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Depth rather than microrelief controls microbial biomass and kinetics of C-, N-, P- and S-cycle enzymes in peatland

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ABSTRACT

The formation of microrelief forms in peatlands - elevated and dry hummocks, depressed wet hollows and intermediate lawns - is controlled by the interaction of water table, nutrient availability and dominant plant communities. This affects the composition and activity of various functional groups of microorganisms. With depth, the change in peat quality from less to more highly processed organic material additionally regulates microbial activity. We hypothesized that microbial biomass and enzyme activities are driven by aeration and by peat quality and therefore (i) they increase from hollows (water saturated/anaerobic) through lawns (intermediate) to hummocks (aerobic) in the top peat and ii) they decrease with depth due to increasing distance from fresh plant-derived inputs and lower oxygen availability. These hypotheses were tested for enzymes catalysing the decomposition of C-, N-, P- and S-containing organic compounds in peat of the three microform types at three depths (15, 50 and 200 cm). Microbial biomass and peat chemical characteristics were compared with enzyme kinetic parameters, i.e. maximal potential activity (V_{max}) and the Michaelis constant (K_m).

Microbial biomass carbon (MBC) and V_{max} of β-glucosidase and *N*-acetyl glucosaminidase increased by 30–70% from hummocks and lawns to hollows in the top 15 cm, contradicting the hypothesis. Similarly, K_m and the catalytic efficiency of enzymes ($K_a = V_{max}/K_m$) were best related to MBC distribution and not to the aeration gradient. With depth, V_{max} of β-glucosidase, xylosidase and leucine aminopeptidase followed the hypothesized pattern in hollows. In contrast, MBC was 1.3–4 times higher at 50 cm, followed by successively lower contents at 15 and 200 cm in all microforms. The same depth pattern characterized the V_{max} distribution of 6 out of 8 enzymes. Phosphatase activity decreased from drier hummock to wetter hollows and the higher activity throughout the peat profile suggested a high microbial demand for P. Enzyme activities and catalytic efficiency in peat were closely linked to the distribution of microbial biomass with depth, which in turn was best explained by P content. From the ecological perspective, these results clearly show that peat decomposition will be accelerated when microbial activity is stimulated e.g. by increased P availability.

1. Introduction

Peatlands are an important source of greenhouse gases (GHG) and play a key role in the global carbon (C) budget (Lai, 2009). Boreal peatlands (> 45°N) cover only 3% of the terrestrial surface but contribute a significant portion of CH_4 (46 Tg CH_4-C yr^{-1}) to the atmosphere and are a steady sink for CO_2 (Limpens et al., 2008; Nilsson et al., 2008). Due to low annual mean temperatures and dominant anoxic belowground conditions, the rate of litter decomposition in peatlands is slow, leading to a net C accumulation (Moore and Basiliko, 2006). Nonetheless, C pools and storages here may become vulnerable due to continuous temperature increase and change of precipitation (IPCC, 2013) as well as eutrophication. According to IPCC (2013), the annual CO_2 emissions from anthropogenic greenhouse gas sources including changes in forestry and other land use systems are predicted to increase by 27 Gt in the upcoming decade. This makes it critically important to understand mechanisms regulating the C balance in peatlands.

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Table 1
Selected hydrolytic enzymes and their function for the degradation of organic matter. For more detailed information, respective references are provided.

Enzymes	Acronyms	Degradation of macromolecules	Potential role	References
β-1, 4-glucosidase	β-GLU	Cellulose	C-cycle	Gong and Tsao, 1979
Cellobiohydrolase	CEL	Cellulose	C-cycle	Gong and Tsao, 1979
Xylosidase	XYL	Hemicellulose	C-cycle	Gong and Tsao, 1979
N-acetyl-β-D-glucosaminidase	NAG	Chitin and bacterial polypeptidoglycan	N-cycle	Allison and Jastrow, 2006
Leucine aminopeptidase	LEU	Protein and peptides	N-cycle	Sinsabaugh et al., 1993
Tyrosine aminopeptidase	TYR	Protein and peptides	N-cycle	Sinsabaugh et al., 1993
Phosphatase	PHO	Nucleic acids, phospholipids and other ester phosphates	P-cycle	Turner et al., 2002; Toor et al., 2003
Sulphatase	SUL	Organic ester sulphates and C-bounded S	S-cycle	Sinsabaugh et al., 1991

The water table level and plant communities shape the surface of peatlands into three main microform types (microrelief sub-units): 1) elevated and relatively dry hummocks, 2) wet depressed hollows and 3) intermediate lawns (Saarnio et al., 1997; Nungesser, 2003; Shen et al., 2006). The specific conditions in each microform affect numerous biochemical processes and microbial parameters (Dorodnikov et al., 2011). Microorganisms use either secreted or membrane-bound digestive enzymes to decompose soil organic matter (SOM). The enzymes differ based on the principal reaction type by which microorganisms catalyse SOM decomposition or the synthesis of new biochemical compounds (Allison and Vitousek, 2005). Hydrolytic enzymes, functioning under aerobic and anaerobic conditions, play an important ecological role: they are mainly responsible for degrading polymeric compounds of plant- and microbial residues (e.g. cellulose, hemicellulose, chitin, peptidoglycan and lignin-prevalent components of SOM, see Table 1). The degradation products are utilized by microorganisms for their metabolism and growth (German et al., 2011).

SOM degradation and the release of C, nitrogen (N), phosphorus (P) and other macro- and microelements (e.g. sulphur (S)) is controlled by SOM quality and environmental factors. Peatlands are nutrient-poor environments, and microbial C mineralization and assimilation is strongly limited by the availability of key nutrients, particularly P and N (Lin et al., 2014b). Nutrient limitation was proven to influence both microbial community composition and enzyme activities (Amador and Jones, 1993; Lin et al., 2014a). Seasonal and weather-related changes of the water table, together with different vegetation communities, are responsible for the surface-specific conditions in the three microforms (Dorodnikov et al., 2011). With peat depth, the abrupt oxygen decrease and the increasing distance from fresh plant-derived deposits promote the development of anaerobic microbial communities (Kotiaho et al., 2013; Deng et al., 2014; Loeppmann et al., 2016). However, little is known about the relationships between peat quality (nutrient contents), microbial biomass and activity as affected by microrelief position and peat depth.

To fill the knowledge gaps, we determined Michaelis-Menten kinetics parameters of several common hydrolytic enzymes contributing to the peat C cycle (β-glucosidase, cellubiohydrolase, xylosidase), N (*N*-acetylglucosaminidase, leucine aminopeptidase, tyrosine aminopeptidase), P and S cycles (phosphatase, sulphatase). We then linked them to microbial biomass carbon in peat of three microrelief forms (hummocks, lawns, hollows) at three depths of a boreal peatland. The substrate-dependent enzyme activity approximated with Michaelis-Menten kinetics provides information about two key parameters: (1) the maximal velocity (V_{max}), i.e. the maximal rate, of an enzyme-mediated reaction at saturating substrate concentrations and (2) the Michaelis constant (K_m), i.e. the substrate concentration at half of the maximal velocity, which represents enzyme affinity to a substrate (German et al., 2011). We hypothesized that microbial biomass C and enzyme activities (i) increase from hollows (water saturated/anaerobic) through lawns (intermediate) to hummocks (dry/aerobic conditions) and (ii) decrease with depth due to increasing distance from fresh plant-derived inputs.

