School of Doctoral Studies in Biological Sciences
University of South Bohemia in České Budějovice
Faculty of Science

Effect of peatland plant dominants on quality and quantity of organic matter in spruce swamp forest soils

Ph.D. Thesis

Mgr. Jiří Mastný

Supervisor:

Ing. Tomáš Picek Ph.D.

Department of Ecosystem Biology, Faculty of Science
University of South Bohemia in České Budějovice

Supervisor specialist:

Assoc. Prof. Mgr. Eva Kaštovská Ph.D.

Department of Ecosystem Biology, Faculty of Science
University of South Bohemia in České Budějovice

Prof. Ing. Hana Šantrůčková, CSc.

Department of Ecosystem Biology, Faculty of Science
University of South Bohemia in České Budějovice

RNDr. Jakub Borovec, Ph.D.

Biology Centre CAS, Institute of Hydrobiology

Mgr. Petra Straková, D.Sc.

Natural Resources Institute Findland (LUKE)
Department of Forest Scienes, University of Helsinki

České Budějovice 2020

This thesis should be cited as:

Mastný J., 2020: Effect of peatland plant dominants on quality and quantity of organic matter in spruce swamp forest soils. Ph.D. Thesis Series, No.1. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 138 pp.

Annotation

The effect of three typical peatland plant species – *Sphagnum fallax*, *Eriophorum vaginatum* and *Vaccinium myrtillus* on soil organic matter quality and quantity (SOM and DOM) was investigated both in the field and under laboratory conditions in the incubation experiments.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 13.12.2019 .....

Jiří Mastný

This thesis originated from Faculty of Science, University of South Bohemia supporting doctoral studies in the Ecosystems Biology study program.

Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

Financial support

This study was supported by the GAČR „Functional diversity of soil microorganisms in spruce swamp forest and its effect on soil DOM“ (13-17398S), the Academy of Findland project (289116) and the SoWa Ecosystem Research Infrastructure funded by MEYS (LM 2015075 and EF16_013/0001782)

Acknowledgments

The greatest thanks go to my supervisors Tomáš Picek and Eva Kaštovská for their patience, guidence and advices. I thank Petra Straková for introducing to the method of FTIR analyses and evaluation of the data and for her great work on papers, Jakub Borovec for organizing of DOM chemical analyses, Hana Šantrůčková for her work on papers and scientific advices, Zuzka Urbanová and Jirka Bárta for great work on the papers, discussions and support, Keith Raymond Edwards for work on papers and language corrections.

I thank Tomáš Hubáček for DOM chemical analyzes and his help with data evaluation and the technicians at our department Katka Kučerová, Dan Vaněk, Ondra Žampach, Ville Narhi, Iveta Chomová, Lenka Čapková, Terezia Říhová for their technical support and chemical analyzes of my samples. and other members of Department of Ecosystem Biology for creating a friendly atmosphere.

Special thanks belongs to Anička and my family for their support. Beer time!

List of papers and author´s contributions

The thesis is based on the following papers:

I. **Mastný J.**, Urbanová Z., Kaštovská E., Straková P., Šantrůčková H., Edwards K.R., Picek T. 2016. Soil organic matter quality and microbial activities in spruce swamp forests affected by drainage and water regime restoration. Soil Use and Management, June 2016, 32, 200–209. (IF = 4.86)

Jiří Mastný took part in designing and running the experiment, in soil and gas sample analyses, He evaluated data and wrote the manuscript.

II. Kaštovská E., Straková P, Edwards K.R., Urbanová Z., Bárta J., **Mastný J.**, Šantrůčková H., Picek T. 2018. Cotton-Grass and Blueberery have opposite effect on peat characteristics and nutrient transformation in peatland. Ecosystems 21: 443-458. (IF = 4.55)

Jiří Mastný took part in designing and running the incubation experiment, in soil and gas sample analyses, and participated on the manuscript revision.

III. **Mastný J.**, Kaštovská E., Bárta J., Chroňáková A., Borovec J., Šantrůčková H., Urbanová Z., Edwards K.R., Picek T. 2018. Quality of DOC produced during litter decomposition of peatland plant dominants. Soil Biology and Biochemistry 121: 221–30. (IF = 5.29)

Jiří Mastný took part in designing and running the incubation experiment, in litter, DOM and gas sample analyses. He evaluated data and wrote the manuscript.

IV. **Mastný J.**, Kaštovská E., Bárta J., Picek T. Microorganisms responsible for priming effect on peatland DOC decomposition induced by root exudates: an incubation study. Manuscript

Jiří Mastný took part in designing and running the incubation experiment, in gas and DOM samples analyses. He evaluated data and wrote the manuscript.

