0.001)**	**66.8 (<0.001)**
Block	3	2.2 (0.525)	1.4 (0.696)	5.2 (0.160)	**20.5 (<0.001)**	1.6 (0.658)	3.4 (0.337)
PFG:WT	2	1.2 (0.546)	0.9 (0.637)	0.6 (0.746)	3.5 (0.173)	1.0 (0.605)	0.1 (0.968)
PFG:Month	8	6.2 (0.623)	4.4 (0.822)	5.3 (0.728)	5.2 (0.738)	8.3 (0.401)	7.8 (0.451)
WT:Month	4	1.2 (0.870)	7.7 (0.105)	3.7 (0.434)	6.9 (0.144)	2.6 (0.635)	1.2 (0.872)
PFG:WT:Month	8	5.2 (0.735)	7.3 (0.503)	9.2 (0.329)	11.4 (0.178)	3.4 (0.905)	1.5 (0.993)
Porewater							
PFG	2	0.2 (0.917)	0.5 (0.773)	0.9 (0.653)	0.3 (0.845)	0.7 (0.715)	1.5 (0.476)
WT	1	0.3 (0.606)	0.3 (0.570)	1.6 (0.207)	2.6 (0.109)	0.2 (0.620)	**4.7 (0.030)**
Month	4	**64.5 (<0.001)**	**497.2 (<0.001)**	**117.7 (<0.001)**	**224.2 (<0.001)**	**221.4 (<0.001)**	**225.7 (<0.001)**
Block	3	**11.5 (0.009)**	0.3 (0.956)	0.9 (0.829)	1.1 (0.780)	2.3 (0.515)	**11.2 (0.011)**
PFG:WT	2	1.0 (0.615)	1.1 (0.580)	0.9 (0.648)	0.2 (0.911)	0.5 (0.769)	0.6 (0.753)
PFG:Month	8	0.5 (1.000)	2.0 (0.982)	3.1 (0.926)	4.2 (0.836)	6.6 (0.581)	5.4 (0.719)
WT:Month	4	0.4 (0.983)	8.5 (0.075)	4.2 (0.379)	5.5 (0.244)	5.2 (0.264)	8.6 (0.071)
PFG:WT:Month	8	0.6 (1.000)	8.1 (0.422)	4.9 (0.774)	5.0 (0.762)	1.9 (0.984)	5.2 (0.742)

df = degrees of freedom; PFG = plant functional group; WT = water table

was significantly greater than in all other months ($P < 0.001$ for all; Table 2). With the exception of CBH in the porewater phase, the activity of all four hydrolytic enzymes within both phases peaked in July ($P < 0.05$ for all; Table 2). In the bulk peat phase, late season (October) activity rates were not significantly different from early season (June) rates for any hydrolytic EEA measured. However, in the porewater phase, September and October EEA rates dropped significantly from June rates ($P < 0.05$ for all; Table 2). When comparing EEAs between phases, we found that the hydrolytic enzymes BG, NAG, and AP in the porewater

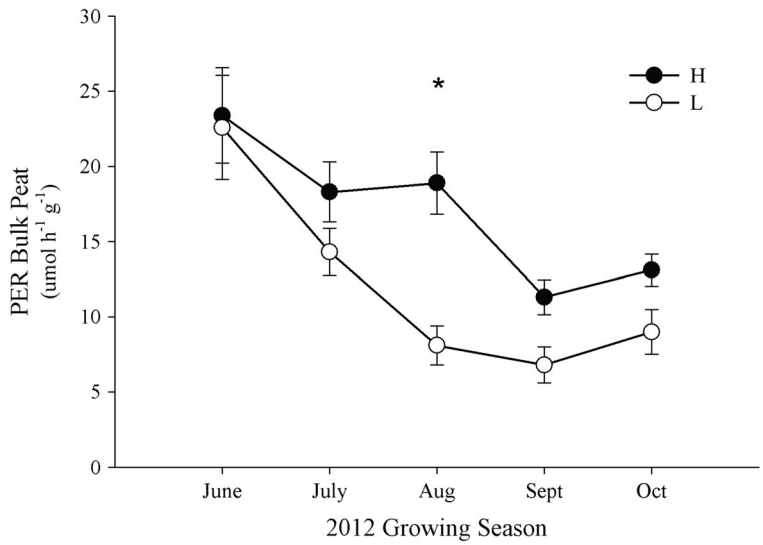

Fig. 2 Differences in seasonal dynamics of peroxidase activity under high (H) and low (L) water table conditions in the bulk peat phase. Mean ± SE are shown. Peroxidase activity based on mean monthly water table treatment ($n = 12$/month). * = $P < 0.05$ for WT contrast in month of August 2012

Table 2 Mean oxidative (μmol h^{-1}) and hydrolytic (nmol h^{-1}) EEA (± standard deviation) for bulk peat and porewater phases over the 2012 growing season (n = 24/month; N = 120/season)