2. Materials and methods

2.1. Site description

Peat samples were collected from the Salmisuo mire complex in eastern Finland (62°47′ N, 30°56′ E). The peatland is an oligotrophic low-sedge *Sphagnum* pine fen (Saarnio et al., 1997; Becker et al., 2008; Dorodnikov et al., 2013). Three microform types were selected based on water table depth and dominant plant species (Becker et al., 2008): elevated hummocks, depressed hollows and intermediate lawns between the two. The most common plant species in hummocks were *Sphagnum fuscum* (Schimp.) Klinggr and *Eriophorum vaginatum*. Lawns were characterized by *S. angustifolium*, *S. balticum* (Russow) C. Jens. and *E. vaginatum*. The dominant plant types in wet hollows were *Sphagnum* species, *Scheuchzeria palustris* and *E. vaginatum* (Saarnio et al., 1997). The average depth of the water table below the studied microforms during the sampling period was -23 ± 5 cm, -5 ± 2 cm and 0 ± 2 cm from the surface of hummocks, lawns and hollows, respectively (Lozanovska et al., 2016).

2.2. Peat sampling

A stainless-steel peat corer (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) was used to collect peat samples. Three replicate sampling plots in three different locations of each microform were randomly selected for collecting peat cores (total nine experimental units). From each plot, peat was collected at three depths: 15, 50, and 200 cm from the surface of microforms for a total samples size of 27 altogether. At each sampling plot, collection was repeated at least 3 times to obtain the necessary amount of peat for each depth. The sample was *Sphagnum* type peat of weakly, partially and highly decomposed state in the top, middle and bottom layers, respectively. Samples were immediately placed in air-tight Whirl-pack plastic bags and after transportation to the laboratory in a cooled thermobox were stored at 4 °C temperature for one week in a dark room. To minimize the oxygen contamination of the samples collected beneath the water tables, samples were kept closed under field-moist (saturated) condition after sampling.

2.3. Peat chemical analyses

The chemical composition of peat samples was determined after drying (60 °C, 2–3 days) and grinding to fine powder by a Fritsch Pulverisette (type 00.502, Oberstein, Germany) equipped with an agate pocket and ball mill. Total C and N contents were measured with an automated CN analyser (Elementar Vario EL Cube, Elementar Analysensysteme GmbH, Hanau, Germany). Total P and S contents were determined with an ICP spectrophotometer (iCAP 6000 series, ASX-520 AutoSampler, Thermo Scientific, USA) after sample digestion in a mixture of nitric and hydrochloric acid (2:1 v:v) by a Digestore Milestone MLS 1200 (Microwave Laboratory System, Sorisole BG, Italy). The elemental composition is given in mg g^{-1}-of peat dry

weight. Peat pH (H_2O) was measured with a pH meter (pH 538, W. Krannich GmbH Co. K.G., Germany).

2.4. Measurement of peat microbial biomass carbon and extracellular enzyme activities

Microbial biomass was measured based on extracted total microbial DNA (Bouzaiane et al., 2007; Fornasier et al., 2014; Semenov et al., 2018). A FastDNA Spin Kit for Soil (BIO 101/Qbiogene, MP Biomedicals) was used according to the manufacturer's protocol for the FastPrep-24 TM instrument (MP Biomedicals, LLC, Santa Ana, CA, USA). The detailed description of the procedure can be found in Semenov et al. (2018). In brief, peat samples were added to lysing matrix tubes containing silica and glass spheres of different diameters and treated with sodium phosphate buffer (Na_2HPO_4; pH 8.0, 0.12 M) and MT buffer (1% sodium dodecyl sulfate – SDS, 0.5% Teepol, and PVP40 with EDTA and Tris). The tubes were subjected to bead beating in the FastPrep® instrument and processed by protein precipitation solution (150 µL of 3 M CH_3COOK and 4% glacial acetic acid). DNA was bound to a DNA binding matrix (1 ml of glassmilk diluted 1:5 with 6 M guanidine isothiocyanate), then washed by a salt ethanol wash solution (SEWS – ultra-pure 100% ethanol and 0.1 M sodium acetate), and finally, eluted in DNase-free water (DES). Purified DNA extracts were stored at −20 °C until analysis.

The quantity of DNA in the extract was determined by a 150-fold dilution of the extract in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). Such a dilution allowed for quantification of total DNA yields even in the presence of co-extracted and co-purified components such as humic acids, cellular debris and organic solvent residues (Bachoon et al., 2001). The amount of extracted DNA was quantified using highly sensitive fluorescent dye PicoGreen (Life Technologies) (Fornasier et al., 2014) based on measured standards and converted to microbial biomass C (µg g^{-1} of dry peat) according to Blagodatskaya et al. (2014).

The activities of eight enzymes catalysing decomposition of cellulose (β-1,4-glucosidase and β-cellobiohydrolase, i.e. cellulase), hemicellulose (xylosidase), chitin and microbial peptidoglycan (*N*-acetyl-β-D-glucosaminidase), proteins (leucine aminopeptidase, tyrosine aminopeptidase), organic P (phosphatase) and S (sulphatase) were included into the assay. Measurements were done using fluorogenically labelled substrates based on 4-methylumbelliferone (MUF) and 7-amino 4 methylcoumarin (AMC) (for full list of enzymes and related substrates see Supplementary Table S1). Enzyme activities were analysed according to the procedure described by Marx et al. (2005) and German et al. (2011). Depending on the enzyme analysis, MUF or AMC standards were prepared using MES (2-(*N*-morpholino) ethanesulfonic acid) and TRIZMA (tris (hydroxymethyl) amino methane) buffer, respectively (Marx et al., 2001).

In brief, 0.5–1.0 g of fresh peat from each microform and depth horizon ($n = 3$) was weighed into a 100 ml glass bottle with 50 ml of deionized sterile water. Each of the samples was ultrasonicated (Sonopuls 2200, Bandelin Electric, Berlin, Germany) for 2 min at a power of 7 kHz by immersion of about 1–1.5 cm of the probe into the peat solution. The mild ultrasonication was applied to homogenize the peat suspension, disperse microbial cells from peat particles, and to release the extracellular enzymes previously immobilized in peat colloids and/or particles into peat solution (De Cesare et al., 2000; Marx et al., 2001). After sonication, the peat solution was stirred during pipetting on a magnetic stirrer. Each of the wells of the black polystyrene 96-well microplate (Brand GmbH, Wertheim, Germany) received 50 µl of peat suspension, 50 µl MES or TRIZMA buffer, and 100 µl of MUF or AMC substrate. In each microplate, eight concentrations of substrates (0, 20, 40, 70, 100, 200, 400, 1000 µM) and four analytical repetitions were prepared for each replicate. The fluorescence was measured on a fluorimeter (Victor^3, Perkin Elmer, Model 4207072, Waltham, MA, USA) using a protocol with excitation at 355 nm and emission at 460 nm (1.0 s).

