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Potential Vulnerability of Deep Carbon Deposits of Forested Swamps to Drought

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Climate warming is resulting in increases in the frequency and intensity of summer droughts in the Great Lakes–St. Lawrence forest region (Ontario, Canada), raising concerns for the fate of C stores. We hypothesized that deeper peat historically existing beneath the water table would produce significant CO_2 efflux during summer droughts. To test this hypothesis, we collected saturated peat cores, partitioned them into depth intervals, incubated the peat under conditions that resulted in peat drying, and monitored daily CO_2 production together with potential drivers of CO_2 production, including peat quality, microbial biomass, and microbial extracellular enzyme activity. Peat CO_2 production (μmol CO_2 min^{-1} g^{-1} dry soil) was highest in the top 30 cm of the peat profile, with the highest production at intermediate volumetric water content (VWC). Peat substrates fuelling CO_2 production had quotients of C to N of <20 and were characterized by more labile forms of C. Microbial biomass C (mg C g^{-1} dry soil) and most microbial extracellular enzymes (nmol g^{-1} h^{-1}) were also highest in the top 30 cm of the peat profile. Activities of microbial extracellular enzymes shifted in their contribution to CO_2 production as the peat dried, with hydrolases positively related to CO_2 under dry conditions (5–35%) and negatively under wet conditions (65 and 85%), with phenol oxidase showing the opposite pattern. Currently, the relatively poor quality (i.e., high C/N) of peat in catotelm limits rapid release of CO_2 with water table declines. However, this substantial C store may be vulnerable to decomposition if constraints on quality are alleviated.

Abbreviations: FTIR, Fourier transform infrared; TLW, Turkey Lakes Watershed; VWC, volumetric water content.

A key uncertainty in C budgets is the degree to which organic forest soils act as a C sink and whether they will continue to be sinks in a warmer climate (Laiho, 2006). Peat forms in wetlands because saturated conditions result in low rates of primary production but even lower rates of decomposition (Päivänen and Vasander, 1994; Moore and Basiliko, 2006). In a warmer climate, higher rates of evapotranspiration may lead to declines in the water table, exposing large volumes of previously saturated peat to aerobic decomposition (Laiho, 2006). While temperature plays an important role in determining peat decomposition rates (Makiranta et al., 2009), constraints induced by microbial nutrient limitation, community composition, and/or enzyme activities may be critical to understanding short- and long-term responses of wetlands to prolonged lowering of the water table (Freeman et al., 1997; Strakova et al., 2011).

Dead plant remains, such as dead moss, leaves, branches, and roots at various stages of decomposition as well as microbial biomass, make up the bulk of the peat. The peat profile is separated into an upper acrotelm layer, a lower catotelm layer, and a middle mesotelm layer (Clymo, 1984; Brown, 1998; Clymo and Bryant,

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2008). The acrotelm is the top portion of the peat where water table fluctuations occur frequently. The catotelm is within the permanently saturated zone beneath the water table. Between these two layers is the mesotelm, an often anoxic layer that is periodically oxic in response to fluctuating water levels. This temporal layering of material creates differences in the peat quality along the profile, with the upper layer containing fresh, labile materials and the lower layer containing decomposition products and recalcitrant materials largely resistant to decay (e.g., lignin, waxes) (Leifeld et al., 2012).

The physical and chemical environment along the peat profile influences microbial dynamics. The acrotelm has fluctuating water tables that create conditions that are variably oxic and anoxic (although not necessarily strongly reducing conditions) (Clymo, 1984; Laiho, 2006). Aerobic metabolism in the acrotelm is predominantly by heterotrophs that use O as an electron acceptor and organic matter as a source of energy and a building block, resulting in efficient breakdown of organic matter (Mitsch and Gosselink, 1986). The catotelm beneath the water table creates an anoxic and reducing environment for microbes (Holden, 2005). Anaerobic metabolism in the catotelm requires electron acceptors other than O, with the acceptor changing as the environment becomes more reducing. Both organic matter and inorganic substrates can be used as energy sources for this type of metabolism; thus, decomposition is less efficient and intermediate metabolites are created (Charman et al., 2007). Along the entire peat profile, extracellular enzymes produced by microbes aid in breaking complex molecules into simpler molecules and are specific in the bonds they cleave (Marx et al., 2001); thus, they can be useful for monitoring microbial activity and uncovering the mechanisms that underlie microbially regulated decomposition processes (Sinsabaugh, 1994).

As water tables in wetlands drop during drought, the depth of the acrotelm will increase, exposing it to O (Gorham, 1991; Schlesinger, 1991; Roulet et al., 1992; Strakova et al., 2011). Lower water tables may result in increases, decreases, or no change in CO_2 production due to soil respiration (Freeman et al., 1993; Moore and Dalva, 1993; Chimner and Cooper, 2003; Blodau et al., 2004; Ellis et al., 2009). Carbon dioxide production may increase because there is a greater mass of peat exposed to oxic conditions (Jaatinen et al., 2008), and/or there is an elimination of the phenolic constraint on hydrolase activity because of the oxic conditions (Fenner et al., 2001). Hydrolase enzymes are strongly inhibited in the presence of high phenolic concentrations or after long exposure to phenolic materials (Freeman et al., 2001). However, phenolic functional groups, such as those found in lignin and humus, can be broken down by phenol oxidase (which oxidizes phenol and consumes O) and peroxidase (which uses H_2O_2 as electron and acceptor) if O is present (Sinsabaugh, 2010). Alternatively, CO_2 production may show no change or decrease despite substrate exposure to oxic conditions because peat quality may be sufficiently low that it cannot be mineralized (Muhr et al., 2011).

In this paper, we examine how CO_2 production along the peat profile changes as it is exposed to drier and aerobic conditions. Specifically, will deeper peat deposits produce greater rates of CO_2 production than surface peat when exposed to drier and aerobic conditions? To answer this question, we conduct a laboratory experiment where we incubate peat collected along different depths in the peat profile and measure CO_2 production under constant temperature and a gradient of decreasing moisture. Specifically, we examine CO_2 production and potential controls on CO_2 production including peat quality, microbial biomass, and microbial extracellular enzyme activity along the profile. Understanding the mechanisms driving CO_2 production at different depths along the profile will allow us to better predict potential responses and feedbacks under future climate conditions.