	June	July	August	September	October
Bulk peat (g^{-1})					
POX	1.5 ± 1.9	1.3 ± 2.1	1.3 ± 1.9	1.9 ± 2.2	1.6 ± 2.6
PER	23.0 ± 11.3	16.3 ± 6.4	13.5 ± 8.1	9.0 ± 4.6	11.1 ± 4.8
BG	3161.5 ± 1384.7	5327.1 ± 2356.7	2347.8 ± 832.5	2660.8 ± 1076.5	2318.5 ± 1157.7
CBH	995.1 ± 644.9	1813.8 ± 1539.7	954.0 ± 415.6	945.2 ± 535.3	838.5 ± 737.3
NAG	1418.4 ± 1365.3	1961.0 ± 1036.9	956.9 ± 600.0	897.6 ± 592.5	910.2 ± 449.8
AP	8752.3 ± 3682.0	15,222.4 ± 6263.1	8371.0 ± 1652.1	8126.7 ± 1765.0	8016.8 ± 3736.5
Porewater (mL^{-1})					
POX	$2.8e^{-3} \pm 3.0e^{-3}$	0.0 ± 0.0	$7.4e^{-4} \pm 6.5e^{-4}$	$2.9e^{-3} \pm 8.9e^{-4}$	$1.1e^{-3} \pm 6.9e^{-4}$
PER	$1.1e^{-2} \pm 2.4e^{-3}$	$1.0e^{-2} \pm 1.5e^{-3}$	$1.7e^{-2} \pm 1.4e^{-3}$	$1.4e^{-2} \pm 1.1e^{-3}$	$1.2e^{-2} \pm 1.0e^{-3}$
BG	$6.6e^{-1} \pm 1.9e^{-1}$	$8.7e^{-1} \pm 3.0e^{-1}$	$5.7e^{-1} \pm 2.4e^{-1}$	$3.6e^{-1} \pm 2.5e^{-1}$	$4.4e^{-1} \pm 1.8e^{-1}$
CBH	$1.4e^{-1} \pm 4.2e^{-2}$	$1.1e^{-1} \pm 3.2e^{-2}$	$8.0e^{-2} \pm 3.4e^{-2}$	$6.7e^{-2} \pm 2.8e^{-2}$	$5.4e^{-2} \pm 1.9e^{-2}$
NAG	$4.2e^{-1} \pm 1.0e^{-1}$	$5.6e^{-1} \pm 1.3e^{-1}$	$3.6e^{-1} \pm 9.1e^{-2}$	$3.1e^{-1} \pm 8.5e^{-2}$	$1.6e^{-1} \pm 1.1e^{-1}$
AP	4.8 ± 1.3	6.2 ± 2.1	3.9 ± 1.9	3.3 ± 1.1	2.4 ± 0.6

phase were significantly correlated to activity rates in the bulk peat (P-value <0.001 for all). However, both oxidative enzymes POX and PER as well as the hydrolytic enzyme CBH in the porewater phase failed to correlate to activity rates in the bulk peat (P-value >0.05 for all).

Hierarchical modeling of expected enzymic latch hypothesis and PFG effects on seasonal EEAs

We found a significant correlation between WT and water content (WC) (Pearson's R = 0.87, P-value <0.001), and selected WC as our predictor variable for all subsequent models as it is directly measured from our bulk peat samples. In Level 1 of our hierarchical model, within the context of the enzymic latch hypothesis, we found that WC had a significant effect on oxidation-reduction potential (ORP) (P-value <0.001; Table 3) with differences in WT manipulations playing an obvious role in regulating ORP values (Fig. 3). However, while WC influenced ORP in Level 1 of our hierarchical model, Ericaceae (Er) biomass also indicated a potential PFG influence on ORP (P-value =0.040; Table 3).

In Level 2 of the enzymic latch hypothesis, we found that ORP significantly influenced total phenolics (P-value =0.008; Table 3). However, we also found that the alternative predictors of temperature and DOC significantly influenced phenolics as well (P-value =0.006, <0.001, respectively; Table 3). No PFG influences on total phenolics were observed.

In Level 3 of the enzymic latch hypothesis, we observed mixed results of phenolic influence on oxidative EEA within the bulk peat and porewater phases, where bulk peat phase POX activity was significantly negatively correlated with total phenolics (P-value =0.015; Table 3; Fig. 4) while porewater phase PER activity was significantly positively correlated (P-value <0.001; Table 3; Fig. 4). Alternatively, porewater phase POX activity and bulk peat phase PER activity were significantly influenced by ORP (P-value <0.001, 0.002, respectively; Table 3). PFG effects were also observed where Ericaceae (Er) biomass was significantly positively correlated with porewater phase POX and PER activity (P-value =0.035, 0.019, respectively; Table 3) and negatively correlated with bulk peat phase PER activity (P-value =0.010; Table 3). The only significant influence of sedge (Sed) biomass was on bulk peat phase POX activity (P-value =0.016) where a positive increase in Sed biomass correlated with a positive increase in POX activity. Furthermore, there were numerous alternative influences where temperature significantly negatively correlated with porewater phase POX and PER activity (P-value <0.001 for both; Table 3) and positively correlated with bulk peat phase PER activity (P-value =0.033; Table 3). DOC positively correlated with both phases of POX activity (P-value

Table 3 Generalized linear mixed effects (GLM) model (type II Wald test with restricted maximum likelihood) for hierarchical model analysis. GLM model factors accounted for a single degree of freedom [a]

Level 1	ORP Slope (*P*-value)
Enzymic Latch Hypothesis	
WC	**-17.109 (<0.001)**
PFG Effect	
Er Biomass [b]	**-0.413 (0.040)**
Sed Biomass [b]	-0.175 (0.724)
Alternatives	
Temp	-6.747 (0.294)

Level 2	Phenolics Slope (*P*-value)
Enzymic Latch Hypothesis	
ORP	**-0.014 (0.008)**
PFG Effect	
Er Biomass [b]	-0.010 (0.249)
Sed Biomass [b]	0.009 (0.667)
Alternatives	
Temp	**-0.764 (0.006)**
DOC	**0.204 (<0.001)**

Level 3	POX bulk peat Slope (*P*-value)	POX porewater Slope (*P*-value)	PER bulk peat Slope (*P*-value)	PER porewater Slope (*P*-value)
Enzymic Latch Hypothesis				
Phenolics	**-0.212 (0.015)**	-6.3e-5 (0.138)	0.229 (0.354)	**3.2e-4 (<0.001)**
ORP	-0.002 (0.517)	**7.2e-6 (<0.001)**	**-0.024 (0.022)**	-3.6e-6 (0.278)
PFG Effect				
Er Biomass [b]	0.009 (0.130)	**7.8e-6 (0.035)**	**-0.063 (0.010)**	**1.3e-5 (0.019)**
Sed Biomass [b]	**0.036 (0.016)**	1.7e-5 (0.058)	-0.088 (0.141)	1.8e-5 (0.195)
Alternatives				
Temp	-0.383 (0.061)	**-5.2e-4 (<0.001)**	**1.212 (0.033)**	**-1.3e-3 (<0.001)**
DOC	**0.049 (0.032)**	**2.8e-5 (0.021)**	-0.141 (0.061)	7.7e-6 (0.707)