2.5. Data processing and estimation of enzyme kinetics (V_{max} and K_m)

The fluorescence reading (relative florescence decomposition min^{-1}, dpm) was converted into nmol MUF/AMC h^{-1} per g dry peat according to the peat-specific standard curve (Marx et al., 2001). The Michaelis-Menten kinetics was applied to analyse enzyme-substrate reactions at increasing substrate concentrations (Michaelis and Menten, 1913).

$$V = (V_{max}*[S])/(K_m + [S]) \quad (1)$$

where V is the best fitted reaction rate as a function of the substrate concentration (S). V_{max} and K_m were calculated after approximating enzyme activities via the Michaelis-Menten Eq. (1) in ModelMaker Version 3.0.3 (ModelMaker, 1997). The K_m mainly describes enzyme activity at low substrate concentrations, which is typical in most soils (Tabatabai, 1994; Nannipieri and Gianfreda, 1998; Davidson et al., 2000; Marx et al., 2001; German et al., 2011). The ratio V_{max} to K_m (K_a) indicates enzyme catalytic efficiency (Tischer et al., 2015). K_a is also known as the specificity constant; this kinetic parameter reflects the intensity and rate of catalytic reactions. Higher K_a indicates better catalytic properties of an enzyme (Moscatelli et al., 2012). K_a was calculated for all enzymes (Supplementary Table S2) and expressed in nmol (MUF/AMC) h^{-1} µmol substrate^{-1}.

2.6. Statistical analysis

Analysis of variance (ANOVA) was conducted using R studio version 3.4.1 (R Core Team, 2017) to evaluate the significant differences ($p < 0.05$) either between microforms of each depth or between depths within one microform. Two-way ANOVA and Tukey HSD post hoc multiple comparisons test were used to evaluate the significance of microform × depth interactions for all variables (Supplementary materials Tables S3–S7). Shapiro-Wilk tests were done with GenStat software (v 16.1) (Payne, 2009) to check normality of data. Levene's test was carried out by R package (function LeveneTest from "DescTool" (Signorell et al., 2016)) to test each variable for the homogeneity of variance across the groups and necessary data transformations were done where applicable. Statistical effects were regarded significant at $p < 0.05$. Linear regressions between selected peat biochemical properties and enzyme activities ($p < 0.05$) were tested by fitting linear model in R using function lm(). All variables are presented as mean values of three replicates with standard error (± SE).

The relationship between K_a of enzymes in three microforms at three depths and the available peat biological (microbial biomass C) and chemical (pH, macroelements content) characteristics were tested by redundancy analysis (RDA) using R-based Multivariate AnalysiS Applications for Microbial Ecology (MASAME), a web-service tool provided by the GUide to STatistical Analysis in Microbial Ecology (GUSTA ME) (Buttigieg and Ramette, 2014). The RDA ordination was presented as a biplot with objects (K_a values) centred. Based on Monte Carlo Permutation ($p < 0.05$), only significant peat biochemical properties were plotted against K_a in the ordination diagram.

3. Results

3.1. Chemical composition of peat and microbial biomass carbon

The pH was acidic under all microforms and increased with depth from 3.9 to 4.6 on average (Table 2). The C and N content increased by 25% and 276%, respectively to the deeper layer in all microforms. Between microforms, C content was similar for each depth horizons, but N content was significantly lower in hummocks at all depths compared to lawns and hollows. The P content was the highest in 50 cm depth, whereas the S and Fe concentrations increased with depth, being 3–4 times greater in the 200 versus top 15 cm for all microforms (Table 2). The stoichiometric ratios of elements in peat strongly decreased from

Table 2
pH, carbon (C), nitrogen (N), C:N ratio, phosphorus (P), sulphur (S) and iron (Fe) contents in peat of three microforms (hummock, lawn, hollow) and three depths (15 cm, 50 cm, 200 cm). Values are the average of three replicates and ± SE within the parentheses. C, N, P, S and Fe were measured as mg g^{-1} of dry peat.

Microforms/depth, cm		pH	Elements content (mg g^{-1})					
			C	N	C:N	P	S	Fe
Hummocks	15	3.90 ^{Ac} (0.11)	448 (3) ^{Ac}	4.6 ^{Cc} (0.4)	98.9 ^{Aa} (17.2)	0.30 ^{Abc} (0.02)	0.45 ^{ABc} (0.06)	0.84 ^{Ab} (0.12)
	50	4.21 ^{Ab} (0.01)	493 (6) ^{Ab}	13.3 ^{Bb} (0.2)	37.1 ^{Ab} (0.5)	0.41 ^{Ca} (0.02)	1.30 ^{Bb} (0.02)	1.14 ^{Ab} (0.03)
	200	4.45 ^{Aa} (0.01)	562 (2) ^{Aa}	16.9 ^{Ba} (0.1)	33.3 ^{Ac} (0.1)	0.34 ^{Cb} (0.01)	1.43 ^{Ba} (0.01)	3.16 ^{Ca} (0.05)
Lawns	15	4.06 ^{Ac} (0.02)	452 (6) ^{Ac}	5.3 ^{Ac} (0.3)	85.4 ^{Aa} (6.9)	0.32 ^{Ac} (0.03)	0.51 ^{Ac} (0.03)	0.46 ^{Bc} (0.03)
	50	4.31 ^{Ab} (0.01)	503 (3) ^{Ab}	15.3 ^{Ab} (0.1)	32.8 ^{Ab} (0.3)	0.49 ^{Aa} (0.01)	1.41 ^{Ab} (0.02)	1.04 ^{Ab} (0.15)
	200	4.59 ^{Aa} (0.01)	566 (6) ^{Aa}	19.1 ^{Aa} (0.2)	29.6 ^{Ac} (0.3)	0.40 ^{Ab} (0.01)	1.54 ^{Aa} (0.03)	3.75 ^{Ba} (0.18)
Hollows	15	4.00 ^{Ac} (0.01)	449 (0) ^{Ac}	5.2 ^{Bb} (0.2)	85.4 ^{Aa} (6.5)	0.27 ^{Bb} (0.04)	0.41 ^{Bc} (0.03)	0.31 ^{Cc} (0.05)
	50	4.21 ^{Ab} (0.01)	508 (0) ^{Ab}	15.1 ^{Aa} (0.3)	33.8 ^{Ab} (1.1)	0.44 ^{Ba} (0.02)	1.15 ^{Cb} (0.05)	1.22 ^{Ab} (0.09)
	200	4.60 ^{Aa} (0.03)	568 (6) ^{Aa}	19.0 ^{Aa} (0.2)	29.9 ^{Ac} (0.2)	0.41 ^{Aa} (0.01)	1.54 ^{Aa} (0.01)	4.16 ^{Aa} (0.12)

Capital letters (^{ABC}) showed significant differences ($p < 0.05$) between three microforms of same depth and small letters (^{abc}) showed significant differences ($p < 0.05$) between three depths within same microforms.

