Vertical Profiles of Soil Properties and Microbial Activities in Peatbog Soils in Slovakia

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Abstract Peatlands are unique ecological communities that represent long-term terrestrial carbon sinks with enormous organic substance supply. Soil organic substance represents the main source of organic carbon in the biosphere, and depending of conditions, it is able to eliminate or sequester greenhouse gasses in the environment. Current methods of peatbogs assessment rely on the use of ecological indicators, but often lack an in-depth analysis of soil parameters. The objective of this study was to examine vertical profiles of selected soil properties (soil reaction, soil organic carbon content, soil moisture) and various types of microbial activity (soil basal respiration, microbial biomass carbon, activity of soil urease, phosphatases and catalase). Soil samples were collected from three sites (centre of peatbog, edge of peatbog and site closely surrounding peatbog) on the peat soil at three depth intervals (0–0.10 m, 0.10–0.20 m and 0.20–0.30 m) in northeast of Slovakia. Enzyme and microbial activities decreased with depth, but significant correlation was found with alkaline phosphatase. Average values of most soil biochemical indices were highest at the centre of peatbog with the exception of urease and catalase activity. Our results indicated that enzymatic and biological potential for organic matter mineralization was strongly correlated to soil pH, soil moisture and soil organic matter content.

Keywords Peatbogs · Chemical properties · Biological properties · Soil enzyme activity · Soil depth

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1 Introduction

Peatlands are one of few long-term terrestrial carbon sinks that play an important role in maintaining water and soil quality. These ecosystems, together with wetlands are highly endangered by consequences of recent climatic processes and anthropogenic influence (Kovács 2013). The interest on the function of peat soils in global carbon turnover has increased due to the threat of global climatic change and studies of sinks and sources for CO₂ and other trace gases relevant for climatic effects (Brake et al. 1999; Golovchenko et al. 2007). Agricultural use of peat soils employs extensive drainage and leads to subsidence of such land, and is the primary cause of degradation and loss of peat resources. Flora, the activity of microorganisms and thermal conditions affect conditions in peat soils. Microorganisms together with flora specify the direction and nature of biochemical processes. Soil microbiota predominantly manage carbon and nitrogen organic fluxes (Chen et al. 2014; Tsoleva et al. 2014), where soil enzymes are associated with microorganisms and their activity in soil plays an important role in catalysing reactions indispensable in life processes of soil microorganisms, decomposition of organic residues, circulation of nutrients as well as forming organic matter and soil structure (Sinsabaugh et al. 1994). Generally, evaluation of microbial parameters in the soil ecosystems is considered to be the best indicator of soil quality that leads to all soil property changes, and thus, early detection of soil degradation (Macci et al. 2012; Romaniuk et al. 2011). The stability of the carbon stock contained in peatlands has recently been questioned and several studies suggest that loss of carbon from soil ecosystems contribute significantly to CO₂ emissions. Field experiments show that CO₂ loss and soil microbial activity in peat soils is seasonal and strongly depends on environmental factors such as temperature, soil water content, peat type (i.e., organic carbon availability) and depth of soil horizon (Bellamy et al. 2005; Kechavarzi et al. 2010). The living and the most active portion in the soil ecosystems are microbes that are responsible for transformation and nutrient storage. In general, peatbogs are thick, and therefore, it is important to understand nutrients and energy flows, and their relationships with microbial activity both in shallower and deeper layers (Senga et al. 2011). Soil organic carbon, respiration rate, microbial biomass content and soil enzymatic activities are among the parameters whose sensitively influences biochemical processes in the soil ecosystem, and therefore, are used in many studies (Brookes 1995; Gavrilenko et al. 2011). Peat soil is understood to be the upper, most active layer of peatbog, in which the roots of recent plants are found, and which is above the long-term average annual level of the groundwater. However, it is difficult to apply the data widely because the organic matter of peat soils in climatic regions varies in composition due to variations in the initial substrate and in the conditions of microbial decomposition (Brake et al. 1999).