Level 4 - Bulk Peat	BG bulk peat Slope (*P*-value)	CBH bulk peat Slope (*P*-value)	NAG bulk peat Slope (*P*-value)	AP bulk peat Slope (*P*-value)
Enzymic Latch Hypothesis				
Phenolics	-13.511 (0.847)	-14.339 (0.700)	9.159 (0.774)	-162.220 (0.341)
PFG Effect				
Er Biomass [b]	**-12.488 (0.017)**	-3.337 (0.257)	-2.773 (0.358)	-17.610 (0.160)
Sed Biomass [b]	**-25.976 (0.045)**	-12.118 (0.097)	-9.295 (0.214)	-47.630 (0.124)
Alternatives				
Temp	**920.381 (<0.001)**	**303.644 (<0.001)**	**369.448 (<0.001)**	**2242.22 (<0.001)**
DOC	2.434 (0.896)	-4.430 (0.664)	-3.247 (0.733)	22.750 (0.614)

Level 4 - Porewater	BG porewater Slope (*P*-value)	CBH porewater Slope (*P*-value)	NAG porewater Slope (*P*-value)	AP porewater Slope (*P*-value)
Enzymic Latch Hypothesis				
Phenolics	-0.008 (0.474)	-1.1e-3 (0.317)	-5.9e-3 (0.193)	-0.060 (0.296)
PFG Effect				
Er Biomass [b]	-4.9e-4 (0.644)	6.3e-5 (0.503)	2.0e-5 (0.967)	0.001 (0.868)
Sed Biomass [b]	4.2e-4 (0.872)	1.0e-4 (0.658)	2.1e-4 (0.862)	0.023 (0.151)
Alternatives				
Temp	**0.149 (<0.001)**	**1.3e-2 (<0.001)**	**7.4e-2 (<0.001)**	**0.954 (<0.001)**
DOC	**0.008 (0.020)**	**1.3e-3 (<0.001)**	6.8e-4 (0.632)	**0.043 (0.020)**

PFG = plant functional group; Temp = temperature; WC = water content; Er = Ericaceae; Sed = sedge; ORP = oxidation reduction potential; DOC = dissolved organic carbon

[a] All factors measured from each bin monthly for July, August, and September ($N = 72$)

[b] Biomass represents 2012 biomass production ($n = 24$) repeated for July, August, and September ($N = 72$)

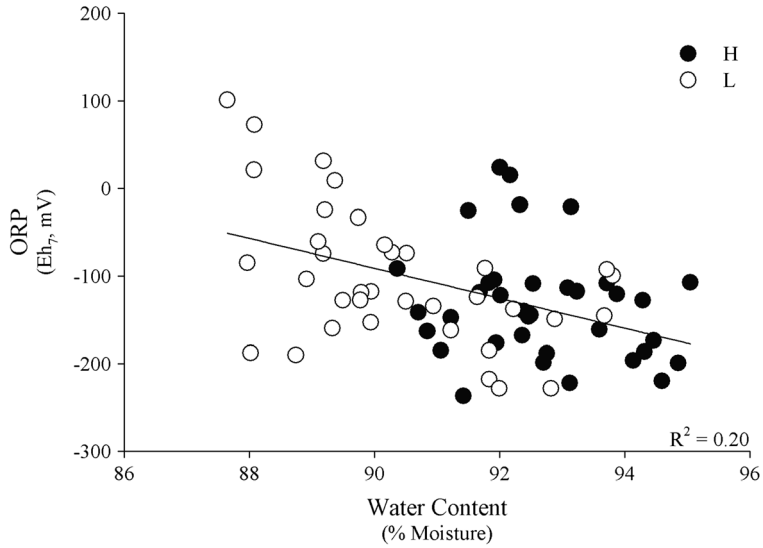

Fig. 3 Bulk peat water content regression analysis on oxidation-reduction potential (ORP) under high (H) and low (L) water table conditions for the months of July, August, and September 2012 (n = 24/month; N = 72/season)

=0.032, 0.021, respectively; Table 3) but had no significant influence on PER activity.

In Level 4 of the enzymic latch hypothesis, total phenolics had no significant influence on the activity of any hydrolytic extracellular enzyme assayed in either the bulk peat or porewater phases (P-value >0.05 for all; Table 3). The only observed PFG influences were within the bulk peat phase of BG activity where both Er and Sed biomass significantly negatively correlated with measured BG EEA (P-value =0.017, 0.045, respectively; Table 3). Temperature was significantly positively correlated with all hydrolytic enzymes in both phases (P-value <0.05 for all; Table 3) and DOC significantly positively correlated with all porewater phase hydrolytic EEAs (P-value <0.05 for all; Table 3) with the exception of NAG EEA (P-value =0.632; Table 3).

Discussion

Water table effects

The enzymic latch hypothesis posits that oxygen availability regulates the activity of extracellular phenol oxidase, which in turn regulates the activity of hydrolytic enzymes by modifying the accumulation of polyphenols in peat soils (Freeman et al. 2001). Our results provide support for some aspects of this hypothesis. For example, redox potential declined with increasing water table and water content, and phenol oxidase lab potential activity increased in porewater, but not bulk peat, as a function of redox potential. Also, total phenolics were negatively related to phenol oxidase activity in bulk peat. We would similarly expect that the in situ activity of phenol oxidase would be higher in peat with higher redox potential because of the requirement of the enzyme for oxygen. Thus in situ phenol oxidase activity should be higher for sites with higher redox potential. However, we found no support for the final prediction of the enzymic latch: there was no indication that total phenolics or water table were related to activity of any hydrolytic enzymes associated with depolymerization of organic carbon (β-glucosidase, cellobiohydrolase, or *N*-acetyl-glucosiminidase).