15 to 50 cm depth, especially for the C to P, S, Fe (Fig. S1a) and the C to N ratios (Fig. S1b). In contrast, a pronounced increase was observed for N:P and S:Fe in hummocks and lawns. From 50 to 200 cm, the stoichiometry pattern either decreased (C:Fe, P:Fe, P:S, S:Fe), did not change (C:S, C:N) or increased (C:P). The N:S and N:Fe stoichiometric ratios were similar for all microforms and depths (Fig. S1b). The C:N ratio was three-fold higher at the top (15 cm) compared to bottom layer (200 cm) in all microforms (Fig. S1b).

The highest MBC content was recorded at 50 cm depth in all microforms (Fig. 1). Except for the hummocks and lawns, where similar values were measured at 15 and 200 cm, in hollows the lowest MBC corresponded to 200 cm peat depth. Between the microforms, the largest MBC content was detected in hollows for all three depths. In the top 15 cm, the MBC followed the pattern hollows > hummocks > lawns, with a 3.5-times difference between the highest and smallest value.

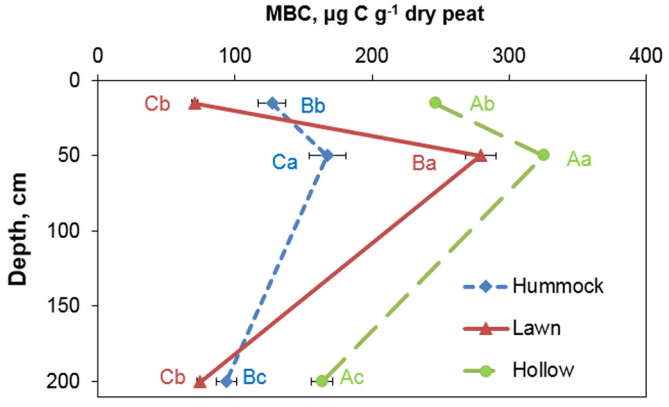

Fig. 1. DNA-based microbial biomass carbon (MBC) content in peat of three microforms (hummocks, lawns, hollows) and three depths (15 cm, 50 cm, 200 cm). Values are the average of three replicates (± SE). Capital letters (ABC): significant differences ($p < 0.05$) between three microforms at the same depth; small letters (abc): significant differences ($p < 0.05$) between three depths within the same microform.

3.2. Effects of microforms and depth on enzyme activities

3.2.1. C-cycle enzymes

β-glucosidase (β-GLU) exhibited the highest activity (V_{max}) at 50 cm in lawns and decreased by 85% in the top- and bottom horizons within the same microform (Fig. 2a). A similar depth pattern was measured in hummocks. In contrast to lawns and hummocks, the β-GLU in hollows gradually decreased by about 70% from 15 to 200 cm depth (Fig. 2a). The Michaelis constant (K_m) followed a reverse pattern compared to V_{max} (Fig. 2b). Accordingly, the lowest K_m was at 15 and 50 cm in all microforms, whereas the highest value was measured at 200 cm in lawns.

The V_{max} patterns of cellobiohydrolase (CEL) were opposite those of β-GLU. The highest activity (256 nM MUF g^{-1} dry matter h^{-1}) was measured in 15 cm depth of hummocks and decreased with depth (Fig. 2c). A similar pattern characterized CEL V_{max} of lawns, although the activity was 33–44% lower as compared to hummocks. V_{max} in hollows was the lowest at 15 cm. Hummocks showed the lowest K_m at 50 cm (Fig. 2d).

Xylosidase (XYL) activities were higher at 50 cm in hummocks compared to lawns and hollows and compared to other depths within hummocks (Fig. 2e). XYL activity was below the detection limit twice: in lawns at 15 cm and hummocks at 200 cm. K_m of XYL in lawns increased with depth, whereas in hollows and hummocks it dropped with depth (Fig. 2f).

3.2.2. N-cycle enzymes

V_{max} of *N*-acetyl glucosaminidase (NAG) was the highest at 50 cm for all microforms, showing the greatest peak in hummocks (Fig. 3a). Lawns and hollows had similar potential activities of N-cycle enzymes. K_m values decreased with depth in hummocks but showed no clear depth-dependence in the other two microforms (Fig. 3b).

Leucine aminopeptidase (LEU) activity was the highest at 15 cm as compared to other depths and decreased in the order hummocks > hollows > lawns (Fig. 3c). For all microforms, V_{max} decreased with depth by 25–48%. K_m values decreased from 15 cm to 50 cm to 200 cm in all microforms (Fig. 3d).

Tyrosine aminopeptidase (TYR) activity showed a depth-dependent pattern similar to NAG; V_{max} was the highest at 50 cm in all microforms (Fig. 3e). The K_m values for lawns were lower than for hummocks and hollows in all depth horizons (Fig. 3f).