List of abbreviations

Al – aluminium

C – carbon

C/N - ratio of C and N

C/P - ratio of C and P

DOM - dissolved organic matter

Fe - iron

MW – molecular weight

N - nitrogen

N/P - ratio of N and P

OM - organic matter

P – phosphorus

S - sulphur

SEC – size exclusion chromatography

SOM - soil organic matter

SRP - soluble reactive phosphorus

SUVA 254 - specific UV absorbance at 254 nm

SUVA 280 - specific UV absorbance at 280 nm

Content

1 General introduction

1.1 Motivation for the work

Peatland ecosystems cover about 2.3 % of the terrestrial area and are located mainly above 45°N (Madgwick and Parish 2008; Yu t al. 2011). Pristine peatlands are sinks for atmospheric carbon (C) and represent about one third of terrestrial C pool (Roulet 2000), with 545 Gt C located in northern peatlands (Yu et al. 2010). Such amount of C was accumulated due to unfavourable conditions for organic matter (OM) decomposition. In peatlands, the OM decomposition is restricted by low litter quality, limited nutrient availability, soil acidity, short vegetation period and especially by anaerobic conditions due to water saturation of the soils (Freeman et al. 2001). In result, peatland soils are highly organic (total OM content > 65 %) with a small proportion of mineral fraction (Charman 2002) resulting in their low bulk density. The peat mostly consists of the partially decomposed remains of plant litter. The SOM quality (the composition, low degradability) is further reflected in the dissolved organic matter (DOM). DOM generally forms very small portion of SOM (commonly around 1 % of the soil organic C). It is the most important source of energy and C for soil heterotrophic microorganisms and thus affects microbial activities and also chemical processes running in the soil profile. In peatlands, DOM amounts and quality are particularly important because peatlands act as substantial sources of DOM leaching to surface waters (Clark et al. 2008; Thacker et al. 2008) and adjacent catchments (Worrall et al. 2003). Although numerous studies about peatland DOM exist, namely the studies about DOM quality are scarce.

Character of peatland ecosystems and their functioning are very sensitive to changes in environmental conditions caused by climate change (Bubier et al. 2003; Holden et al. 2004; Aurela et al. 2007). Reduced rainfall during growing season together with increasing temperature may result in a decrease of water table levels and consequently to temporarily enhanced OM decomposition (Moore and Dalva 1993). It is evidenced by increased CO_2 emissions (Shurpali et al. 1995; Alm et al. 1999; Lafleur et al. 2003) and the most importantly by enhanced activity of phenol oxidase, the enzyme, which is responsible for decomposition of recalcitrant phenolic compounds in peat (Freeman et al. 2001). A long-term water level drawdown in peatlands causes shifts in plant community composition. Vascular plants and forest mosses increase their cover at the expense of *Sphagnum* (Weltzin et al. 2000;

Wiedermann et al. 2007; Elmendorf et al. 2012; Dieleman et al. 2015). Since *Sphagnum* litter commonly decomposes slower than the graminoid and woody shrub litter (Moore et al. 2007), such vegetation change may increase OM decomposition rate and accelerate C loss from peatlands. Moreover, vascular plants release significant amounts of low-molecular organic compounds – root exudates to the soil, which increase amounts of simple, non-aromatic compounds in the DOM (Crow and Wieder 2005; Robroek et al. 2016). Such input of labile compounds to the DOM provides energy to microbial community, stimulates its growth and associated production of extracellular enzymes. It can potentially enhance decomposition of pre-existing native, more complex OM. The phenomenon is named a positive priming effect (Kuzyakov et al. 2000). As a result, C balance of peatland ecosystem could shift from C sink to C source. Therefore, determining the impact of peatland vascular plants on SOM, DOM and peat microbial community is crucial.