This work considers differences in soil properties due to vertical profile and different sampling sites. Our study, therefore, had two goals: (1) to assess the relationships between soil microbial indices and some physical and chemical properties; and (2) to investigate activity of soil microbial community from the surface to the deeper layers by examining vertical profiles of peat soil in the investigated area.

2 Materials and Methods

The study was carried out on the peatland in the region of north-eastern Slovakia in Liptovská Teplička (48°57.83' N; 20°06.24' E). The area of Liptovská Teplička is situated in the Low

Tatras National Park at an altitude ranging from 846 to 1492 m a.s.l.. Climatically, the area is situated in the mild cold zone with an average temperature during the period of vegetation growth (i.e., months IV to IX) 10–12 °C and an average annual precipitation of 700–1200 mm (Fazekašová et al. 2013). Samples for determination of chosen soil properties were taken in spring time at two sites from peat soil (centre of peatbog and edge of peatbog), and one site that closely surrounded our observed peatbog from different depths (0–0.10 m, 0.10–0.20 m and 0.20–0.30 m). Soil samples were air dried, sieved (sieve with 2 mm size opening), homogenized prior to analyzing of measured soil properties and soil enzyme activity. Soil pH was determined by the potentiometric method in 0.01 M CaCl₂ solution. Organic carbon content was determined by the Tiurin's method (Fiala et al. 1999) and soil moisture was determined by drying soil samples in the oven at 105 °C until constant weight. Activity of acid and alkaline phosphatase was determined according to Grejtovský (1991) and urease activity according to Khaziev (1976). Activity of phosphatases were colorimetrically determined as a phenol release after the incubation of soil samples with phenyl phosphate solution and acetate buffer (for acid phosphatase), or borate buffer (for alkaline phosphatase) for 3 h at 37 °C. Phenol release was provided by spectrophotometer at 510 nm. Activity of soil urease was colorimetrically determined as an ammonia release after the incubation of soil samples with urea solution for 24 h at 37 °C. Ammonium determination was carried out by spectrophotometer at 410 nm. Basal respiration, soil microbial biomass carbon and activity of soil catalase were performed on freshly collected soil samples sieved through 2 mm mesh size. Soil basal respiration was measured as the amount of carbon dioxide released from 50 g of fresh soil at 25 °C for an incubation period of 24 h which was absorbed in 25 mL 0.05 N NaOH. The amount of carbonate was determined by the titration with 0.05 N HCl after precipitation of carbonates by 5 mL of 0.5 N BaCl₂ (Isermeyer 1962, in Alef and Nanniperi 1995). Soil microbial biomass carbon was determined using the microwave irradiation method according to Islam and Weil (1998). 10 g oven-dried equivalent (ODE) of field moist soil adjusted to 80 °C water-filled porosity was irradiated twice by microwave (MW) energy at 400 J g⁻¹ ODE soil to kill the microorganisms. The time settings and MW oven power depended on the total amount of soil in the MW oven. After cooling, soil samples were extracted with 25 mL of 0.5 M K₂SO₄ and carbon content in the extract was quantified by the oxidation with K₂Cr₂O₇/H₂SO₄, measuring the absorption at 590 nm using a spectrophotometer. The same procedure was done with a non-irradiated sample. The microbial biomass carbon was then calculated as $C_{mic} = (C_{irradiated} - C_{non-irradiated}) / K_{ME}$, whereby Islam and Weil (1998) recommended the extraction efficiency factor $K_{ME} = 0.213$. Activity of soil catalase was measured 10 min after adding 20 mL of 3 % H₂O₂ was added to 10 g of fresh soil sample based on the volume of discharged oxygen, compared to the respective sample where microbial activity was suppressed by overheating at 180 °C, to account for H₂O₂ dissociation caused by soil Fe and Mn oxides (Khaziev 1976).

Obtained data were tested by mathematical-statistical methods from which regression analysis was used (the Statgraphic software package).