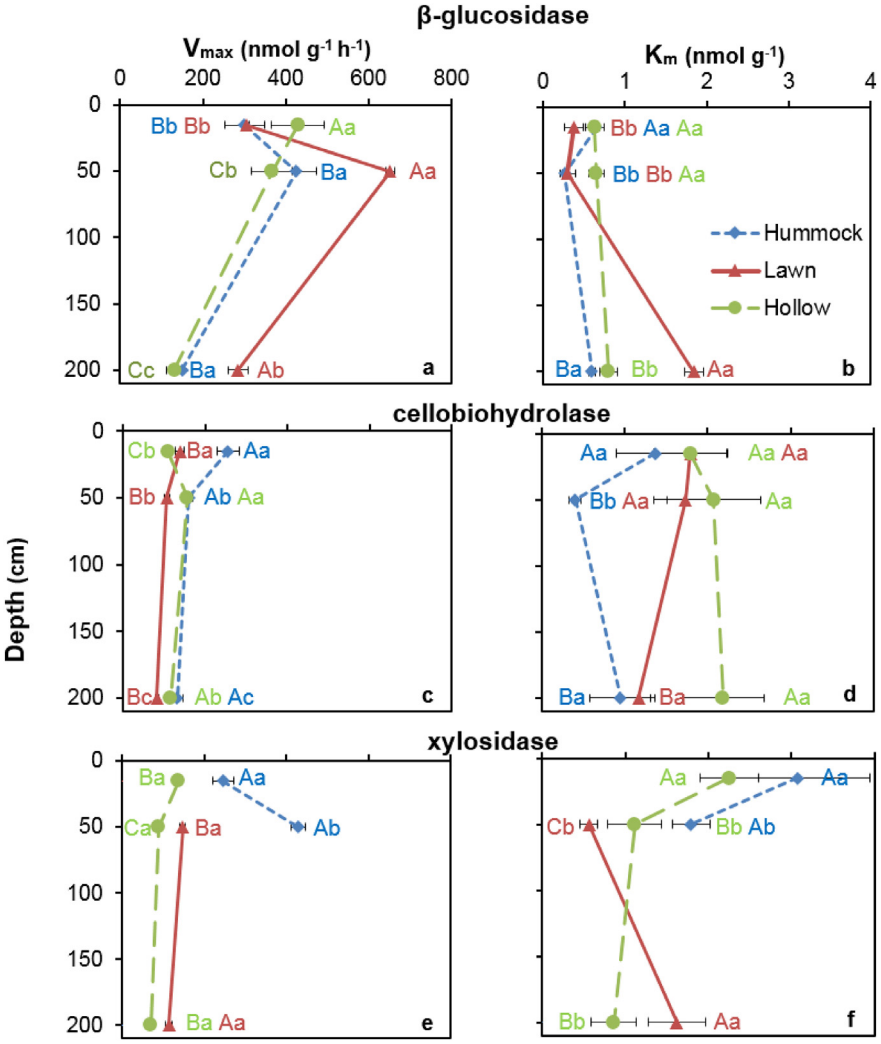

Fig. 2. Maximal potential activity (V_{max}) and Michaelis constant (K_m) of enzymes responsible for the C cycle: β-glucosidase (a & b), cellobiohydrolase (c & d) and xylosidase (e & f) in three microforms (hummocks, lawns, hollows) and three depths (15 cm, 50 cm, 200 cm). Values are the average of three replicates (± SE). Capital letters (ABC): significant differences ($p < 0.05$) between three microforms at the same depth; small letters (abc): significant differences ($p < 0.05$) between three depths within the same microform.

3.2.3. P- and S-cycle enzymes

The highest V_{max} of phosphatase (PHO) was under hummocks in all depth horizons, whereas 1.5–3.0 times lower values were measured in lawns and hollows (Fig. 4a). The lowest V_{max} of PHO was detected in hollows at 15 and 50 cm depths. With depth, values peaked at 15 and 50 cm (hummocks, lawns) as well as at 200 cm (hollows). K_m values were higher in the top peat (15 cm) of hummocks and lawns than at 50 or 200 cm depths (Fig. 4b).

Similar to PHO, the sulphatase (SUL) V_{max} was the highest in all three hummock depths as compared to other two microforms (Fig. 4c). SUL activity decreased in the order of hummocks > hollows > lawns by 14–77%, depending on depth. Within microforms, the highest V_{max} corresponded to 15 cm (lawn) and 50 cm (hummocks, hollows), with the lowest activities at 200 cm in all microforms (Fig. 4c). The K_m values showed similar depth-dependent patterns for hummocks and lawns, being the highest at 15 cm but lowest at 50 cm (Fig. 4d). In contrast, the K_m of hollows was significantly higher at 50 cm than at the other two depths.

3.3. Catalytic efficiency of enzymes and peat characteristics

Peat biological and chemical variables explained 69.2% of the overall variance of the catalytic efficiency (K_a). K_a of C-, N-, P-, and S-cycle enzymes was positively related to the MBC (Fig. 5). The effects of peat pH on K_a were both positive (increasing pH corresponded to higher K_a values of PHO, XYL, β-GLU and TYR) and negative (NAG, LEU, CEL, SUL). Among the measured peat macronutrients, total P concentration strongly regulated K_a of TYR, PHO, XYL and β-GLU. A strong negative correlation was found between peat organic C and K_a of CEL, NAG, SUL and LEU. The redundancy analysis revealed that K_a was best explained by the peat characteristics and MBC in hummocks and lawns at 50 cm depth (Fig. 5).

4. Discussion

4.1. Microbial biomass distribution between and within peatland microforms

Based on the differences in the water table level and vegetation on microforms, we hypothesized that the MBC content would decrease in the order: hummocks > lawns > hollows. However, the MBC pattern differed from the expected: hollows > lawns > hummocks (Fig. 1). Higher MBC from hollows could be attributed to the abundance of anaerobic microbial communities (methanogens) arising from higher water table (Lai, 2009). Both vegetation types and depth of water table have been shown to influence microbial activity and slight alterations of the plant community composition/water table drawdown can have profound effect on microbial processes such as methanogenesis