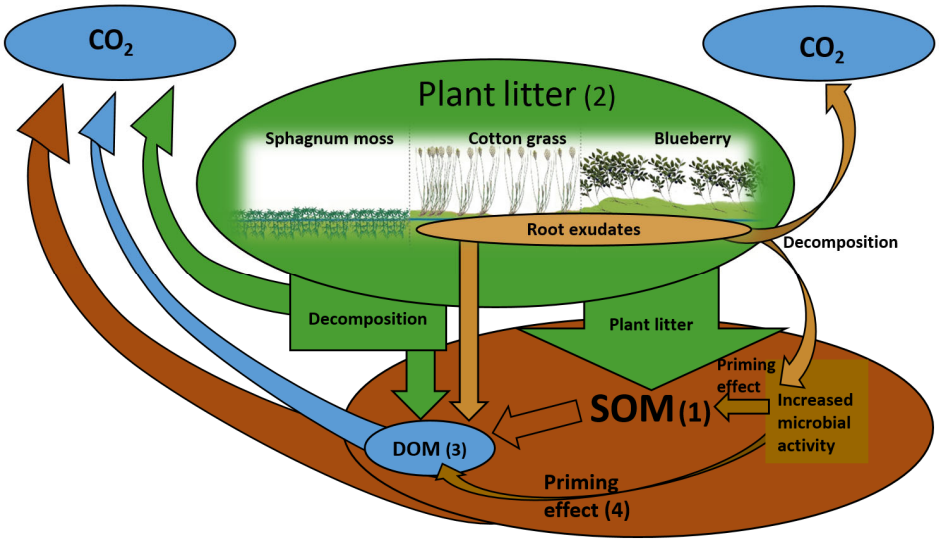


Figure 1: The scheme of studied parameters (characteristics and processes) within peatland ecosystem with the numbers in brackets referring to the particular articles included in the thesis.

Four studies included in this thesis are focused on the effect of water level drawdown and specific effects of peatland plant dominants on SOM and DOM characteristics and on the activity of soil microbial community (Fig. 1). The four

specific studies increase our understanding of possible future changes in the peatland ecosystem functioning connected namely with direct and indirect effects of water level drawdown. The first article compared vegetation, SOM and DOM quantity and quality among pristine, long-term drained and rewetted peatlands with the aim to identify qualitative OM and microbial characteristics which are sensitive to water level changes and could serve as indicators of degraded peat and/or of rewetting efficiency (1). The second study targeted common plant dominants of spruce swamp forests formed on acidic, nutrient-poor subsoils – *Sphagnum fallax, Eriophorum vaginatum* and *Vaccinium myrtillus*, including characterization of their biomass composition, nutrient resorption efficiency, litter decomposition rate and related effects on SOM characteristics, microbial biomass and enzymatic activities *in situ* (2). The follow-up experiments held in laboratory conditions enabled us a detailed study of decomposing plant litter as an important source of DOM, which quality was studied during the ongoing litter decomposition (3). The second laboratory experiment was targeting the effect of root exudates on recalcitrant peatland DOM decomposition and on microbial community composition (4). Its main aim was to estimate whether the larger input of root exudates connected with potential spread of vascular plants due to an ongoing climate change could significantly enhance decomposition of recalcitrant OM and affect dynamics of C cycling in peatlands (4).

1.2 Peatland vegetation

The vegetation is the primary source of SOM and DOM and its quality is affected by production of plant litter and root exudates, and by changing of soil chemistry (e.g. by acidification, transport of oxygen etc.). Boreal peatland vegetation is usually dominated by *Sphagnum* mosses and supplemented by vascular plants with various ground coverage depending on the distinct peatland type. Vascular plants with different functional traits are typical inhabitants of bogs (Dorrepaal et al. 2007). Vascular plant species, which commonly inhabit peatlands, can be grouped in several functional types based on similar functional traits like life form, nutrient acquisition strategy, nutrient resorption efficiency, growth rate, biomass allocation and composition and other physiological and morphological characteristics. The vascular plant functional types commonly occurring in peatlands are graminoids,

ericoids and trees (and in minority also forbs). The representatives of the individual functional types studied in the frame of this thesis are characterized below.

1.2.1 *Sphagnum* (peat) mosses

Sphagnum mosses, including dozens of species, are dominant vegetation in acidic boreal peatlands because they are well adapted to acidic, waterlogged and nutrient-poor conditions with capability to create and maintain such environment themselves. *Sphagnum* mosses contribute significantly to the C fixation in peatlands, since they often have a ground cover of 80-100 %. The mean productivity ranges from 200 g m^{-2} $year^{-1}$ to 350 g m^{-2} $year^{-1}$ (Gunnarson 2005). *Sphagnum* litter is the key for the peat formation because it decomposes very slowly. Low decomposability of *Sphagnum* tissue is mainly caused by *Sphagnum*-specific cell wall pectin-like polysaccharides (sphagnan) (Hájek et al. 2011) and by polyuronic and sphagnum acids (Painter 1991). All these compounds readily bound with enzymes and can inhibit their activity (Painter 1991; Verhoeven and Toth 1995; Verhoeven and Liefveld 1997). The reactive carboxylic acid groups of sphagnan can form recalcitrant humic substances with proteins via abiotic Maillard reactions (Painter 1983, 1991). Further, enzymatic inhibition is strengthened by *Sphagnum*-induced acidic conditions (Williams et al. 2000) and by anoxic conditions, which lead to the enzyme latch mechanism. By this mechanism the low concentration of oxygen constraints the activity of enzyme phenol oxidase, which is responsible for degradation of phenolic compounds. Phenolics then accumulate in the soil solution and inhibit activities of hydrolytic enzymes. This chain effect results in overall lower decomposition of peatland organic matter (Freeman et al. 2001; Fenner and Freeman 2011).