Previous studies of peatland water table effects on EEAs have found contradictory results. Sun et al. (2010) found phenol oxidase and β-glucosidase were more active under high vs. low water table conditions, whereas Fenner et al. (2005) found suppressed phenol oxidase activity under high water table conditions and later found a significant increase in phenol oxidase activity under severe drought conditions (Fenner and Freeman 2011; Fenner et al. 2011). A possible explanation for these differences under water table manipulations might be differences in the duration of dry and wet conditions (several weeks to 1 year or more), where short-term waterlogged conditions could cause priming effects after long-term drought (Williams et al. 2000; Xiang and Freeman 2009; Sun et al. 2010). In the present study, water table levels were only below our sampling depth for the August to October sampling dates, so any systemic inertia in EEAs might have masked the community response.

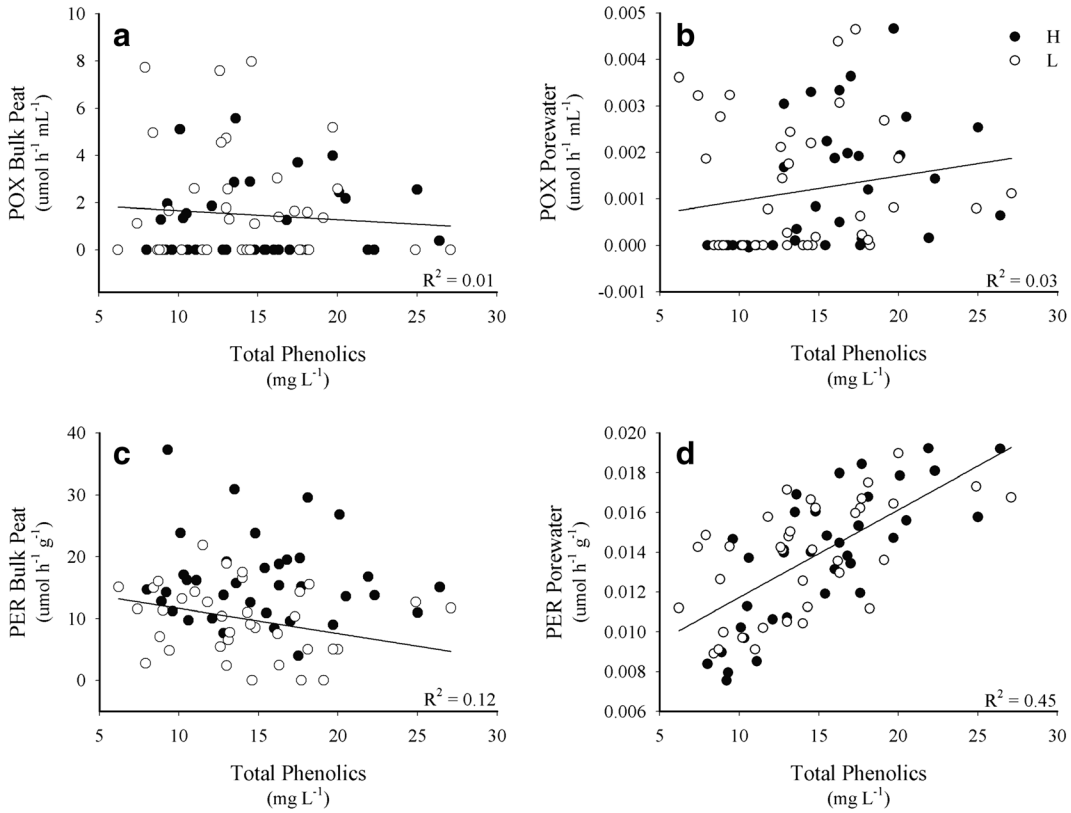

Fig. 4 Total phenolics regression analyses on phenol oxidase (**a, b**) and peroxidase (**c, d**) in the bulk peat (**a, c**) and porewater (**b, d**) phases under high (**H**) and low (**L**) water table conditions for the months of July, August, and September 2012 (n = 24/month; N = 72/season)

In contrast with phenol oxidase, peroxidase did respond to water table treatments. However, the sign of the response was the opposite of that hypothesized. The greater mid-summer decline in peroxidase activity in the low water table treatment is surprising because peroxidase activity was expected to be greater under more oxidizing conditions. Under both water table conditions, peroxidase activity was highest in June when water tables were highest and declined thereafter. Extracellular peroxidase activity is most often attributed to Basidiomycete and Ascomycete fungi (Higuchi 1990; Rabinovich et al. 2004; Sinsabaugh 2005, 2010) and extracellular lignin- and Mn-peroxidases are believed to play an important role in degradation of lignin and perhaps phenolics (Sinsabaugh 2010). Peroxidase activity in microorganisms is induced by a variety of factors, including oxidative stress (Brown et al. 2007) and presence of phenolic compounds (Sinsabaugh 2010). The observed mid-season decline in peroxidase activity under more oxygen-rich low water table conditions (see Fig. 2) would seem to indicate that oxidative stress is not causal. Soils were flooded under high water table treatment (see Fig. 1), which rapidly drives the disappearance of free oxygen. However, somewhat counter intuitively, hypoxia commonly leads to oxidative stress via the production of reactive oxygen species (Bai et al. 2010), so it is possible that in the present study the flooded condition leads to oxidative stress, keeping peroxidase activity high, and that when water tables drop the oxidative stress is alleviated and hence peroxidase activity declines in the low water table treatments. It is also possible that observed peroxidase activity was associated not only with microorganisms, but also with vascular plant roots present in our samples. The extremely fine roots of Ericaceae and sedges would be susceptible to lysis during soil prep for extracellular enzyme assays and so could contribute to the total extracellular pool of enzymes. Root-associated peroxidase activity can increase in hypoxic flooded roots (e.g., Lee and Lin 1995) especially in the early stages of hypoxia (Bai et al. 2010).