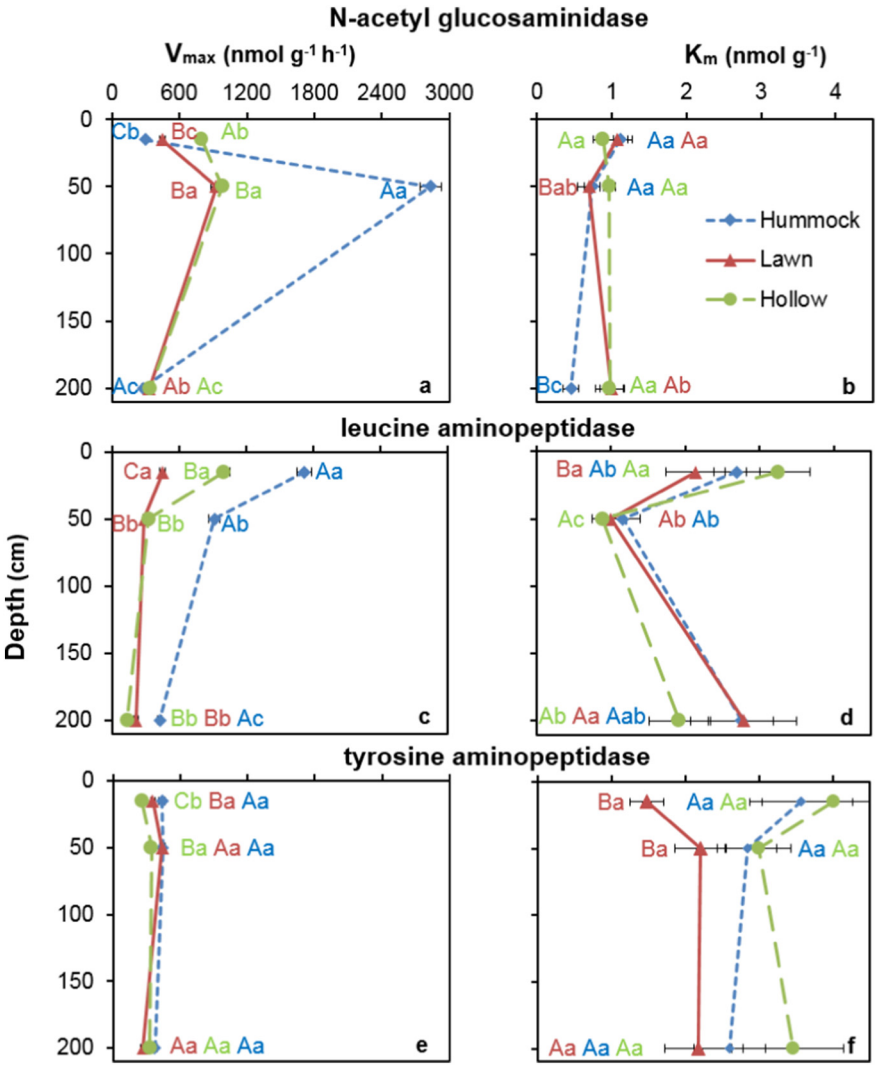

Fig. 3. Maximal potential activity (V_{max}) and Michaelis constant (K_m) of enzymes responsible for the N cycle: *N*-acetyl glucosaminidase (a & b), leucine aminopeptidase (c & d) and tyrosine aminopeptidase (e & f) in three microforms (hummocks, lawns, hollows) and three depths (15 cm, 50 cm, 200 cm). Values are the average of three replicates (± SE). Capital letters (ABC): significant differences ($p < 0.05$) between three microforms at the same depth; small letters (abc): significant differences ($p < 0.05$) between three depths within the same microform.

(Strakova et al., 2011; Preston et al., 2012). For example, the hollows-adjacent vascular plant *Scheuchzeria palustris* contributed 4 times more to methanogenesis from recent photosynthates (based on ^{14}C-labeling) than hummocks- and lawns-adjacent *Eriophorum vaginatum* (Dorodnikov et al., 2011). However, if water table fluctuates so that the aerobic conditions are prevailing in peat long enough, the putative anaerobes - methanogens - will be damaged and recovery of CH_4 production takes a long time even there are substrates available (Waddington et al., 1996). Apart of the methanogenesis, the potential difference in rhizodeposition, e.g. C input, between microforms, can be more relevant for the microbial abundance as the oxygen availability.

Microbial biomass did not gradually decrease in any microform with depth. Rather, MBC increased between 15 and 50 cm depth (Fig. 1). The stoichiometric ratios of the sets of elements revealed a sharp change from the top peat to 50 cm depth. This was the best depicted by ratios between C and N, P, S and Fe (Fig. S1). Seemingly, 50 cm was the depth where organic remnants and root deposits of vascular plants were combined with sufficient nutrients to stimulate substrate decomposition and growth of (anaerobic) microorganisms (Artz et al., 2008). In contrast, oligotrophic, oxygen-rich conditions occurred at the top of the peat profile of hummocks and lawns with wider C:N ratio, as depicted by the distribution of N, P and S with depth (Table 2) and by the generally higher stoichiometric ratios of elements in the top peat (Fig. S1). Moreover, increased competition with plants for the available nutrients strictly regulated the abundance of microorganisms. Lower MBC in the top peat layer as compared to deep peat may also be related to higher aerobic biomass turnover in upper peat than anaerobic turnover in deeper layers.

Below 50 cm (down to 200 cm) the easily decomposable organic substrates became scarce (Strakova et al., 2011). The low availability of such easily decomposable substrates could induce the replacement of the bacterial community by archaeal groups/fermenting anaerobes within the microbial biomass under anoxic condition (Preston et al., 2012). The increasing fraction of not only methanogens but also fermenting anaerobes with peat depth shifts the capacity to produce extracellular enzymes (Preston et al., 2012, Gittel et al., 2014, Lin et al., 2014a, 2014b) with the release of organic acids and hydrogen in deep peat layers. These fermentation products can then (partly) be used in methane production (Yavitt and Seidman-Zager, 2006). This potential shift from aerobic to anaerobic microbial community composition and functionality, may affect the enzymatic system in contradiction to hypothesized trend (see below).

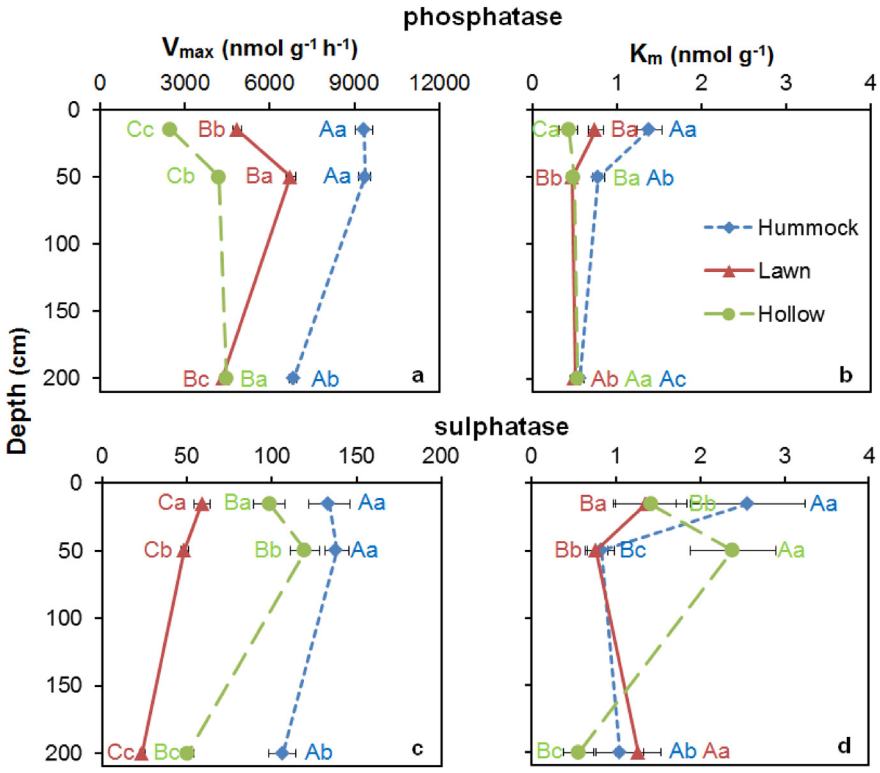

Fig. 4. Maximal potential activity (V_{max}) and Michaelis constant (K_m) of enzymes responsible for P and S cycles: phosphatase (a & b) and sulphatase (c & d) in three microforms (hummocks, lawns, hollows) and three depths (15 cm, 50 cm, 200 cm). Values are the average of three replicates (± SE). Capital letters (ABC): significant differences ($p < 0.05$) between three microforms at the same depth; small letters (abc): significant differences ($p < 0.05$) between three depths within the same microform.