MATERIALS AND METHODS

Site Description

This study was performed in a hardwood swamp (1.5 ha) within a small (6.3 ha) catchment of the Turkey Lakes Watershed (TLW) called c38 (47°03′ N lat; 84°25′ W long) that is part of the Great Lakes–St. Lawrence forest region (Jeffries et al., 1988). The Great Lakes–St. Lawrence forest region is Canada's second-largest forest region. With the exception of a 322 km gap where the boreal region touches the north shore of Lake Superior, this forest stretches from southeastern Manitoba to the Gaspé Peninsula. Hardwood swamps are very common within this forest region (National Wetland Working Group, 1997) and have been shown to be disproportionate in their importance to C cycling relative to their area extent (Webster et al., 2008a).

The TLW has significant relief (400 m) with complex topographic configurations underlain by greenstone bedrock with small occurrences of granite. Glacial till covers the bedrock and Haplorthod (Spodosol) soils have developed with dispersed pocks of Haplohemist organic soils (Histosols) in bedrock-controlled depressions and adjacent to streams and lakes. Hardwood swamps form in bedrock depressions and contain overstories that are mixtures of black ash (*Fraxinus nigra* Marshall), eastern white cedar (*Thuja occidentalis* L.), red maple (*Acer rubrum* L.), balsam fir [*Abies balsamea* (L.) Mill.], yellow birch (*Betula alleghaniensis* Britton), and tamarack [*Larix laricina* (DuRoi) K. Koch] (Wickware and Cowell, 1985). The wetland understories are composed of the seedlings and saplings of the overstory trees, various ferns, herbs [e.g., marsh marigold (*Caltha palustris* L.), threeseeded sedge (*Carex trisperma* Dewey), and jewelweed (*Impatiens capensis* Meerb.)], and a mix of sphagnum and feather mosses {e.g., toothed sphagnum (*Sphagnum cuspidatum* Ehrh. Ex Hoffm.) and knights plume moss [*Ptilium crista-castrensis* (Hedw.) De Not.]}. The rest of the watershed is covered by an uneven aged, old growth forest dominated by sugar maple (*Acer saccharum* Marshall) (>90%), with minor occurrence of yellow birch (*Betula alleghaniensis* Britton) (7%) and other species (1%).

The TLW climate is continental with an average annual precipitation of 1189 mm and temperature of 4.6°C, for the period of 1981 to 2010. Stream discharge peaks during spring snowmelt and again during autumn storms. Droughts are occurring with increasing frequency during the summer. For example, water tables of hardwood swamps within the watershed that typically

decline to approximately 15 cm in the summer have been dropping to about 100 cm during recent summer droughts (Creed, unpublished data, 2005–2011). Climate models for the region (CRCM3.6.1) predict that summer droughts will continue, if not intensify (Environment Canada, 2012).

Peat Collection

Peat samples were collected from the hardwood swamp with catchment c38 in December 2007. This time period for sampling was chosen to follow the autumn leaf fall that recharges the system with fresh litter. At this time of year, the water table is also at the surface, which ensures that all of the samples began field wet and fully recharged with dissolved organic matter. Peat cores were collected by inserting a 1-m Jeglum box corer (7.6- by 7.6-cm area) into the ground in an area free of fallen trees and roots. Triplicate cores were taken with total depths of 45, 50, and 80 cm, with care given to wiping the corer clean between samples. Bulk density and C to N ratio data from previous work confirm that cores are homogeneous at lower depths (Creed, unpublished data, 2008). The top 10 cm were divided into two 5-cm increments, and the rest were divided into 10-cm increments. Each increment was placed into a labelled plastic bag, sealed, and stored in a fridge at 4°C for 4 wk until incubation commenced.

Peat Preparation

Peat from the triplicate cores were composited to ensure adequate mass for sampling. Composited soil samples were separated into seven depth increments, starting below the litter and fibric horizon, within the hemic layer at 0 to 5, 5 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50, and 50 to 60 cm. Woody debris was removed from the composited samples because we wanted to focus on the peat only and the woody debris may alter the physical and chemical properties of the peat. The peat was placed into 2-L beakers, homogenized by hand, and made into slurries by combining the peat with distilled, deionized water to make a consistent paste (i.e., peat was wet but not fluid). For the 0- to 5-cm sample, no deionized, distilled water was added because whatever snow and ice that came along with the sample was sufficient to create a slurry consistent with the other depths. For the other samples, 463 ± 74 mL of water was added to each beaker.

Once the slurries were thoroughly mixed by hand, 150 ± 1 g of slurry was allocated into three 1-L glass jars creating three replicates for each depth for CO_2 measurements and an average of 400 ± 8 g of slurry was added to a fourth 1-L glass jar from which material was progressively sampled throughout the incubation to perform analyses on microbial biomass and enzyme activity.

Peat Moisture Water Content

The VWCs of the samples were tracked throughout the duration of the incubation experiment (i.e., up to 24 d). For the jars where there was no destructive sampling (i.e., the CO_2 jars), each time a measurement was taken, the jar with slurry was weighed, and at the end of the incubation, the jar with slurry was dried at 105°C for a minimum of 48 h until the mass stopped changing. For the jars where there was destructive sampling (i.e., the microbial biomass and enzyme activity jars), each time a subsample was taken, a separate 5-g subsample was taken, and its wet weight and then dry weight (after being dried at 105°C for a minimum of 48 h) measured. Volumetric water content (%) was then determined (Eq. [1]):

$$\mathrm{VWC}=\left\{\frac{[\text{wet weight (g)}-\text{dry weight (g)}]}{[\text{density of water (g/mL)}*\text{initial V (mL)}]}\right\}100 \quad [1]$$

The jars were incubated in a Conviron Environmental Chamber set to a temperature of 15°C (reflecting a peak temperature of peat monitored at a 5- to 10-cm depth in the field [Webster et al., 2008b]) and relative humidity of 40% to allow the slurries to dry out slowly. While inside the incubator, the jars remained uncapped between measurements and were placed on a shaker table set to 70 rpm to allow continuous shaking to reduce the chance of microsites forming within the slurries that could potentially skew CO_2 measurements (Creed, unpublished data, 2008). The incubation experiment was terminated when there was no more measurable change in weight of the samples (i.e., VWC < 2%; times varied among depths, from about 12 d for surface peat to about 24 d for deeper peat).