1.2.2 Vascular plants

Morphology and ecology of vascular plants strongly differ from that of *Sphagnum* mosses. Vascular plants divide photosynthesis and the acquisition of mineral nutrients between shoots and roots, whereas in *Sphagnum* these two functions take place over the entire living plant surface. Moreover, vascular plants affect SOM dynamics through rhizodeposition, releasing easily decomposable compounds from

roots directly into the soil profile and thus affecting decomposition of SOM and plant litter including dead *Sphagnum* mosses.

Most incremental growth of vascular plants is restricted to a few months in the earlier part of the vegetation period, while *Sphagnum* mosses are able to grow as long as the moss surface is not frozen and the capitula in the apex of the moss is provided with the necessary moisture. This difference in growth phenology facilitates the co-existence of both vascular plants and *Sphagnum* mosses, which brings internal peatland variability (Chapin et al. 2000). This variability is further supported by interactions between vascular plants and *Sphagnum* mosses. The vascular plants as efficient photosynthesizers may shade *Sphagnum* which results in its locally reduced productivity (Hayward and Clymo 1983, Murray et al. 1989), whereas on other places the aboveground shoots of vascular plants are often observed being overgrown by *Sphagnum* (Backe´us 1985, Redbo-Torstensson 1994). It results in a small-scale mosaic of microhabitats with a high vascular plant cover and with a high moss cover. Such mosaic pattern is very characteristic for the plant communities in most peat forming ecosystems.

The litter decomposability is determined by the content of recalcitrant compounds and by elemental stoichiometry (Berg and McClaugherty 2003; Mooshammer et al. 2014). Generally, rate of plant biomass decomposition decreases with increasing content of lignin, polyphenols and waxes and increasing lignin/N and C/N ratios. Initial rate of plant biomass decomposition is also affected by other main nutrients like P and S and their availability to the microbial community (Berg and McClaugherty 2003). Therefore, decomposition of vascular plant litter differs between species but also between roots and leaves of the certain species. Generally, roots decompose slower than leaves due to their higher content of lignin-like compounds and lower nutrient content (Crow et al. 2009). Although *Sphagnum* does not contain lignin, its biomass is usually less decomposable than that of vascular plants (Moore et al. 2007; Kool and Heijmans 2009) due to a content of other decomposition-inhibiting compounds such as sphagnan (Painter 1991).

Vascular plant species, which commonly inhabit peatlands, can be grouped into several functional types according to their life-form characteristics and use of resources, which determine their function in ecosystem (Chapin et al. 1996,

Dorrepaal et al. 2007). The major plant functional types of peatlands, except dominant peat mosses, are graminoids (grasses, sedges), ericoids, in minority forbs and also trees, occurring in forested types of peatlands like boggy forests and spruce swamp forests. In the studies included in this thesis, we focused on two species of vascular plants, *Eriophorum vaginatum* and *Vaccinium myrtillus*, the common peatland species and typical representatives of graminoids and ericoids, respectively.

1.2.2.1 Eriophorum vaginatum as a representative of graminoids

Cotton-grasses (*Eriophorum* sp.) are typical graminoids, which occur in peatlands. Cotton-grass is a non-mycorrhizal peatland vascular plant well adapted to flooded nutrient poor environment, which tends to favour water tables no deeper than 30–40 cm below the peat surface (Lavoie et al. 2005). Cotton-grass effectively immobilises nutrients in stems and leaves (Shaver et al. 1986). The leaves die back each year but may remain on the plant for several years. It results in a formation of dense tussocks, which can persist for over hundred years and in which a growth of new leaves is supported almost entirely by nutrient retranslocation from senescing leaves (Lavoie et al. 2005).

Cotton-grass also forms deep root system (Wein 1973), which transports oxygen and easily decomposable exudates to anoxic organic soil layers (Chapin et al. 1993) and is known to renew practically all its fine roots annually (e.g. Chapin et al. 1979). Cotton-grass efficiently takes up N in various forms from nearby soil (Chapin, et al. 1993) and significantly supports the presence of both aerobic and anaerobic microorganisms such as methanotrophs and methanogens (Chroňáková et al. 2019) via the intensive root exudation (Edwards et al. 2018).