Of the hydrolytic enzymes assayed, only acid-phosphatase activity in porewater showed significantly greater activity under low water table conditions. These results correspond with previous findings that show phosphatase activity varies seasonally and is greatest when the peat is not waterlogged (Kang and Freeman 1999). However, the differences in acid-phosphatase response to water table treatments between the bulk peat and porewater suggest a divergence in root or microbial enzymes to treatments. Interestingly, within the Ericaceae treatment, acid-phosphatase EEA in the porewater phase was lowest for most dates, whereas in the bulk peat phase, acid-phosphatase activity in the Ericaceae treatment was substantially higher for most dates, although not significantly so. This might point to a plant community effect on the location of EEAs.

The fact that none of the carbohydrate-depolymerizing enzymes responded significantly suggests a limited cellulolytic microbial response to water table drawdown in the present study. However, this is inconsistent with our results from cellulose decomposition assays (Potvin et al. *unpublished results*), which clearly show that cellulose decomposition is greatly accelerated in the zone just above the water table. It may be that the pool of available cellulose is limited in the peat because of protection by the recalcitrant *Sphagnum* tissues (such as the hummock forming Sphagna; Turestsky et al. 2008), hence there is limited induction of cellulolytic enzymes.

Plant functional group effects

When plant functional groups were treated as continuous variables they appeared to differ in their impact on enzyme activity. The positive relationship of Ericaceae biomass with phenol oxidase in porewater is consistent with the known enzymic potential of Ericaceae, and is consistent with enzyme use to mobilize phenolic-bound nitrogen in proteins (Read et al. 2004). The negative relationship of Ericaceae biomass with peroxidase activity in bulk peat is consistent with the Gadgil effect (Gadgil and Gadgil 1971), because there is no clear evidence that ericoid mycorrhizas use peroxidases to degrade lignocellulosic material (Cairney and Burke 1998) whereas white rot fungi do. Therefore, suppression of free-living white rot fungi by Ericaceae would be expected to lead to a decline in peroxidase activity. Consistent with this, sedge biomass did not affect peroxidase activity. The decline in β-glucosidase activity as a function of Ericaceae biomass is also consistent with the Gadgil effect, because this class of enzymes is broadly used by heterotrophs to mobilize sugars from polymeric glucose chains, so could be indicative of a broad suppression of microbial activity. Ericaceae abundance was also negatively associated with redox potential, perhaps as a consequence of consumption of oxygen by non-aerenchymous Ericaceae roots. This should constrain the in situ activity of phenol oxidases.

In contrast with Ericaceae, sedge abundance had been expected to prime microbial activity in the peat because they are non-mycorrhizal and transport oxygen into the peat, both of which should favor rhizosphere microbial communities. Consistent with this, sedge abundance was positively associated with phenol oxidase activity in bulk peat. However, sedge abundance was also associated with a decline in β-glucosidase activity, which is more consistent with a suppressive effect on microbial activity rather than a priming effect. This is in contrast with previously observed increases in decomposition of soil organic matter in the presence of aerenchymous graminoids (e.g., Wolf et al. 2007) and requires further investigation to understand causal linkages.

Seasonal trends

We observed consistent strong seasonal patterns of potential EEAs that could not be accounted for as a direct result of our treatments. The significant seasonal shift in potential EEAs observed in our study reflects similar results found in previous studies across multiple ecosystem types (e.g., Bonnett et al. 2006; Wallenstein et al. 2009; Bell et al. 2010; Weedon et al. 2014; Hargreaves and Hofmockel 2014). Explanations for these observed seasonal effects, characterized by early season spikes in EEAs, relate to the rapid turnover of microbial biomass during spring-thaw (Schmidt et al. 2007) which causes feedbacks to carbon availability, mobilization, and/or uptake in step with temporal dynamics of plant nutrient uptake and rhizodeposition (Jaeger et al. 1999). Consistent with the latter model, adjusting our measured EEAs for natural shifts in in situ soil temperature alone did not eliminate our observed changes in seasonal EEAs. This is not surprising, as the highest laboratory rates for most enzymes occurred in July, the month with the highest peat temperature. Therefore, any $Q_{10} > 1$– i.e., any increase in EEA with temperature– will only increase the July peak. Weedon et al. (2014) proposed that soil temperatures act on extracellular enzyme pools indirectly by driving the seasonality of

carbon and nutrient availability. Such findings relate well to previous studies that have failed to find a direct relationship between temperature and potential EEAs, when Q_{10} is >1 (Bell et al. 2010; Henry 2012). Root priming of microbial activity (Kuzyakov 2010) is one possible mechanism driving increased microbial activity during July, however if that were the case we might expect positive effects of sedge or Ericaceae abundance on enzyme activities, yet our analysis indicated a negative relationship between sedge and Ericaceae abundance and β-glucosidase activity. Further mechanistic studies may be necessary to elucidate the direct causes of observed seasonal shifts in potential EEAs.

Implications of phase activity correlations for future extracellular enzyme assay methods

Results from this experiment indicate that the relative rates of hydrolytic EEAs in the porewater phase were two orders of magnitude lower than bulk peat activity, yet the porewater phase tracked most bulk peat phase potential hydrolytic EEAs well across dates. We contend that significant correlations of most hydrolytic EEAs between the bulk peat and porewater phases suggest that organisms in both phases respond similarly to seasonal variation in resources and conditions. In contrast, the lack of significant positive correlations for potential oxidative EEAs between the bulk peat and porewater phases suggests differences exist between these phases for substrate pools, microbial composition, oxygen availability, and myriad other influences. Phenol oxidase and peroxidase enzymes are two important classes of enzymes capable of degrading recalcitrant phenolic substrates such as lignin (McLatchey and Reddy 1998) and the majority of oxidative EEAs are attributable to lignolytic fungi (Criquet et al. 2000; Thormann et al. 2002; Baldrian 2006). Differences in microbial communities between phases could contribute to changes in overall potential extracellular enzyme production because organic soils are more likely to be dominated by filamentous fungi (Latter et al. 1967; Williams and Crawford 1983; Thormann 2006), while bacteria and microfungi likely dominate the porewater phase because the fine Nitex netting on the piezometers used to collect porewater samples should exclude most filamentous fungi and peat particles. These differences in microbial composition between the two phases may differentially influence phenol oxidase and peroxidase activity, as fungi in the peat have a greater capacity to produce oxidative enzymes that break down lignin and lignin-like substances (Bending and Read 1997), which could explain the lack of correlation of potential oxidative EEAs between phases. Consistent with this, the one hydrolytic extracellular enzyme assay with no significant correlation between phases over the measured growing season was cellobiohydrolase, which is likely to be more strongly expressed in fungi involved in depolymerization of complex plant cell wall material. Future studies must be implemented to determine if these potential functional differences are due to compositional differences in the microbial communities between bulk peat and porewater phases as suggested.