Peat Carbon Dioxide Production

Incubation methods were adapted from Bourbonnière and Creed (2006). The headspace (850 mL) of three 1-L jars containing 150 mL of slurry was sampled for CO_2 every 24 h. At Time 0 of sampling, jars were sealed with a flat disk lid fitted with a two way Luer-Lok valve. Headspace air was sampled using a 30-mL syringe that attached to the Luer-Lok valve. The syringe was flushed twice with headspace air, returning the air back and forth into the jar, to mix the headspace air, and then a 30-mL sample was taken. The syringe needle was flushed with 5 mL of the sample before injecting a 12-mL evacuated exetainer with the remaining 25 mL. This procedure has been shown to minimize contamination from atmospheric CO_2 while not incurring significant pressure differentials (R.A. Bourbonnière, personal communication, 2008). Two headspace samples were collected: the first immediately after sealing each jar and the second 40 min later, the time interval it took for each jar to be sampled in sequence.

The concentration of CO_2 in the headspace samples was determined using gas chromatography on an SRI model 8610C (SRI Instruments, Torrance, CA) using flame ionization detection. A CO_2 standard was run after each batch of 10 samples to establish a calibration factor that was used to convert the area of the CO_2 peak into a concentration. The volume of CO_2 in the headspace of the jar at each sampling time was then calculated with molar volume corrected for temperature and pressure. Rate of CO_2 production over the sample time was calculated by taking the difference in concentration of CO_2 (Time 40 − Time 0) and dividing it by the time elapsed (40 min). The cumulative CO_2 production for each jar over the incubation period was calculated by extrapolating flux during the 40-min measurement

to a daily production, and then summing the daily production for each day of the incubation period. Outlier values that were negative, indicating that a headspace sample was lost due to an improperly sealed exetainer, were removed from the time series of CO_2 production.

Peat Quality

At the start of the experiment, a 250-g wet weight subsample from each of triplicate peat cores was dried at 50°C to a constant weight for 1 wk. The subsamples were then ground up with mortar and pestle, after removing all grass, roots, and/or debris, and passed through a 2-mm sieve. Samples were analyzed for total C and N concentrations using a Carlo-Erba NA2000 analyzer (Milan, Italy). One gram was also taken to determine the dominance of labile vs. refractory compounds in peat through Fourier transform infrared (FTIR) spectroscopy (Artz et al., 2008). Scans of dried soil samples were taken using Bruker Tensor 27 FTIR (Bruker Optics, Billerica, MA) with a Golden Gate ATR sample introduction system (Specac Ltd, Slough, UK) with resolution of 4 cm^{-1}, with 50 scans over a range of 4000 to 500 wavenumbers (cm^{-1}). Minor differences in the amplitude and baseline were corrected by normalizing the data through subtraction of the sample minimum, followed by division by the average of all data points per sample.

Microbial Biomass

Microbial biomass was determined when peat reached VWCs of 85, 65, 35, and 15 and 5% using the well-established $CHCl_3$ fumigation-extraction method (Vance et al., 1987). Extracted C in fumigated and non-fumigated samples was measured on Shimadzu TIC–TOC analyzer (TOC 5000A, Shimadzu Corp., Tokyo, Japan) and N was measured on the Antek 9000 (Antek Instruments, Houston, TX). Total microbial biomass was determined by calculating the difference in soluble C and N (mg C or N per g soil) between the fumigated and non-fumigated samples.

Microbial Enzyme Activity

Similar to the microbial biomass sampling, enzyme assays were run on a 5-g subsample of slurry from the fourth jar. Samples were placed into a blender, mixed with 125 mL of 0.1 M MES buffer (pH 5.73, similar to the soil pH at the site) and spun for 1 to 2 min to homogenize.

We quantified activities of hydrolytic enzymes (β-glucosidase, cellobiosidase, N-acetyl glucosaminadase, sulfatase, and phosphatase) and oxidative enzymes (phenol oxidase and peroxidize).

Hydrolase enzymes were measured using MUB-linked substrates. Two-hundred microliter aliquots of slurry were dispensed into each of eight microplate wells. Additional wells were used for negative control (200-μL buffer + 50-μL substrate), blank (200-μL sample + 50-μL buffer), standard (200-μL buffer + 50-μL MUB), and quench (200-μL sample + 50-μL substrate). Fifty microliters of prepared MUB substrate was added to the wells containing the 200 μL of slurry. Plates were incubated in the dark at room temperature for 0, 30, 60, 90, and 120 min. Once the sample reached the incubation time, the pH was raised above 7.5 to measure fluorescence by adding a 10-μL aliquot of 0.5 N NaOH to each well (including assays, standards, negative controls, blanks, etc.) at the termination of incubation. Fluorescence was measured on a SpectraMax M2e microplate reader (Molecular Devices, Sunnyvale, CA) at 365 nm excitation and 450 nm emission.

Phenol oxidase and peroxidize were measured using L-DOPA substrate using a procedure similar to the hydrolase except that incubation was done using clear 96 well plates. Since the procedure measures absorbance, only a negative control and the blank were required, and NaOH was not needed to stop the reaction. For peroxidize, 10 μL of 0.3% H_2O_2 was also added to each well to alleviate peroxide limitations. Measurements were taken at the same incubation times using SpectraMax M2e microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm.

Enzyme activity rates (nmol g^{-1} h^{-1}) for hydrolases and phenol oxidase were calculated as nmol substrate converted per hour per gram of dry sample. Peroxidase activity is the difference in activity between the phenol oxidase and the peroxidase assay samples.

Statistical Analysis

Quadratic regressions were performed to predict CO_2 production from soil VWC, and an inverse first order polynomial regression was used to predict cumulative CO_2 using the molar C/N quotient. A one-way ANOVA and post-hoc Holm–Sidak tests were used to determine differences in cumulative CO_2 production with depth, and Pearson product moment correlations were performed to examine the relationship between chemical functional groups to C/N and between enzyme activities to water contents. All statistical analyses were performed using SigmaPlot (ver. 12; Systat Software Inc., 2008).