3 Results and Discussion

Generally, the pH of peat soils is very low and could vary between 2.7 up to 7.0 (Brake et al. 1999; Grodnitskaya et al. 2013), but our study found a slight tendency of pH to be higher at our sites and varied between 6.5 and 7.0 on peat soil and 7.2 on the surroundings site (Table 1).

Lower soil reaction was found in the centre of peatbog at deeper profiles. Content of organic carbon ranged from 3.16 to 29.41 % and the highest amount of this parameter was measured in the centre of peatbog, where its value increased with depth (Table 1). These sites are considered to be the most active in synthesis and decomposition of organic matter, and microbial activity is at high level. A similar trend was observed with soil moisture content and these three parameters had a significant effect on many other soil biological parameters, which is confirmed by several studies (Brockett et al. 2012; Duguma et al. 2010; Speir et al. 2003). Generally, many authors studied soil microbial activity in terms of depth of soil profiles. They found that microbial parameters decrease with the soil depth (Brake et al. 1999; Raiesi and Riahi 2014; Senga et al. 2011; Speir et al. 2003), which was also shown in our study. Activity of soil enzymes at the two peat sites and the site that surrounded the peat soil was higher in the shallower layers. Enzymes in the soil ecosystem might be produced by micro-organisms and also by higher-order plants. High activity of enzymes in surface layers may be a result of the presence of higher plant roots. The relationship between soil microbial enzyme activities and humic substances, or other soil parameters, has not yet been fully clarified. Soil enzyme activity levels are indicators of microbial status and soil health, and can be useful in predicting the impact that nutrient loading has on ecosystems, such as peatbogs, wetlands or aquatic ecosystems (Prenger and Reddy 2004; Sardans et al. 2008). Soil phosphatases play a very important role in the mineralisation processes of organic phosphate substrates and in catalysing decomposition of organic phosphorus. They differ in pH that is influenced by many factors, and thus, are recognized as acidic and alkaline phosphatases (Kumar et al. 2011). Activity of acidic and alkaline phosphatases were highest at the site that was located in the centre of the peatbog, but surprisingly, the activity of urease and catalase showed highest values at the site which surrounds the peatland (Table 1). The most extended enzyme in microbial, plant and animal cells is urease that is very stable and catalyses the urea hydrolysis. Bandick and Dick (1999) showed that high content of soil urease could be mainly caused by high organic carbon content that protects microbial biomass and by other physico-chemical properties (Fazekašová et al. 2012; Moreno et al. 2001). In our study, the activity of soil urease was lower in the peat soil than at sites surrounding the peatbog. This could be due to the high soil moisture content in the peat soil which, according to several authors, rapidly decreases microbial activity in the soil ecosystem (Brockett et al. 2012). A similar trend was observed

Table 1 Characteristics of peat soil in studied sites

Site	Depth	pH	Mois	C _{ox}	Ure	ACP	ALP	Cat	C _{res}	C _{mic}
Centre of peatbog	0.0–0.10 m	6.9	35.3	20.28	0.25	465.26	348.56	1.34	255.59	720.82
	0.10–0.20 m	6.5	44.5	25.55	0.21	452.06	320.68	1.12	213.18	853.62
	0.20–0.30 m	6.6	49.7	29.41	0.12	384.99	302.56	0.55	206.99	834.66
Edge of peatbog	0.0–0.10 m	6.8	21.4	11.19	0.29	288.48	343.71	1.36	192.72	531.22
	0.10–0.20 m	7.0	15.9	12.78	0.17	436.22	431.93	1.40	178.33	417.32
	0.20–0.30 m	6.9	16.1	11.56	0.09	256.53	210.97	0.49	148.92	397.82
Site surrounding peatbog	0.10–0.20 m	7.2	12.7	3.22	0.49	325.41	341.10	2.41	136.13	341.44
	0.20–0.30 m	7.2	11.2	3.16	0.37	321.98	337.30	1.51	139.87	341.44