1.2.2.2 Vaccinium myrtillus as a representative of ericoids

Generally, ericoids are more resilient to drought than sedges (Weltzin et al. 2000; Weltzin et al. 2003) and they occupy microhabitats in peatlands with lower water table than *Eriophorum*, which are characteristic by prevailing aerobic conditions. Blueberry as other ericoids is associated with ericoid mycorrhizal fungi, which belong mainly to the *Ascomycetes* phylum (Read 1996; Smith and Read 1997). Ericoid mycorrhizal fungi are well known for their high extracellular enzymatic

activities and the associated ability to use organic forms of N and P (Smith and Read 1997). They are able to mobilize N and P and support plant nutrient uptake in environments with severe nutrient limitation (Cairney and Meharg 2003; Read et al. 2004). They also have the ability to decompose lignin, cellulose, chitin, starch, pectin and gelatin (Rice and Currah 2001). However, the symbiosis is energetically very demanding. Therefore, a considerable portion of C assimilated by ericoids is transported belowground and translocated directly to the mycorrhiza (Smith and Read 1997). Ericoid shrubs have higher contents of phenolics and lignin in the litter than graminoids (Hobbie 1996; Dorrepaal 2007) causing their lower tissue decomposability.

1.3 Microbial community in peatlands

High water table level, which is often close to the soil surface, and the ability of *Sphagnum* to store water lead to prevailing anoxic conditions. Anoxia together with the *Sphagnum* ability to acidify environment via the proton efflux create harsh conditions that constrain microbes to slow metabolic pathways e.g. primary and secondary fermentation and methanogenesis (Moore and Basiliko 2006). Long-term anaerobic conditions are also unfavourable for the growth and development of rich fungal community. According to the studies of Winsborough and Basiliko (2010) and Myers et al. (2012) it can be generalized that bacteria are more active and more important for C, N and P cycling than fungi across many peatland types. Generally, peatlands contain large microbial populations of wide metabolic diversity, still the specific environmental conditions favour certain microbial groups (Williams and Crawford 1983). The most frequent bacterial phyla in bogs are *Proteobacteria* and *Acidobacteria* with a good adaptations to low pH. *Acidobacteria* are able to grow under oligotrophic conditions (Philippot et al. 2010) and are known for their ability of the cellulose and aromatic compounds degradation (Ausec et al. 2009; Pankratov et al. 2011). The other important bacterial groups typical for bogs are *Actinobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Chloroflexi* and *Firmicutes*. These are mostly groups of bacteria, which are adapted to the shifts between oxic and anoxic conditions or to perennially anoxic conditions. Under long-term anoxic conditions, methanogenic *Archaea* produce CH_4, which production generally increases from poor bogs to rich fens with spruce swamp forests in between

(Urbanová and Bárta 2014). Most of the produced CH_4 is usually oxidized to CO_2 by methanotrophs (several genera from *Alphaproteobacteria* and *Gammaproteobacteria* (Knief 2015)), which are most active close to the interphase of the anoxic and oxic layers (Sundh et al. 1995; Laine et al. 2012). CH_4 is thus emitted to the atmosphere when its production is higher than the consumption (Le Mer and Rodger 2001).

Fungal communities vary among peatland types according to the dominant litter type (Trinder et al. 2009) mainly due to different plant litter chemistry (Thormann 2006). The most common fungal groups in peatlands are *Ascomycetes* (also forming mycorrhiza with ericoids) followed by *Basidiomycetes*, *Zygomycetes* and *Chytridiomycetes* (Thormann and Rice 2007). Generally, *Basidiomycota* are the most efficient decomposers of lignin, while *Ascomycota* degrade wide range of compounds from simple sugars to lignocellulose complexes (Kirk et al. 2008). However, commonly isolated fungi from peatlands have a limited capacity to decompose the most complex polymers (Thormann et al. 2001, 2002).

1.4 SOM in peatlands

The peatland ecosystems represent about one third of the terrestrial C pool (Roulet 2000). These vast stores of C in northern peatlands have accumulated due to the remarkably slow decomposition rates of peat OM, with *Sphagnum* mosses being the most important peat-forming species (Rydin and Jeglum 2013). The C accumulation always involves retention/immobilization of nutrients (particularly of N and P) in the peat (Damman 1988) leading to their reduced availability for plants (Scheffer et al. 2001).

1.4.1 Peatland SOM composition

Plant inputs are the main source of OM in the soil. Differently from soils of other ecosystems, peatland soils are characterized by a high SOM content and thus high C concentration (approx. 50 %) due to slow decomposition rate and very low contribution of mineral particles to the soil matrix. The SOM generally consists of a range of plant fragments of all sizes at various stages of OM decomposition and microbial products formed during their transformation. It contains carbohydrates (cellulose, hemicellulose and sugars), nitrogenous compounds (proteins, amino

acids and amino sugars), polyphenols (lignin, tannins), lipids (waxes, resins), humic and fulvic acids. The peatland SOM - the peat, is mainly formed by undecomposed and only partly-decomposed plant remains. Therefore, its chemical composition is similar to that of the original plant species from which it is derived. It is also poor in nutrients, thus having higher C/N and C/P ratios in comparison to SOM in other types of ecosystems (grasslands, forests, arable soils) since the peatland plants are efficient in nutrient resorption and immobilization in their biomass.