RESULTS

Peat Carbon Dioxide Production

The total CO_2 produced per gram of peat decreased with increasing depth, except for the first 5 cm (Fig. 1, 2). Carbon dioxide production had a unimodal response to VWC, such that CO_2 peaked at moderate soil moisture (i.e., about 40–50%) for each depth (Fig. 1, 2). Regressions of CO_2 production as a function of VWC were significant at each depth ($p < 0.05$), but the variance explained ranged from 0.09 to 0.72, with the deeper peat layers having lower r^2 values (Fig. 2). Cumulative CO_2 production declined with increasing depth (Fig. 3).

Peat Quality

Peat quality was not uniform along its profile. The amount of C and N decreased slightly with depth (Table 1), and the C/N quotient shifted from relatively high quality at the surface (molar C/N quotient of 20.3 ± 0.5 for the top 30 cm) to lower quality at depth (molar C/N quotient of 28.2 ± 1.8 for depths greater than 30 cm) (Fig. 4a; Table 1). Fourier transform infrared scans suggest that the decline in the C/N quotient was due both to an increase in more recalcitrant functional groups [complex polysaccharides, carboxylate and carboxylic structures,

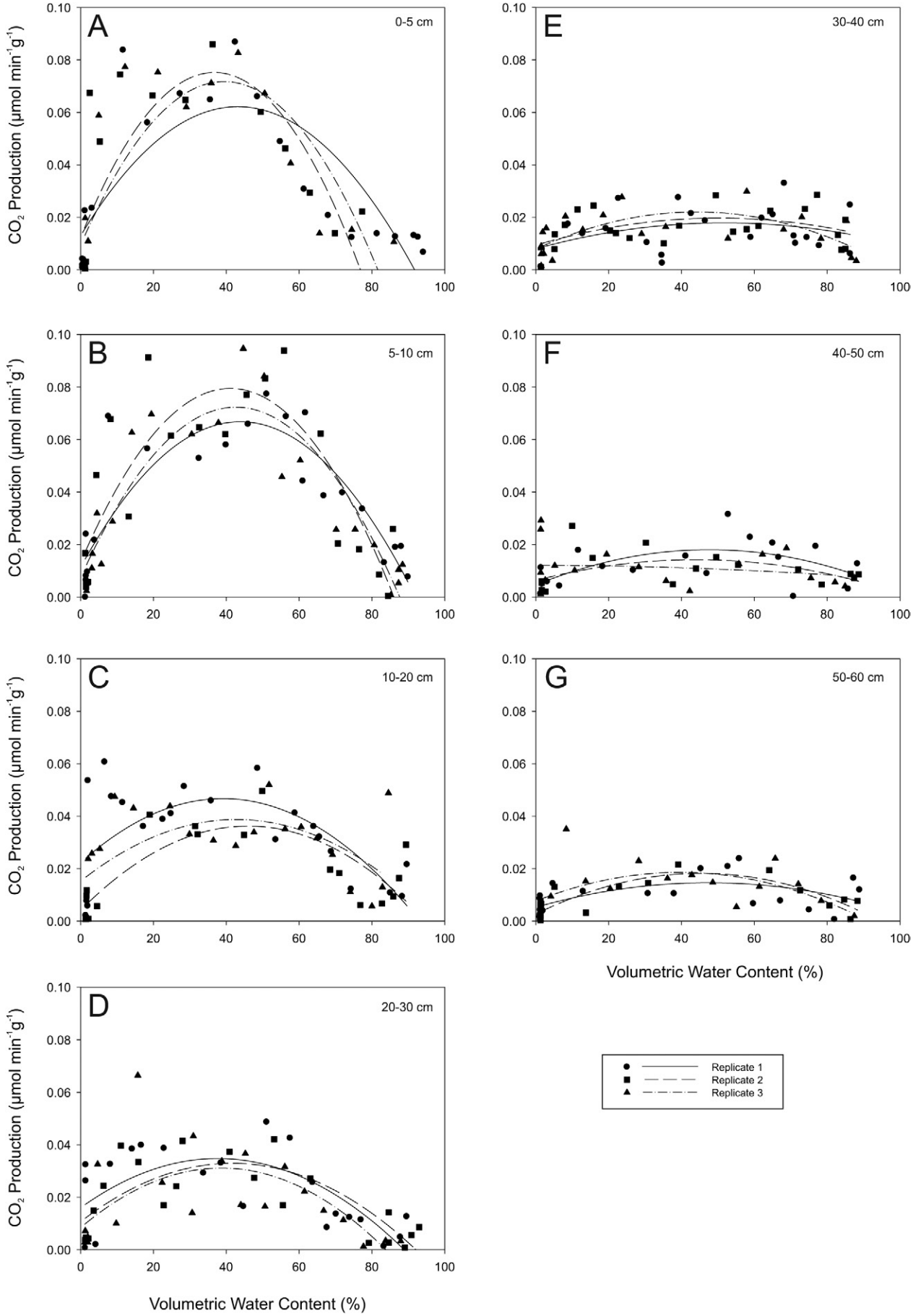

Fig. 1. Carbon dioxide production curves fitted by quadratic function for three replicate soil samples from each soil depth interval [(a) 0–5, (b) 5–10, (c) 10–20, (d) 20–30, (e) 30–40, (f) 40–50, and (g) 50–60 cm] across a gradient of volumetric water contents.

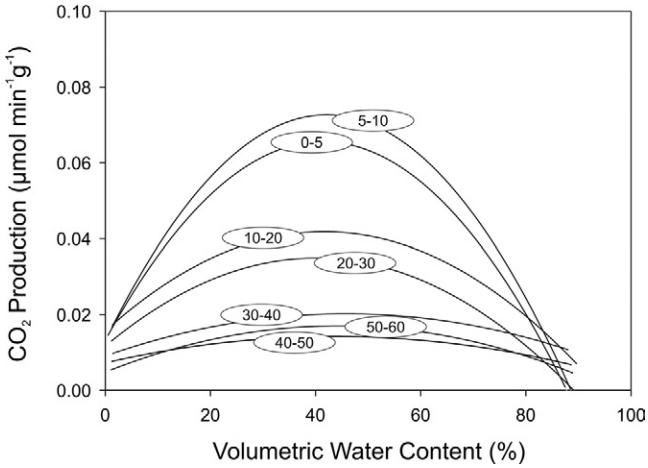

Fig. 2. Carbon dioxide production curves fitted by quadratic functions for each soil depth interval (as indicated by increment in oval) across a gradient of volumetric water contents. Coefficients of determination are 0.57 (0–5 cm), 0.72 (5–10 cm), 0.37 (10–20 cm), 0.48 (20–30 cm), 0.21 (30–40 cm), 0.09 (40–50 cm), and 0.35 (50–60 cm). All curves are significant at p < 0.001, except 40–50 cm (p = 0.029).