Moisture (Mois) [%], Organic carbon content (C_{ox}) [%], Urease activity (Ure) [mg NH₄⁺-N g⁻¹ d⁻¹], Acid phosphatase activity (ACP) [mg P g⁻¹ 3 h⁻¹], Alkaline phosphatase activity (ALP) [mg P g⁻¹ 3 h⁻¹], Catalase activity (Cat) [mL O₂ g min⁻¹], Basal respiration (C_{res}) [μg CO₂ g h⁻¹], Microbial biomass carbon (C_{mic}) [μg C g⁻¹]

with the catalase activity that decomposes hydrogen peroxide and it reflects the biochemical activity in soil (Gömöryová 2004). Literature data point out that among soil physical and chemical properties, the strongest connections with enzymatic activity are indicated by organic carbon and nitrogen contents (Dick et al. 1996). Bielińska et al. (2003) and Blońska (2010) showed high microbial activity in bog soils, which was also proven in our study. This was associated by the authors to the content of organic carbon, total nitrogen and phosphate. Soil respiration is considered to be a well-established parameter for monitoring decomposition in the soil, but it is highly variable and can fluctuate widely depending on substrate availability, organic matter and moisture content (Truu et al. 2009), which agreed with our findings. Soil respiration reflects the overall biological activity and rates of mineralization processes. The obtained soil basal respiration rate at the different sites on our peat soil is shown in Table 1. Carbon mineralization is highly sensitive to temperature and moisture changes and gives this parameter variable rates between the seasons (Gömöryová 2004; Zhang et al. 2009). In the peat soil, the respiration rate decreased with increasing soil moisture. Excess of soil water prevents gas exchange, reduces oxygen content in the soil air that makes anaerobic conditions for microscopic fungi, actinobacteria and aerobic bacteria (Fuhrer 2003). This parameter shows the highest values on the site which is localized at the centre of peatbog, where carbon mineralization is very fast and decreases with increasing depth. These results agree to those of other authors (Maková et al. 2011; Klose et al. 2004), and from various types of ecosystems.

The similar trend was observed after determination of soil microbial biomass carbon and these findings were alike to those of Kechavarzi et al. (2010). Soil microbial biomass is primary factor responsible for the soil ecosystem decomposition of organic matter, nutrient transformation and energy flow. Similar to soil respiration, it is very variable to changes in the environment and thus these two parameters are considered for soil quality and health evaluation (Dick et al. 1996; Macci et al. 2012). Because of the moisture and high organic carbon content of peatbogs, these soils provide a unique and variable environment for microorganisms; despite this fact, the soil microbial activity is very high. The high organic matter content of these soils results from the accumulation of partially decayed plant and animal remains under waterlogged conditions (Robert and Tate 1980).

A correlation analysis identified significant relationships between several soil parameters (Table 2). Soil moisture was positively correlated with soil organic matter, microbial biomass and soil respiration. In contrast, soil moisture was negatively correlated with soil pH and catalase activity. The study by Rokosch et al. (2009) showed negative correlation with other enzymatic activities. However, Miralles et al. (2012) showed a positive correlation between soil moisture and many enzymatic activities that was not seen in our study. This finding presents the need for studying soil parameters and their relationship in the soil ecosystem. It is not surprising that soil moisture is correlated with many of the soil parameters we measured because of influence on the accumulation and cycle of soil organic matter. Anaerobic conditions occur under saturated conditions, and by limiting microbial decomposition, they enhance organic matter and organic accumulation. Tate and Terry (1980) studied soil microbial activity in histosols and found correlation between enzymatic activity and soil moisture, and concluded that moisture could be a limiting factor for microbial activity. Soil biological indices were positively correlated with soil organic carbon, while they were only negatively correlated with soil reaction measured in this study. Similar findings by other authors (Gömöryová et al. 2009; Maková et al. 2011) showed a very close relationship between chemical and biological soil parameters. Wide variations in the values of soil pH cause changes in physical and chemical soil properties, and thus, significantly influence microbial carbon and mineralization