From fresh litter input into the surface, active acrotelm layer, is ca 80–90 % gradually mineralized, while the rest ca 10-20 % of the plant input finally contributes to the peat accumulation and reaches deeper, permanently water-saturated catotelm layer (Clymo 1984). Peat chemistry changes from acrotelm to the catotelm as a result of the degree of decomposition. Contents of carbohydrate, carboxyl-C and other oxygenated functionalities decrease, whereas contributions of aliphatic-C, aromatic C and microbial mucilage increase (Cocozza et al. 2003; Delarue et al. 2011; Klavins et al. 2008; Zaccone et al. 2007).

The OM is present in the soil either in a free form or is stabilized by various physical and chemical mechanisms, including physico-chemical bounding to clay particles, an occlusion in soil aggregates and biochemical recalcitrance (Six et al. 2002; Dodla et al. 2012). The SOM stabilization enhances its potential persistence in the soil. To characterize the SOM and estimate its stability, physical and chemical fractionation methods have been used. They separate soil matrix together with the present SOM into various pools (fractions) differing in the amount, composition and stability of C. Physical fractionation methods separate the soil into putative size or density fractions (Beare et al. 1994; Kemper and Rosenau 1986; Six et al. 2000; Sohi et al. 2001). They commonly involve an application of various disaggregating treatments (dry and wet sieving, slaking), dispersion to disrupt soil aggregates (mostly ultrasonic vibration in water), followed by density separation and sedimentation (von Lützow et al. 2007 and citations therein). The most common physical fractionation method of Six et al. (2000a) starts with a disruption of macroaggregates (>250 μm) into fractions occluded within them and then separates coarse particulate organic matter, occluded microaggregates (53–250 μm) and occluded silt + clay (< 53 μm). Physical fractionation methods are established for mineral soils, in which

significantly represented mineral particles enable aggregate formation and thus physical and physico-chemical stabilization of the SOM (Six et al. 2000). However, they are not suitable for highly organic soils with naturally low content of mineral particles like peatland soils because they do not consider biochemical recalcitrance, which is the main stabilization mechanism for SOM in peatlands (Olk and Gregorich 2006).

Chemical fractionations enable advanced chemical characterization of the SOM pools and can be used to elucidate molecular-level interactions within SOM (Olk and Gregorich 2006). Chemical procedures are based on the extraction of SOM in aqueous solutions with and without electrolytes, in organic solvents, on the SOM hydrolysability with water or acids, and the resistance of SOM to oxidation (von Lützow et al. 2007). However, some of the chemical fractionation procedures are not suitable for peat soils (e. g. oxidation with NaOCl and $Na_2S_2O_8$), because organic materials poor in minerals are only partly attacked by the bleaching agents (Chefetz et al. 2002; von Lützow et al. 2007). Chemical fractionation methods commonly used for the characterization of peatland SOM are sequential cold-water and hot-water extractions, which separate easily hydrolysable C pools, followed by an acid hydrolysis to determine residual stable SOM. The SOM extracted by cold water is usually used as the approximation of DOM (Gregorich et al. 2003). Hot-water extractable SOM is frequently used as a measure of potentially bioavailable SOM. This readily decomposable C pool can range from 1 % to 5 % of total SOM (Davidson et al. 1987; Schulz, 2002; Sparling et al. 1998). Acid hydrolysis removes compounds that are supposed to be potentially biodegradable, e.g. proteins, nucleic acids or polysaccharides, leaving behind a fraction of recalcitrant biomacromolecules (e.g. cutans and suberans). Commonly used OM hydrolysing agents are trifluoroacetic acid (TFA), sulphuric acid (H_2SO_4) and hydrochloric acid (HCl) (e.g. Poirier et al. 2003; Quenea et al. 2006) or a hydrolysis using HCl in a single step (e.g. Paul et al. 2001; Plante et al. 2006).

Trifluoroacetic acid is used to quantify non-cellulosic neutral and acidic sugars (Amelung et al. 1996), while sulphuric acid hydrolysis is used for estimation of acid-soluble lignin in soils (Sarkanen and Ludwig 1971; Ehrman 1996). According to the study of Six et al. (2002), the hot acid hydrolysis using 6 M HCl in a single step is the simplest and best available technique for defining a stable SOM fraction in

soil (represented by the residual SOM fraction remaining in the soil after the hydrolysis).