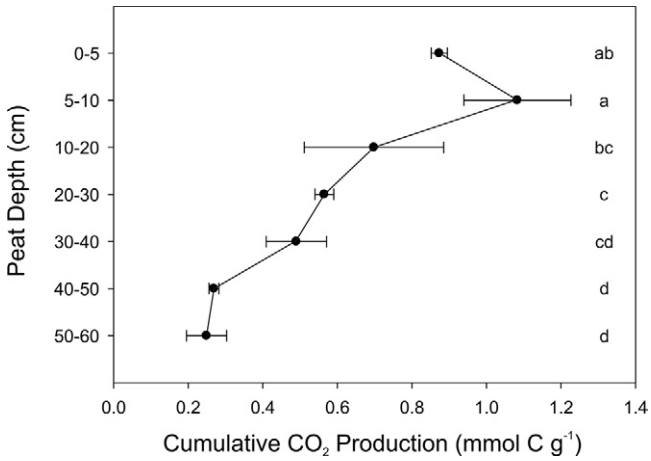

Fig. 3. Average cumulative CO_2 (±SD; mmol C g^{-1}) produced over the course of the incubation at each depth in the peat. Letters indicate significantly differences ($p < 0.05$) among depths.

phenolics (lignin) and aliphatic structures at 1450 wavenumber, and symmetric and antisymmetric fats] and a decrease in a more labile functional group (cellulose) (Fig. 4b and 4c; Table 2). The C/N quotient was a good indicator of peat quality, with many compounds correlated with C/N quotient (Table 2). Lignin and lignin–phenolic backbone showed no clear pattern with depth, although lignin and other aromatic structures showed a distinct peak at a 30- to 40-cm depth (Fig. 4d).

Microbial Biomass and Enzyme Activity

Microbial C biomass decreased with depth and was highest at moderate VWC (15, 35, and 65%) (Fig. 5). Peroxidase and phenol oxidase activity rates were low and did not show any consistent pattern with depth or with VWC (Fig. 6). Hydrolase activity rates decreased with depth, were highest at the wettest (85%) VWC above 30 cm, and below 30 cm were relatively constant irrespective of VWC (Fig. 6).

Drivers vs. Constraints on Peat Carbon Dioxide Production

Higher cumulative CO_2 production corresponded to higher peat quality (low C/ N ratios, Fig. 7, $r^2 = 0.79$). At lower VWCs (5–35%), CO_2 production was positively correlated to hydrolase activities but negatively correlated to phenol oxidase activity (Table 3). The opposite response was observed at higher VWCs (65–85%) (Table 3).

DISCUSSION

Declines in water tables that will accompany droughts are not likely to lead to more CO_2 production in hardwood swamps in the short term. Although there will be a greater volume of peat exposed to oxic conditions, the low quality of peat at depth will constrain CO_2 increase, a conclusion supported by others (e.g., Yavitt et al., 1997; Muhr et al., 2011) in other peatland systems. This experiment was unique in that CO_2 production was simultaneously examined along with peat quality, microbial biomass, and enzyme activity to develop a better understanding of mechanisms of C mineralization with declining in water table during droughts induced by climate warming.

Mechanisms of Peat Carbon Dioxide Production at Different Depths

High CO_2 production was maintained in the upper acrotelm layer because of high quality of C substrate, high microbial biomass, and high hydrolase activity due to the presence of O. High CO_2 production and microbial biomass in this zone of forested swamps is, thus, consistent with findings of Silvola et al. (1996), Blodau and Moore (2003), and Blodau et al. (2004) for other northern peatlands. High peat quality in this layer is related to low C/N (approximately 20), with the presence of cellulose as a more labile functional group in the upper layers. Variability in peat CO_2 production within the acrotelm layer is driven primarily by moisture content, which affects both the solubility of substrate and amount of O. Optimal CO_2 production has been shown to occur under aerobic and moist conditions (Davidson et al., 1998; Borken et al., 2003; Webster et al., 2008b; Henry, 2012), which this study also confirms. Hydrolase enzymes were most active in the

Table 1. Bulk density, total C, N, C/N by weight, and C/N by molecular weight in the peat before the incubation. One subsample was taken from each of the original slurries to be used in the calculations.

Soil depth	*n*	Bulk density	Carbon	Nitrogen	C/N	C/N (molar)
cm		g cm^{-3}	mg g^{-1}	mg g^{-1}	mg g^{-1}:mg g^{-1}	
0–5	1	0.061	4.47	0.27	16.36	19.10
5–10	1	0.072	4.64	0.26	17.55	20.48
10–20	1	0.088	4.70	0.27	17.51	20.43
20–30	1	0.072	4.54	0.26	17.75	20.71
30–40	1	0.099	4.64	0.21	22.45	26.20
40–50	1	0.099	4.75	0.19	24.62	28.72
50–60	1	0.098	4.43	0.18	25.36	29.59

upper 30 cm, likely because of the availability of cellulose and the high peat quality at these depths. All hydrolase enzymes were most active in moist conditions (85%) because labile materials were readily dissolved.