4.2. Effects of microtopography and peat depth on enzyme activities and catalytic efficiency

4.2.1. C-cycle enzymes

The distribution of enzyme activities (V_{max}) in the top peat between microforms (Fig. 2a, c, e) showed an unexpected pattern: there was no clear relationship between V_{max} and the naturally established degree of aeration from drier hummocks to wetter hollows. With regard to depth, we expected to have higher V_{max} at the top layer and decreasing values at deeper layers. Such a trend was recognized in hollows but only partly in hummocks and lawns (Fig. 2a, c, e). Interestingly, β-GLU was strongly positively related to MBC in all microforms, although for XYL and especially CEL the relationship with MBC was weak (Fig. S2).

It is important to note, we observed either no significant relationship or even a negative pattern between C content and C-acquiring enzymes (Fig. S3, left y-axis). Along with the potential changes in microbial community structure, the feasible explanation is related to changes in the quality of decomposable organic matter (Blagodatskaya et al., 2014). The increase of C content with peat depth is usually accompanied by the relative increase of lignin but decrease of cellulose and hemicellulose (Hargreaves and Hofmockel, 2014). Although we did not measure these constitutional compounds of SOM, deeper peat is older and has typically lower level of labile carbon. Thus, microbial communities at deeper layers have relatively limited access to easily-available substrate (Artz et al., 2008). So, the lack of relationship or the decrease of V_{max} of β-GLU, XYL and CEL with peat C content may reflect a change in the peat quality between microforms and with depth, at least between the top 50 cm layer and 200 cm.

The relationship between K_m and MBC is a functional property of microbial communities (Tischer et al., 2015; Razavi et al., 2016). An overall negative relation (Fig. S2) corresponds to an increased substrate affinity with increasing microbial biomass and can be related to competition between microorganisms for organic substrates. However, the relationship between K_m of C-enzymes and peat C content was rather multidirectional (Fig. S3, right y-axis). Therefore, contrasting substrate quality between top and deeper layers – along with a possible shift in the microbial community – can be responsible for changes in enzyme catalytic properties and enzyme kinetics (Bodelier and Dedysh, 2013; Loeppmann et al., 2016).

4.2.2. N-cycle enzymes

NAG activity peaked in all microforms at 50 cm and the V_{max} decreased from hummocks to lawns (Fig. 3a). Since fungal chitin and bacterial peptidoglycan are abundant components of microbial cell walls, the increased NAG activity in hummocks may reflect the fast turnover and re-utilization of microbial biomass. These results are in line with the higher abundance of actinobacteria (Gram-positive bacteria containing a thick peptidoglycan layer) in the upper 40 cm of hummocks compared with lawns and hollows of several boreal peatlands (Kotiaho et al., 2013). Along with actinobacteria, fungi may additionally contribute to higher NAG activity in more aerated environments (e.g. hummocks) versus anoxic hollows (Jaatinen et al., 2007). NAG activity could also be induced under a variety of scenarios leading to microbial N limitation, including chemical sequestration of N through humification of peat organic matter (Wang et al., 2015). No clear relationship, however, was detected between peat N content and N-cycle enzymes (Fig. S5). Hence, the availability of other nutrients such as P may primarily control the microbial activity.

The activity of LEU gradually decreased with depth and followed a similar pattern in hummocks, hollows and lawns (Fig. 3c). This was the only tested enzyme that agreed with the hypothesized depth-dependent decrease. This pattern may reflect the effect of fresh aboveground plant remnants at the top of peat profile. They contain 2–5 times more proteins/amino acids than root litter and are easily decomposable and thus often decreased in processed organic matter (Fuchsman, 1980). There was a strong negative correlation between LEU and peat N concentration in all microforms (Fig. S5). The reduced LEU activities corresponded to an increase in the inorganic N source (i.e. NH_4^+, data not presented) with depth. We therefore conclude that the availability of inorganic N regulates the secretion of LEU enzymes by microorganisms (Currey et al., 2010).

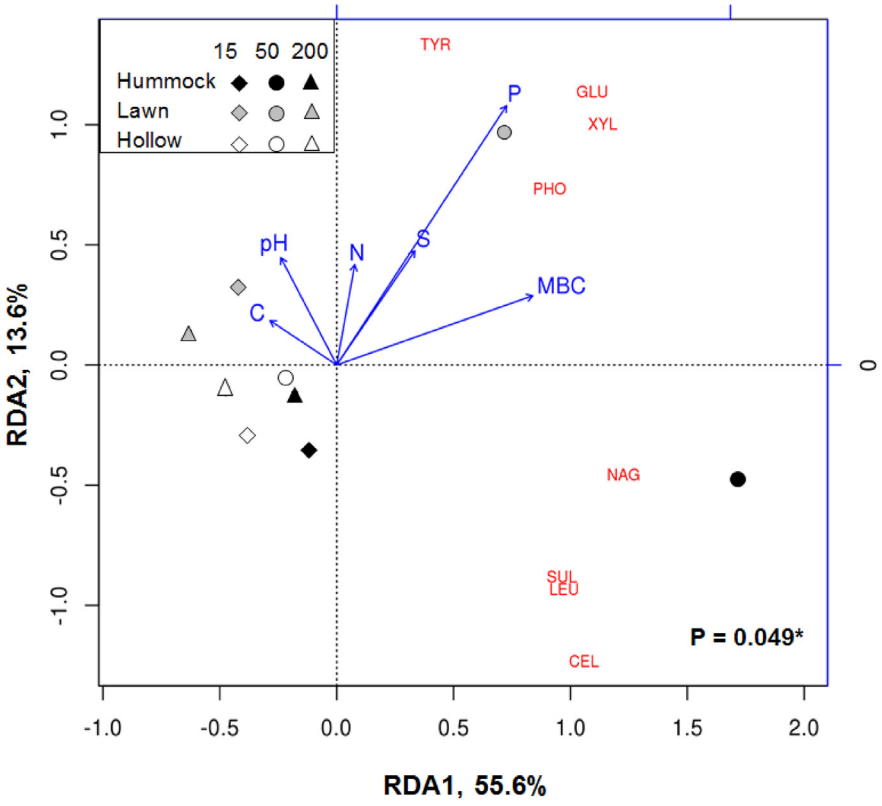

Fig. 5. Biplot of a redundancy analysis (RDA) showing a relationship between catalytic efficiency ($K_a = V_{max}/K_m$) of peat C-cycle enzymes (β-glucosidase – GLU, cellobiohydrolase – CEL, xylosidase – XYL), N-cycle enzymes (*N*-acetyl glucosaminidase – NAG, leucine aminopeptidase – LEU, tyrosine aminopeptidase – TYR) and P-, S-cycle enzymes (phosphatase – PHO, sulphatase – SUL) of three microform types (hummocks, lawns, hollows) at three depth horizons (15 cm, 50 cm, 200 cm) and the selected peat characteristics – microbial biomass C (MBC), pH, macronutrients (C, N, P, S). The first two axes explain 69.2% of the variability in the K_a data. Significance of the permutation test for RDA under the reduced model was determined at $p < 0.05$. K_a was measured as nmol (MUF/AMC) h^{-1} μmol substrate^{-1}.