Conclusions

Our examination after two years of climate change treatments found partial support for the enzymic latch hypothesis, with links between peat water content and redox potential as well as phenolic concentrations with phenol oxidase activity. However, we found a notable absence of any significant effect of total phenolics on carbohydrate depolymerizing hydrolytic enzymes. The significant decrease in peroxidase activity under low water table conditions suggests that flooding stress rather than O_2 availability might drive peroxidase activity in these ecosystems. The only extracellular enzyme assay that showed a significant positive effect of lowered water table was the nutrient mobilizing extracellular enzyme acid-phosphatase. There is also some support for plant functional group effects on oxidative and hydrolytic enzymes, generally consistent with expectations of suppressive effects by Ericaceae, but showing little evidence of expected priming effects by sedges.

The strong seasonal pattern, with a peak in hydrolytic EEAs early in the growing season, is not consistent with a simple temperature driven model of potential EEA, but rather is more supportive of activity driven by biotic demand for resources during the period of rapid plant growth. The divergence of bulk peat and porewater EEA for oxidative enzymes and cellobiohydrolase, and agreement for other enzymes suggest functional diversity in these two phases may be linked to differences in utilization of recalcitrant substrates by microbial communities and requires further exploration.

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References

Artz RRE, Anderson IC, Chapman SJ, Hagn A, Schloter M, Potts JM, Campbell CD (2007) Changes in fungal community composition in response to vegetational succession during the natural regeneration of cutover peatlands. Microb Ecol 54:508–522

Bai T, Li C, Ma F, Feng F, Shu H (2010) Responses of growth and antioxidant system to root-zone hypoxia stress in two *malus* species. Plant Soil 327:95–105

Baldrian P (2006) Fungal laccases – occurrence and properties. FEMS Microbiol Rev 30:215–242

Bell TH, Klironomos JN, Henry HAL (2010) Seasonal responses of extracellular enzyme activities and microbial biomass to warming and nitrogen addition. Soil Sci Soc Am J 74:820–828

Belyea LR (1996) Separating the effects of litter quality and microenvironment on decomposition rates in a patterned peatland. Oikos 77:529–539

Bending GD, Read DJ (1997) Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycol Res 101(11):1348–1354

Bier AW (2009) Introduction to oxidation reduction potential measurement. Hach Company, Lit No. 2072

Blodau C, Basiliko N, Moore TR (2004) Carbon turnover in peatland mesocosms exposed to different water table levels. Biogeochemistry 67:331–351

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nat Commun 1(48):1–11

Bonnett SAF, Ostle N, Freeman C (2006) Seasonal variations in decomposition processes in a valley-bottom riparian peatland. Sci Total Environ 370:561–573

Breeuwer A, Robroek BJM, Limpens J, Heijmans MMPD, Schouten MGC, Berendse F (2009) Decreased summer water table depth affects peatland vegetation. Basic Appl Ecol 10: 330–339

Brix H (1997) Do macrophytes play a role in constructed treatment wetlands? Water Sci Technol 35(5):11–17

Brown SM, Campbell LT, Lodge JK (2007) *Cryptococcus neoformans*, a fungus under stress. Curr Opin Microbiol 10 (4):320–325

Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A (2013) Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol Biochem 58:216–234

Cairney JWG, Burke RM (1998) Do ecto- and ericoid mycorrhizal fungi produce peroxidase activity? Mycorrhiza 8:61–65

Choi JH, Kang H, Park SS (2009) Comparison of enzyme activities in vegetated and nonvegetated sediments. J Environ Eng 135:299–305

Churchill AC, Turetsky MR, McGuire AD, Hollingsworth TN (2015) Response of plant community structure and primary productivity to experimental drought and flooding in an Alaskan fen. Can J For Res 45:185–193

Criquet S, Farnet AM, Tagger S, Le Petit J (2000) Annual variations of phenoloxidase activities in an evergreen oak litter: influence of certain biotic and abiotic factors. Soil Biol Biochem 32:1505–1513

Deppe M, Knorr KH, McKnight DM, Blodau C (2010) Effects of short-term drying and irrigation on CO_2 and CH_4 production and emission from mesocosms of a northern bog and an alpine fen. Biogeochemistry 100:89–103

Fenner N, Freeman C (2011) Drought-induced carbon loss in peatlands. Nat Geosci 4:895–900

Fenner N, Freeman C, Reynolds B (2005) Hydrological effects on the diversity of phenolic degrading bacteria in a peatland: implications for carbon cycling. Soil Biol Biochem 37:1277–1287

Fenner N, Williams R, Toberman H, Hughes S, Reynolds B, Freeman C (2011) Decomposition 'hotspots' in a rewetted peatland: implications for water quality and carbon cycling. Hydrobiologia 674:51–66

Finzi AC, Sinsabaugh RL, Long TM, Osgood MP (2006) Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. Ecosystems 9: 215–226

Flessa H, Fischer WR (1992) Plant-induced changes in redox potentials of rice rhizospheres. Plant Soil 143:55–60