Carbon dioxide production was low in the catotelm even under drier (more oxic) conditions because of the low substrate quality. Fourier transform infrared scans indicated that the decline in peat quality with depth was due both to an increase in more recalcitrant functional groups (e.g., complex polysaccharides, fats, phenol, and lignin), consistent with increasing humification (Artz et al., 2008) and a decline in the more labile cellulose. This is consistent with Charman et al.'s (1994) observation that the change in C/N along a peat profile is attributed to older organic material made up of recalcitrant C and hydrophobic material such as lignin and waxes that are much more complex in structure. The poor quality of the deep peat likely reflects that labile material was consumed before entering the anaerobic zone and/or that additional chemical (e.g., condensation) and biologically (e.g., fermentation) reactions have degraded material after entering the anaerobic zone (Charman et al., 1994; Holden, 2005), which would be consistent with the observed presence of lignin and other aromatic structures at a 30- to 40-cm depth.

Low microbial activity further constrained CO_2 production in deep peat. Both microbial C biomass and the activities of all five hydrolase enzymes were low at these depths for all VWCs, likely because the labile substrate cellulose was low in this layer as well. While microbes are capable of decomposing more recalcitrant C, the acquisition of energy from such substrates is not adequate to sustain long-term activity (Fontaine et al., 2007). However, some CO_2 was produced at depths greater than 30 cm. Although the activity levels were quite low at all depths, there was a positive correlation between phenol oxidase activity and CO_2 production. Lignase activity did not appear to be affected by depth, likely because there was not much variation in the lignin substrates with depth, though there was a slight peak in the 30- to 40-cm interval. A potential artefact of the experimental design is that the layers of peat were physically separated from each other; thus, potential "colonization" of lower peat layers on drying (e.g., expansion of aerobic decomposer communities into deeper peat deposits [Thormann, 2011]) could not be evaluated. Sinsabaugh (2010) acknowledged that there may be other complex interactions between the environmental and microbial communities along the peat profile. For example, episodic rain events might aid in redistributing microbes within the peat profile.

This study highlights the importance of chemical, physical, and biological conditions within the mesotelm layer, as the interface layer between the high and low CO_2 producing environment. The primary control on peat chemistry within the mesotelm will be the length of time from when litter is deposited to when it enters the anaerobic zone of the catotelm (Laiho, 2006). If the time from deposition to incorporation into the catotelm is short, there would be less mass loss and potentially higher quality substrate present when the water table drops. In contrast, if it takes a long time for material to reach the anaerobic zone, labile

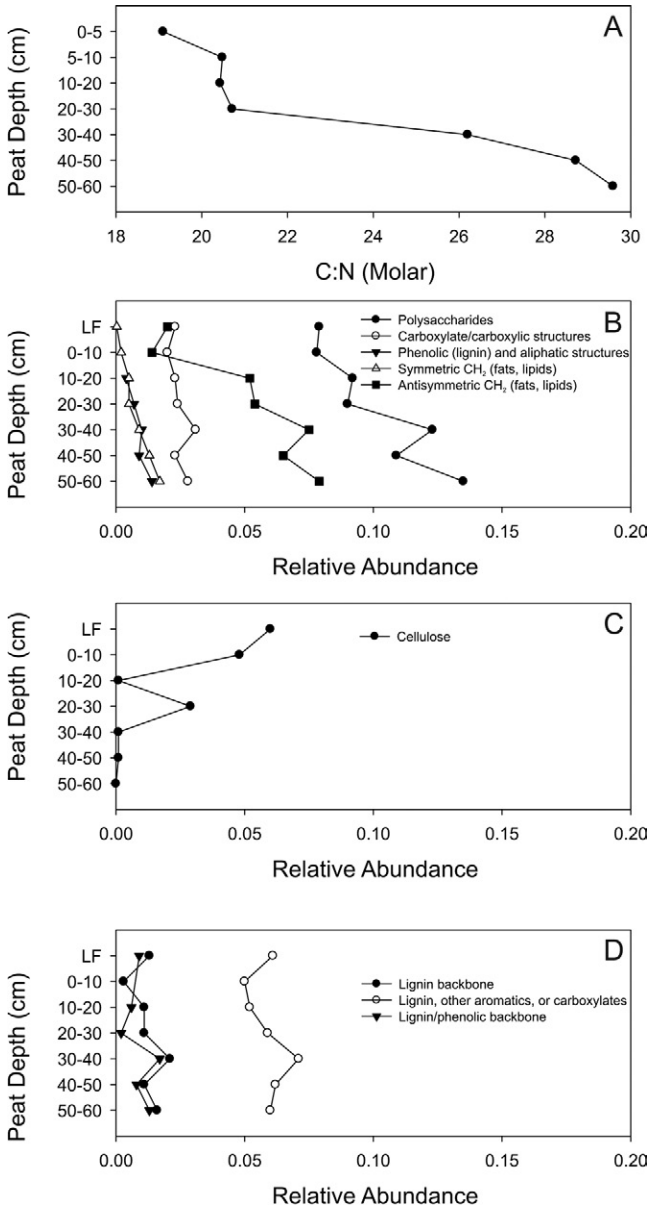

Fig. 4. (a) Ratio of molar C/N at each depth and relative absorbance of functional groups that (b) increase, (c) decrease, or (d) show no clear pattern with depth. Functional groups are based on peaks in wavenumbers as described by Artz et al. (2008).

material would have already been consumed by the time the water table drops, leaving only refractory material.

The results suggest other factors within the mesotelm besides peat quality may also influence the rate of decomposition, including microbial community composition, the removal of inhibitory substances, the supply of an external source of high quality C to prime the process, and the supply of other potentially limiting nutrients such as N and P (Clymo and Bryant, 2008). Although it was neither the intent nor design of this experiment to test for these different constraints, it is useful to consider the mechanisms that may help to overcome poor peat quality.

In terms of microbial community composition, fungi have been recognized as the principal decomposer of organic matter in the acrotelm in peatlands, with bacteria being less important (Thormann, 2006a, b). Fungi, with higher C/N (Myers et

Table 2. Relative intensity for different functional groups at different peat depths and their Pearson product moment correlation to C/N. The functional groups are determined from the median wavenumber of the peaks across depths, with respect to the wavenumber for the functional group as defined by Artz et al. (2008).