4.2.3. *P- and S-cycle enzymes*

In contrast to C- and N-cycle enzymes, the V_{max} of PHO (averaged across all depths) decreased in the order hummocks > lawns > hollows. This agreed with the hypothesized pattern (Fig. 4a). PHO activity strongly correlated with MBC in hummocks and lawns (Fig. S2). Microorganisms require initial hydrolysis by PHO to take up P from organic sources such as plant litter or microbial residues. The increased PHO activity thus suggested microbial P mining under low P conditions in all microforms (Shackle et al., 2000). Study by Margalef et al. (2017) reported that boreal peatlands are extremely rich in organic matter in which P is trapped and thus unavailable to both plants and microbes. PHO activity was greater than C-, N-, and S-cycle enzymes throughout the peat profile, highlighting a strong P limitation. Therefore, P limitation in the studied peatland appeared to be a driving force for the observed distribution of microbial biomass and its activity (see below).

SUL activity was lower in all microforms than C-, N- and P-acquiring enzymes. This was accompanied by the lack of any significant relationship between V_{max} of SUL and total S content (Fig. S6, bottom left). Similarly, no clear pattern was detected between peat S content and K_m in any microform (Fig. S6, bottom right). These results suggest either the availability of inorganic S sources (Wieder and Lang, 1988) or generally low S requirements by microbial metabolism, irrespective of microtopographic position or oxygen access.

4.3. *Effects of microtopography and peat depth on catalytic efficiency of enzymes*

The variation in catalytic efficiency (K_a) was largely explained by MBC and P concentration (Figs. 5 and 6). In peatlands, this phenomenon depends on the heterogeneous distribution of nutrients and labile organic substrates in the profile. Inputs of fresh plant materials lead to a high content of easily available C sources in nutrient-poor (N, P, S) top peat. With increasing depth, C sources become more recalcitrant and nutrient contents increase (Table 2). The optimal combination of C and nutrient availability was met in 50 cm depth below hummocks and lawns, whereas such conditions occurred in the top 15 cm of hollows. The correspondingly higher microbial biomass compared to other depths coincided well with the peat P content (Fig. 6). Therefore, the catalytic efficiency of the microbial enzymatic system was strongly P limited. P limitation causes microbial resource allocation to P acquisition (Lin et al., 2014b). With decreasing P limitation, decomposers apparently invest in C-acquiring enzymes that increase the rate of organic matter decomposition (Zak et al., 2010; Strakova et al., 2011). From the ecological perspective, peat SOM decomposition may be accelerated by stimulated microbial activity – if the availability of P in peatlands increases. This has significant implications towards C gains and losses from peatland ecosystems and global C budget. Further studies are warranted to determine the effect of P availability in peats to understand the changes in the stoichiometric relationships, microbial activity and thus the response to environmental changes.

5. Conclusions and outlook

The effects of microrelief forms in a boreal peatland (hummocks, lawns, hollows) and the corresponding biophysical conditions (plant communities and water table level) on the distribution and activities of peat microbial biomass can be summarized as follows:

- Aeration gradient from drier hummocks to wetter hollows did not affect microbial biomass (MBC) and potential enzyme activities (V_{max}) in the top 15 cm of peat. Rather, MBC content controlled Michaelis constant (K_m) and the catalytic efficiency of enzymes (K_a).
- The hypothesized gradual decrease of V_{max} with depth was measured for C- and N-acquiring enzymes in water-saturated hollows. MBC, however, was highest at 50 followed by 15 and 200 cm depth in all microforms.
- Phosphorus (P) availability was the main driver regulating microbial catalytic efficiency. The relatively high phosphatase activities compared to C-, N- and S-acquiring enzymes throughout peat profile suggested a high metabolic demand for P.

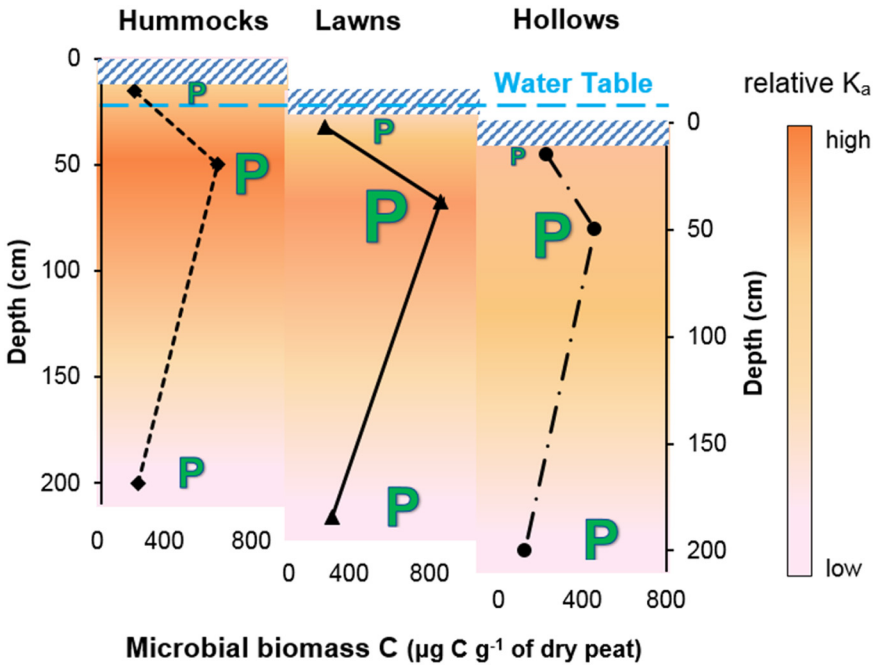

Fig. 6. Relative catalytic efficiency (K_a) of all enzymes (gradient color background) as related to microbial biomass C and total peat P content of three microform types at 15, 50 and 200 cm depths. Dashed blue line indicates the water table level. Font size demonstrates the relative difference in P content between microforms and with depth. Diagonal pattern area represents the top peat (0–15 cm), where the catalytic efficiency of enzymes may substantially differ from the proposed K_a distribution in the peat profile. K_a was measured as nmol (MUF or AMC) h^{-1} µmol substrate^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

While the water table (i.e. aeriation) controls the formation of peatland microforms, there were no direct effects on microbial biomass and functioning. Changes in activity and catalytic efficiency of enzymes with peat depth were mainly linked to nutrient and substrate availabilities, P in particular. This indicates that peatland ecosystems are highly vulnerable to changes in nutrient supply. Anthropogenic P inputs through leaching of fertilizers or atmospheric deposition may stimulate microbial activity and thus increase peat decomposition. A change in the nutrient and C balance of this biome eventually would feed back on the global climate system.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2018.03.006.

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