1.4.2 SOM transformations in peatlands

In the pristine peatland ecosystems, the primary productivity exceeds the decomposition rate. Decomposition rate depends on the availability of oxygen which is closely connected with the water table level, on the microbial activity in the peat, the soil temperature, the type of vegetation and its chemical composition, and the chemical characteristics of the peat (Moore and Dalva 1993; Updegraff et al. 1996; Yavitt et al. 1997). Common conditions in the bogs like high humidity, low pH and oligotrophic conditions slow down decomposition processes and result in SOM accumulation. Dominant peatland vegetation type – *Sphagnum* mosses strongly contribute to slow decomposition rates by producing highly recalcitrant litter (Straková et al. 2010; Pinsonneault et al. 2016) inhibiting enzymatic and microbial activity (Painter 1991; Verhoeven and Toth 1995; Verhoeven and Liefveld 1997). Differently, other plant functional types such as graminoids may stimulate decomposition processes by producing comparatively easily decomposable litter (Bombonato et al. 2010; Straková et al. 2010) and root exudates, and by aerenchymatic oxygen transport in roots causing aeration of the rhizosphere (Chanton and Whiting 1996).

Ways and rates of transformation of plant inputs (in the form of litter and rhizodeposition) are controlled by litter and SOM chemical composition, temperature (Conant et al. 2011; von Lützow and Kögel-Knabner 2009) and by oxygen availability. Anaerobic conditions due to high water table level strongly reduce decomposition rate, while any disturbance causing water level draw-down allows oxygen to enter the peat column and results in oxidative microbial degradation of the peat and a release of stored C to the atmosphere. Therefore, the efficient protection of SOM in peatlands is primarily dependent on the maintaining of anaerobic conditions under which SOM is accumulated (Bradford et al. 2016; Davidson and Janssens 2006).

1.5 Dissolved organic matter (DOM)

DOM represents only small proportion (0.04–5 %) of the total SOM yet being the most mobile and the most active SOM fraction. It serves as a source of substrate and energy for the osmotrophic soil microbial community (Bosatta and Ågren 1991), plays very important role in the cycling of C, N and P and contributes to soil forming processes (Jansen et al. 2003). DOM also affects soil processes such as metals solubility, transport and toxicity by chelation (Schnitzer and Khan 1972), transport of organic pollutants (Carter and Suffet 1982), forming of colloidal particles (Tipping 1986) and pH buffering (Oliver et al. 1983).

In the peatlands, DOM is characterized by its low biodegradability in comparison to DOM from other ecosystem types (Tfaily et al. 2013) and the DOM leaching from peatlands is an important component in the C cycle of adjacent catchments (Worrall et al. 2003) or surface waters (Clark et al. 2008; Thacker et al. 2008).

1.5.1 Sources of DOM

Besides the processed and stabilized solid SOM (Guggenberger et al. 1994a), plant litter is considered as the important source of DOM in the soil (Kalbitz et al. 2000; Kalbitz et al. 2007; Müller et al. 2009; Sanderman et al. 2008). Other important DOM sources are root and microbial exudates, decomposing microbial and animal necromass, metabolic by-products and the lysis of microorganisms (Guggenberger et al. 1994a; McDowell 2003; McDowell et al. 1998). Although very low proportion of labile compounds originating in root exudates occurs in DOM (Jones et al. 2004), the exudate flux to the DOM pool could be extraordinarily high due to its fast turnover driven by assimilation and decomposition by microbial community (Yano et al. 2000; van Hees 2005). Each plant species has the specific composition of root exudates, which create unique belowground microbiomes (Haichar et al. 2008). Therefore, changes in plant community composition may cause changes in microbial community composition which can be followed by changes in dynamics of C cycle.

1.5.2 DOM composition

DOM is a heterogeneous mixture of soluble organic compounds. Similarly to the SOM, the chemical characterization of DOM is very complicated. DOM can be

divided according to its decomposability into labile and recalcitrant DOM (Marschner and Kalbitz 2003). Labile DOM contains simple carbohydrate compounds, low molecular weight (MW) organic acids, amino acids, amino sugars and low molecular-weight proteins (Guggenberger et al. 1994b; Kaiser et al. 2001; Qualls and Haines 1992). These compounds can be directly utilized by microorganisms and thus do not require a degradation by extracellular enzymes (Lynch 1982), while degradation of recalcitrant compounds requires special extracellular enzymes. Recalcitrant dissolved compounds are mainly alkyls and aromatics which accumulate during decomposition of OM (Baldock et al. 1992; Kögel-Knabner et al. 1992). Namely, the recalcitrant DOM is composed of polysaccharides (breakdown products of cellulose and hemicellulose), other plant compounds (lignin derived compounds), microbial derived degradation products and supramolecular associations of small molecular mixtures of plant and microbial derived components (Marschner and Kalbitz 2003; Sutton and Sposito 2005; Hardie et al. 2007). The supramolecular associations are special “structures” bound by relatively weak dispersive forces, such as hydrophobic interactions or H bonds (Sutton and Sposito 2005) instead of covalent linkage (Piccolo 2002).