	Poly-saccharides	Lignin backbone	Phenolic (lignin) and aliphatic structures	Carboxylate/ carboxylic structures	Phenolic (lignin) and aliphatic structures	Lignin/ phenolic backbone	Lignin, other aromatics, or carboxylates	Symmetric CH_2 (fats, lipids)	Antisymmetric CH_2 (fats, lipids)	Cellulose
Wavenumber										
Artz et al., 2008	1030–1080	1265	1371	1426	1450	1513–1515	1600–1650	2850	2920	3340
Median wavenumber	1027	1262	1382	1414	1452	1511.5	1592	2850	2919	3293
Relative intensities at different peat depths										
0–10 cm	0.078	0.003	0.002	0.02			0.05	0.002	0.014	0.048
10–20 cm	0.092	0.011	0.002	0.023	0.004	0.006	0.052	0.005	0.052	0.001
20–30 cm	0.09	0.011	0.002	0.024	0.007	0.002	0.059	0.005	0.054	0.029
30–40 cm	0.123	0.021		0.031	0.01	0.017	0.071	0.009	0.075	0.001
40–50 cm	0.109	0.011		0.023	0.009	0.008	0.062	0.013	0.065	0.001
50–60 cm	0.135	0.016	0.002	0.028	0.014	0.013	0.06	0.017	0.079	0
Correlation to C/N										
r value	0.89	0.58	–0.39	0.57	0.87	0.66	0.63	0.96	0.75	–0.66
p value	0.02	0.23	0.52	0.24	0.06	0.22	0.18	0.003	0.09	0.16

al., 2012), break down high C/N material and produce specialized lignin degrading enzymes (Paul, 2007). The abundance of fungal taxa capable of producing the enzymes needed to degrade complex polyphenolic polymers must be specifically considered (Thormann, 2011). Furthermore, a source of N alleviates N limitations for microbes degrading high C/N material (Paul, 2007). For example, Fierer et al. (2003) found that the addition of N to subsurface soils generated a four-fold increase in soil organic C mineralization rates (Fierer et al., 2003) and CO_2 emissions (Gerdol et al., 2007). Phosphorus may also be required (Fierer et al., 2003); however, the mechanisms controlling cycling and availability of P within wetlands are poorly understood. Other molecules, such as phenols, limit decomposition by inhibiting hydrolytic enzymes (Freeman et al., 2004). Water table draw down invoked decreases in phenolic concentrations by promoting phenol oxidase activity that removes phenols (Ellis et al., 2009), although this activity is transient (Bell and Henry, 2011). Once phenols are removed, hydrolase enzyme activities are not inhibited. Finally, inputs of labile materials can help to prime the decomposition process. There is a strong coupling between root exudation, soil organic matter decomposition (Bengtson et al., 2012), and increased microbial respiration (Fontaine et al., 2007). Thus, we suggest that further experimentation should specifically test for the presence and relative importance of these mechanisms or additional mechanisms that may help to overcome quality limitations within the mesotelm at a variety of wetland types.

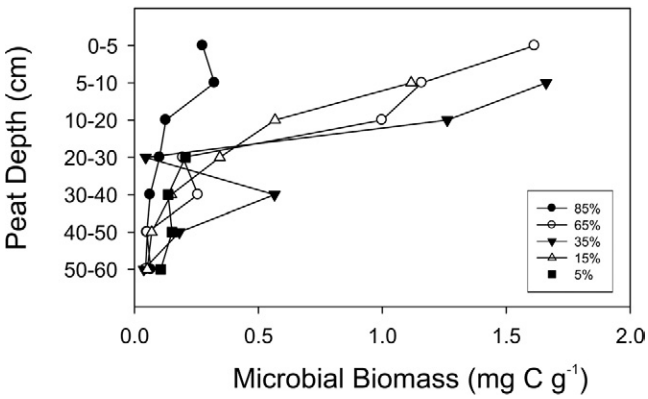

Fig. 5. Changes in microbial C (mg C g^{-1}) with depth and changing moisture conditions.

Predicted Changes in Carbon Dioxide Production with Declining Water Table over Time

Although declines in water tables that will accompany warmer climate are not likely to lead to more CO_2 production in the TLW swamps in the short term, over the long term the response will be complex. Constraints on decomposition at the mesotelm boundary between upper and lower peat, may be removed, making C available for decomposition. For example, increased atmospheric N deposition or concomitant changes in vegetation may increase available N (N fixating plants or leafy, green deciduous plants or grasses) (Basiliko et al., 2006). Also, fungi degrade low quality, high C/N material, produce lignin degrading enzymes, and are likely to increase under drier conditions (Peltoniemi et al., 2009). Furthermore, ectomycorrhizae are likely to increase under drier conditions, and they may play a dual role as decomposers in organic-rich environments (Jaatinen et al., 2008). Finally, increases in temperature and CO_2 concentrations increase belowground C allocation, requiring consideration of priming effects on decomposition (Bengtson et al., 2012) by labile root exudates or litter (Strakova et al., 2011). Thus, short-term changes in CO_2 production represent a disturbance in an ecosystem adapted to the pre-water level lowering conditions, while long-term changes are likely to result from several adaptive mechanisms of the ecosystem to the new hydrological regime (Laiho, 2006).

CONCLUSIONS

Droughts are resulting in greater range of water table fluctuations in swamps, exposing deep deposits to conditions that could result in rapid mineralization of C pools. However, we