Table 2 Correlation between physico-chemical soil properties and soil biological indices

Parameter	Depth	pH	Mois	C _{ox}	Ure	ALP	Cat	C _{res}	C _{mic}
Depth	–	ns	ns	ns	ns	–0.743*	ns	ns	ns
pH	ns	–	–0.927**	–0.783*	ns	ns	0.747*	–0.771*	–0.921**
Mois	ns	–0.927**	–	0.904**	ns	ns	–0.738*	0.857**	0.946**
C _{ox}	ns	–0.783*	0.904**	–	ns	ns	ns	0.809*	0.898**
Ure	ns	ns	ns	ns	–	ns	0.857**	ns	ns
ALP	–0.74*	ns	ns	ns	ns	–	ns	ns	ns
Cat	ns	0.747*	–0.738*	ns	0.857**	ns	–	ns	ns
C _{res}	ns	–0.771*	0.857**	0.809*	ns	ns	ns	–	0.922**
C _{mic}	ns	–0.921**	0.946**	0.898**	ns	ns	ns	0.922**	–

** $p < 0.01$, * $p < 0.05$

Moisture (Mois) [%], Organic carbon content (C_{ox}) [%], Urease activity (Ure) [mg NH₄⁺-N g^{–1} d^{–1}], Acid phosphatase activity (ACP) [mg P g^{–1} 3 h^{–1}], Alkaline phosphatase activity (ALP) [mg P g^{–1} 3 h^{–1}], Catalase activity (Cat) [mL O₂ g min^{–1}], Basal respiration (C_{res}) [μg CO₂ g h^{–1}], Microbial biomass carbon (C_{mic}) [μg C g^{–1}]

of soil organic matter. The most significant impact on soil microbial indicators, except soil moisture, has the organic carbon content (Duguma et al. 2010; Gavrilenko et al. 2011; Raiesi and Riahi 2014), something also seen in our study. The same authors showed that the high correlation coefficient between soil organic carbon and soil enzymatic activity confirms the stability of enzymes mediated by organic matter in soils. Nowadays, there is an ongoing discussion about correlations between soil urease activity and other parameters of biological activity in soil ecosystem. Alef and Nannipieri (1995) and Miralles et al. (2012) found a close correlation of soil urease with soil respiration and microbial biomass carbon, which was also observed in our study; however, other authors have not observed this (Klose et al. 2004). There was a positive correlation between urease and catalase activity, but on the other hand, depth of soil profile negatively correlated only with one soil enzyme (alkaline phosphatase). In terrestrial ecosystems, some of biological soil parameters have proven to be reliable indicators of soil health and quality, and can be considered as early indicators of changes in soil properties due to land management. In these systems, soil microbial biomass, carbon mineralisation and enzyme activity often decrease as a result of anthropogenic disturbances (Gil-Sotres et al. 2005). These biological and microbial indices are generally positively correlated with soil organic matter, something also observed in our study. Positive correlation between phosphatases and soil reaction shows increasing microbial populations, because these enzymes are not produced by plant roots (Javoreková et al. 2011).

4 Conclusions

It is very important to monitor soil biological parameters at different soil profiles, which present an objective overview about soil status, health and quality, and a way to find out the distribution of microbial communities and their relationship to other soil parameters. There was a clear depth effect on soil properties, but statistically significant effect was found on alkaline phosphatase. The most significant differences in soil properties were observed between the sites of peat soils and the site that surrounded the studied peatland. The main

limiting factors of soil microbial activity are the soil reaction, soil moisture and soil organic carbon content. The microbial biomass, and also various enzymatic and process activities, are well proven indicators of soil status and are widely used in soil microbial ecology studies as easy to measure and informative parameters. Peat soils are considered to be a unique environment for soil microbes because of the moisture and high content of soil organic matter.

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