Freeman C, Liska G, Ostle NJ, Lock MA, Reynolds B, Hudson J (1996) Microbial activity and enzymatic decomposition processes following peatland water table drawdown. Plant Soil 180:121–127

Freeman C, Liska G, Ostle NJ, Lock MA, Hughes S, Reynolds B, Hudson J (1997) Enzymes and biogeochemical cycling in wetlands during a simulated drought. Biogeochemistry 39: 177–187

Freeman C, Ostle N, Kang H (2001) An enzymic 'latch' on a global carbon store. Nature 409:149

Freeman C, Ostle NJ, Fenner N, Kang H (2004) A regulatory role for phenol oxidase during decomposition in peatlands. Soil Biol Biochem 36:1663–1667

Gadgil RL, Gadgil PD (1971) Mycorrhiza and litter decomposition. Nature 233:133

Gorham E (1991) Northern peatlands – role in the carbon-cycle and probable responses to climatic warming. Ecol Appl 1: 182–195

Groisman PY, Knight RW, Easterling DR, Karl TR, Hegerl GC, Razuvaev VN (2005) Trends in intense precipitation in the climate record. J Clim 18:1326–1350

Hargreaves SK, Hofmockel KS (2014) Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochemistry 117:67–79

Henry HAL (2012) Soil extracellular enzyme dynamics in a changing climate. Soil Biol Biochem 47:53–59

Higuchi T (1990) Lignin biochemistry: biosynthesis and biodegradation. Wood Sci Technol 24:23–63

Hilbert DW, Roulet N, Moore T (2000) Modelling and analysis of peatlands as dynamical systems. J Ecol 88:230–242

Holzapfel-Pschorn A, Conrad R, Seiler W (1986) Effects of vegetation on the emission of methane from submerged paddy soil. Plant Soil 92:223–233

Inderjit MAU (2002) Can *kalmia angustifolia* interference to black spruce (*picea Mariana*) be explained by allelopathy? For Ecol Manag 160:75–84

Jaeger CH, Monson RK, Fisk MC, Schmidt SK (1999) Seasonal partitioning of nitrogen by plants and soil microorganisms in an alpine ecosystem. Ecology 80:1883–1891

Jassey VEJ, Chiapusio G, Gilbert D, Buttler A, Toussaint ML, Binet P (2011) Experimental climate effect on seasonal variability of polyphenol/phenoloxidase interplay along a narrow fen-bog ecological gradient in sphagnum fallax. Glob Chang Biol 17:2945–2957

Jassey VEJ, Chiapusio G, Gilbert D, Toussaint ML, Binet P (2012) Phenoloxidase and peroxidase activities in sphagnum-dominated peatland in a warming climate. Soil Biol Biochem 46:49–52

Joanisse GD, Bradley RL, Preston CM, Munson AD (2007) Soil enzyme inhibition by condensed litter tannins may drive ecosystem structure and processes: the case of kalmia angustifolia. New Phytol 175:535–546

Kalbitz K, Schmerwitz J, Schwesig D, Matzner E (2003) Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma 113:273–291

Kane ES, Mazzoleni LR, Kratz CJ, Hribljan JA, Johnson CP, Pypker TG, Chimner R (2014) Peat porewater dissolved organic carbon concentration and lability increase with warming: a field temperature manipulation experiment in a poor-fen. Biogeochemistry 119:161–178

Kang H, Freeman C (1999) Phosphatase and arylsulphatase activities in wetland soils: annual variation and controlling factors. Soil Biol Biochem 31:449–454

Kang H, Kim SY, Fenner N, Freeman C (2005a) Shifts in soil enzyme activities in wetlands exposed to elevated CO_2. Sci Total Environ 337:207–212

Kang H, Freeman C, Park SS, Chun J (2005b) N-acetylglucosaminidase activities in wetlands: a global survey. Hydrobiologia 532:103–110

Knorr KH, Blodau C (2009) Impact of experiemental drought and rewetting on redox transformations and methanogenesis in mesocosms of a northern fen soil. Soil Biol Biochem 41: 1187–1198

Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. Soil Biol Biochem 42:1363–1371

Lafleur PM, Moore TR, Roulet NT, Frolking S (2005) Ecosystem respiration in a cool temperate bog depends on peat temperature but not water table. Ecosystems 8:619–629

Latter PM, Cragg JB, Heal OW (1967) Comparative studies on the microbiology of four moorland soils in the northern pennines. J Ecol 55:445–464

Lee TM, Lin YH (1995) Changes in soluble and cell wall-bound peroxidase activities with growth in anoxia-treated rice (*Oryza sativa* L.) coleoptiles and roots. Plant Sci 106(1):1–7

Lipson DA, Wilson RF, Oechel WC (2005) Effects of elevated atmospheric CO_2 on soil microbial biomass, activity, and diversity in a chaparral ecosystem. Appl Environ Microbiol 71(12):8573–8580

McLatchey GP, Reddy KR (1998) Regulation of organic matter decomposition and nutrient release in a wetland soil. J Environ Qual 27:1268–1274

Megonigal JP, Rabenhorst M (2013) Reduction-oxidation potential and oxygen. In: DeLaune RD, Reddy KR, Richardson CJ, Megonigal JP (eds) Methods in biogeochemistry of wetlands, SSSA book series 10. Madison, WI, pp. 71–85

Munster U (1991) Extracellular enzyme activity in eutrophic and polyhumic lakes. In: Chrost RJ (ed) Microbial enzymes in aquatic environments. Springer, New York, pp. 96–122

Munster U (1993) Concentrations and fluxes of organic substrates in the aquatic environment. Antonie Van Leeuwenhoek 63: 243–274

Peacock M, Jones TG, Airey B, Johncock A, Evans CD, Lebron I, Fenner N, Freeman C (2015) The effect of peatland drainage and rewetting (ditch blocking) on extracellular enzyme activities and water chemistry. Soil Use Manag 31:67–76