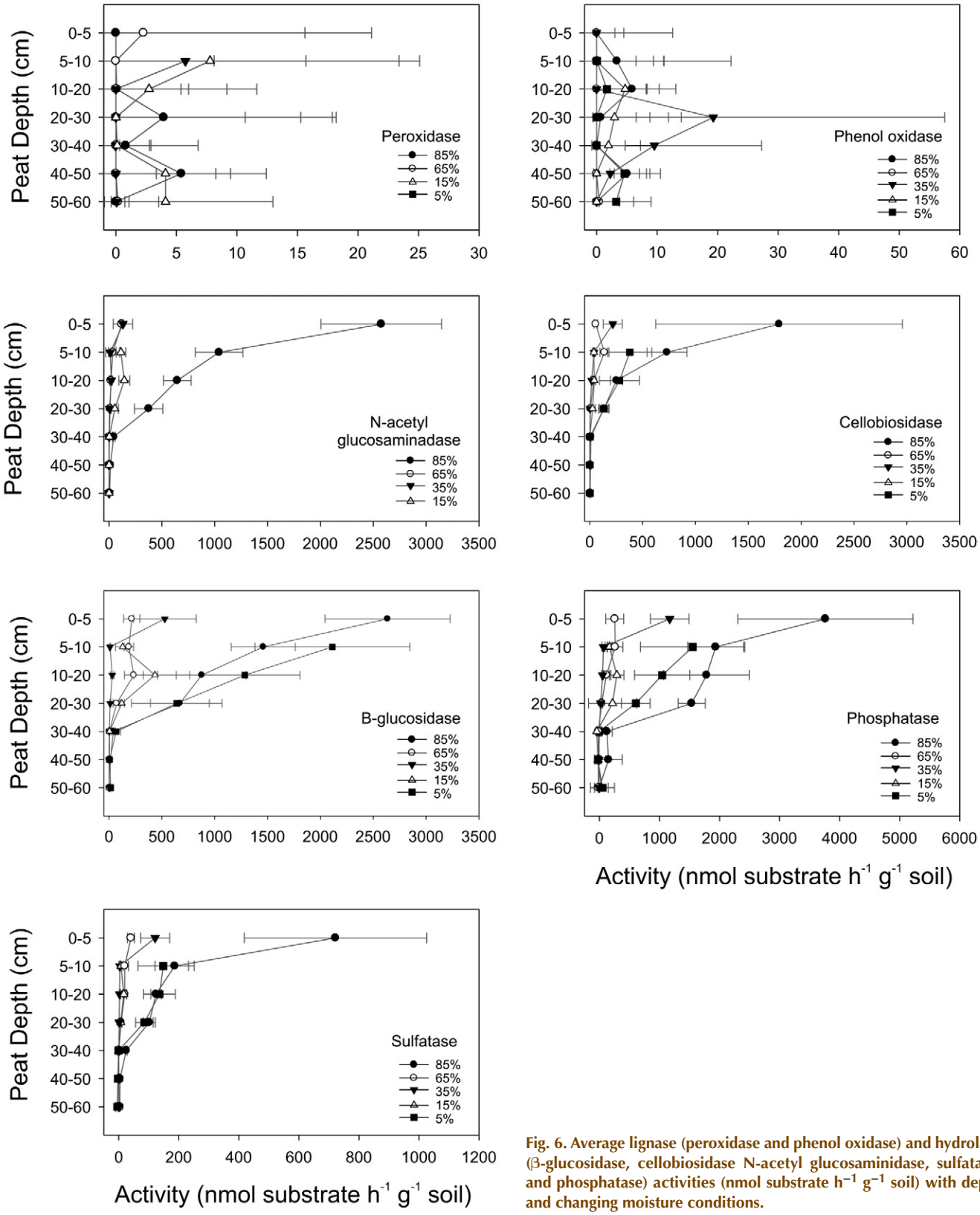

Fig. 6. Average lignase (peroxidase and phenol oxidase) and hydrolase (β-glucosidase, cellobiosidase N-acetyl glucosaminidase, sulfatase, and phosphatase) activities (nmol substrate h^{-1} g^{-1} soil) with depth and changing moisture conditions.

found that swamp peat below about 30 cm (approximately the boundary between the acrotelm and catotelm) was not of sufficient quality to allow rapid mineralization to CO_2 under simulated drought. The C/N ratio was a good (and easily measured) metric of peat quality, reflecting changes in molecular functional groups (e.g., increases in recalcitrant functional groups such as complex polysaccharides, some lignins, and fats and decreases in the labile functional group of cellulose) along the peat profile. Knowledge of peat C/N and water table depth could be an indicator of wetland vulnerability to climate-induced water table lowering. For example, wetlands with peat deposits of low C/N

Table 3. Pearson correlations (*r*) between enzyme activity rates and CO_2 production at different peat water contents (%).

	Volumetric water content (%)				
Enzyme	5	15	35	65	85
Sulfatase	0.963***	0.803*	0.139 ns†	−0.329 ns	−0.989***
N acetyl glucosaminidase	na	0.929***	0.249 ns	−0.622 ns	−0.970***
Cellobiosidase	0.983***	0.943***	0.279 ns	0.263 ns	−0.973***
β-glucosidase	0.984***	0.706*	0.145 ns	0.062 ns	−0.927***
Phosphatase	0.990***	0.829**	0.178 ns	−0.067 ns	−0.888***
Phenol oxidase	−0.143 ns	0.383 ns	−0.592 ns	0.226 ns	0.453 ns
Peroxidase	0.374 ns	0.337 ns	na	−0.666*	0.844**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† ns, Nonsignificant at the 0.05 probability level.

and large declines in water table are at higher risk for increased CO_2 production than wetlands with small declines in water table. Application of such an indicator would serve to identify wetlands of high conservation value or in need of mitigation. We caution, however, that this study considers only short-term responses to water table lowering. If constraints on CO_2 production (e.g., including N, microbial composition, removal of substances that inhibit enzymes, and sources of high quality C) are relieved, these C stores could be unlocked over the long-term. Further work is required to determine which of these constraints operating within the mesotelm are most important in unlocking deep peat C stores.

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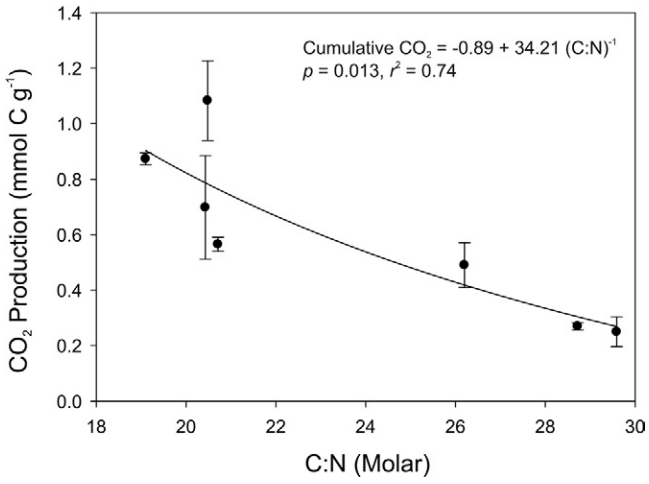

Fig. 7. Average cumulative CO_2 (±SD; mmol C g^{-1}) as a function of peat C/N at different depths.

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