Potvin LR, Kane ES, Chimner RA, Kolka RK, Lilleskov EA (2015) Effects of water table position and plant functional group on plant community, aboveground production, and peat properties in a peatland mesocosm experiment (PEATcosm). Plant Soil 387:277–294

Rabinovich ML, Bolobova AV, Vasilchenko LG (2004) Fungal decomposition of natural aromatic structures and xenobiotics: a review. Appl Biochem Microbiol 40:1–17

Read DJ (1991) Mycorrhizas in ecosystems. Experimentia 47: 376–391

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? New Phytol 157:475–492

Read DJ, Leake JR, Perez-Moreno J (2004) Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Can J Bot 82:1243–1263

Roulet N, Moore T, Bubier J, Lafleur P (1992) Northern fens: methane flux and climatic change. Tellus 44B:100–105

Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biol Biochem 34: 1309–1315

Schmidt SK, Costello EK, Nemergut DR, Cleveland CC, Reed SC, Weintraub MN, Meyer AF, Martin AM (2007) Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. Ecology 88:1379–1385

Sebestyen S, Dorrance C, Olson DM, Verry ES, Kolka RK, Elling AE, Kyllander R (2011) Long-term monitoring sites and trends at the marcell experimental forest. In: Kolka RK, Sebestyen SD, Verry ES, Brooks KN (eds) Peatland biogeochemistry and watershed hydrology at the marcell experimental forest, 1st edn. CRC Press, Boca Raton, FL, pp. 15–72

Shackle VJ, Freeman C, Reynolds B (2000) Carbon supply and the regulation of enzyme activity in constructed wetlands. Soil Biol Biochem 32:1935–1940

Sinsabaugh RL (2005) Fungal enzymes at the community scale. In: Dighton J, White Jr JF, White J, Oudermans P (eds) The fungal community: its organization and role in the ecosystem, 3rd edn. CRC Press, Boca Raton, FL, pp. 349–360

Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol Biochem 42:391–404

Sinsabaugh RL, Lauber CL, Weintraub MN, et al. (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11:1252–1264

Strack M, Waller MF, Waddington JM (2006) Sedge succession and peatland methane dynamics: a potential feedback to climate change. Ecosystems 9:278–287

Sun X, Xiang W, He L, Zhao Y (2010) Impacts of hydrological conditions on enzyme activities and phenolic concentrations in peatland soil: an experimental simulation. Front Earth Sci China 4(4):463–470

Thormann MN (2006) The role of fungi in boreal peatlands. In: Wieder RK, Vitt DH (eds) Boreal peatland ecosystems. Springer-Verlag, Berlin, pp. 101–123

Thormann MN, Currah RS, Bayley SE (2002) The relative ability of fungi from *sphagnum fuscum* to decompose selected carbon substrates. Can J Microbiol 48:204–211

Toberman H, Freeman C, Evans C, Fenner N, Artz RRE (2008) Summer drought decreases soil fungal diversity and associated phenol oxidase activity in upland calluna heathland soil. FEMS Microbiol Ecol 66:426–436

Trettin CC, Laiho R, Minkkinen K, Laine J (2006) Influence of climate change factors on carbon dynamics in northern forested peatlands. Can J Soil Sci 86:269–280

Turestsky MR, Crow SE, Evans RJ, Vitt DH, Wieder RK (2008) Trade-offs in resource allocation among moss species control decomposition in boreal peatlands. J Ecol 96:1297–1305

Turunen J, Tomppo E, Tolonen K, Reinikainen A (2002) Estimating carbon accumulation rates of undrained mires in Finland – application to boreal and subarctic regions. The Holocene 12:69–80

Vasander H, Kettunen A (2006) Carbon in boreal peatlands. In: Wieder RK, Vitt DH (eds) Boreal peatland ecosystems. Springer-Verlag, Berlin, pp. 165–194

Verhoeven JTA, Toth E (1995) Decomposition of carex and sphagnum litter in fens: effect of litter quality and inhibition by living tissue-homogenates. Soil Biol Biochem 27:271–275

Wallenstein M, McMahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Glob Chang Biol 15:1631–1639

Weedon JT, Aerts R, Kowalchuk GA, van Bodegom PM (2014) No effects of experimental warming but contrasting seasonal patterns for soil peptidase and glycosidase enzymes in a sub-Arctic peat bog. Biogeochemistry 117:55–66

Weltzin JF, Pastor J, Harth C, Bridgham SD, Updegraff K, Chapin CT (2000) Response of bog and fen plant communities to warming and water-table manipulations. Ecology 81(12): 3464–3478

White JR, Shannon RD, Weltzin JF, Pastor J, Bridgham SD (2008) Effects of soil warming and drying on methane cycling in a northern peatland mesocosm study. J Geophys Res 113: G0A06

Whiting GJ, Chanton JP (1993) Primary production control of methane emissions from wetlands. Nature 364:794–795

Williams RT, Crawford RL (1983) Microbial diversity of Minnesota peatlands. Microb Ecol 9:201–214

Williams CJ, Shingara EA, Yavitt JB (2000) Phenol oxidase activity in peatlands in New York state: response to summer drought and peat type. Wetlands 20(2):416–421

Wolf AA, Drake BG, Erickson JE, Megonigal JP (2007) An oxygen-mediated positive feedback between elevated carbon dioxide and soil organic matter decomposition in a simulated anaerobic wetland. Glob Chang Biol 13 (9):2036–2044

Xiang W, Freeman C (2009) Annual variation of temperature sensitivity of soil organic carbon decomposition in north peatlands: implications for thermal responses of carbon cycling to global warming. Environ Geol 58(3): 499–508

Yavitt JB, Williams CJ, Wieder RK (2005) Soil chemistry versus environmental controls on production of CH_4 and CO_2 in northern peatlands. Eur J Soil Sci 56:169–178

Yu Z, Loisel J, Brosseau DP, Beilman DW, Hunt SJ (2010) Global peatland dynamics since the last glacial maximum. Geophys Res Lett 37:L13402. doi:10.1029/2010GL043584