DOM composition depends on the DOM source. Plant-derived DOM contains approximately 10 % proteins, 30-50 % carbohydrates, 15-25 % lignin-like compounds (Hatakka 2001), lipids (Killops and Killops 2005) and other macromolecules. Root exudates contributing to the DOM are mainly composed of low MW and easily decomposable organic compounds such as organic acids, sugars, amino acids with a high proportion of organic nitrogen (Jones et al. 2009) and secondary metabolites. The specific composition of plant-derived DOM differ between plant species. Plant derived DOM contains mainly pentoses, while microbially processed DOM mainly hexoses (Oades 1984) and compounds like carbohydrates, proteins, amino sugars, lipids (Kögel-Knagner 2002; Kalbitz et al. 2003a; Miltner et al. 2009) and uncharacterized molecules (Marschner and Kalbitz 2003).

DOM composition also largely varies in time due to changes in plant and microbial DOM production, consumption and its transport. DOM isotope analysis showed that microorganisms use the plant-derived DOM mainly in energy metabolism and very

little is used for biosynthesis (Malik et al. 2013). Similarities in isotope values and composition between microbial biomass and low MW DOM up to 10 kDa suggest that this DOM fraction is a footprint of microbial activity, while the DOM fraction larger than 10 kDa is mostly composed of recent plant-derived C (Malik et al. 2013).

1.5.3 Selected methods of DOM characterization

According to the structural composition, DOM can be divided into hydrophobic and hydrophilic acids and hydrophilic neutral fractions (Dai 1996). For this purpose, an aliquot of DOM is acidified to pH 2 with 1M HCl and pumped through columns filled with Amberlite XAD-8 resin. The effluent represents hydrophilic fraction. Hydrophobic fraction is then eluted from the column by 0.1M NaOH. The hydrophobic acid fraction is rich in lignin-derived aromatic compounds and only low amount of nutrients (P, N, and S) is organically bound on this fraction (Priha and Smolander 1999). The hydrophilic acid fraction is mainly composed of carboxylic C and is enriched in organically bound nutrients (Jandl and Sollins 1997). According to Piccolo (1998), **the hydrophobicity** of OM controls its accumulation. These fractions differently interact with soil surfaces and organic contaminants. Compounds in a hydrophobic acid fraction interact strongly with Al/Fe oxides and hydroxides and with hydrophobic organic pollutants (Priha and Smolander 1999; Priha et al. 2001). Hydrophobic acids are less mobile and less available to microbial decomposition (Qualls and Haines 1992) than the compounds from hydrophilic fractions. The decomposability of DOM fractions increase in order: the hydrophobic acid fraction < hydrophilic acid fraction < the hydrophilic neutral fraction (Jandl and Sollins 1997).

DOM can also be divided into the **size fractions** according to its MW. For that purpose, size exclusion chromatography (SEC) is used. SEC is a physical fractionation method based on molecular size of compounds, when the bigger the molecules, which cannot penetrate the small pores in the stationary phase in the column, have shorter retention time and leave the column earlier than smaller molecules (Jones et al. 2012). The particular MW DOM fractions can be further analysed for contents of various elements (C, N, P, Al, Fe and others), for their spectroscopic characteristics (like aromaticity, a presence of functionalities etc. using UV/VIS detectors) or for more detailed biochemical composition (e.g. by liquid

chromatography with a subsequent compound identification). The DOM is usually divided into two to four MW fractions (Mueller et al. 2000; Her et al. 2002; Landry and Tremblay 2012; Kiikkila et al. 2014). Kiikkila et al. (2014) divided DOM into >100 kDa, 10−100 kDa, 1−10 kDa and <1 kDa MW fractions (typical compounds contained in the particular fractions are shown on Fig. 2). While the smallest MW fraction (<1kDa) contains only the simple compounds, which can originate from root exudation, microbial cell lysis or from cleaving activities of extracellular hydrolytic enzymes, the middle size fractions (1-100 kDa) carry a footprint of microbial activity combined with compounds of plant origin. Larger MW DOM fractions up to 10 kDa are mostly derived from the old SOM as suggested by the presence of highly processed material (Malik et al. 2013), while MW fraction >10 kDa consists mostly of recent plant-derived C (Malik et al. 2013) and supramolecular compounds (Picolo 2002).

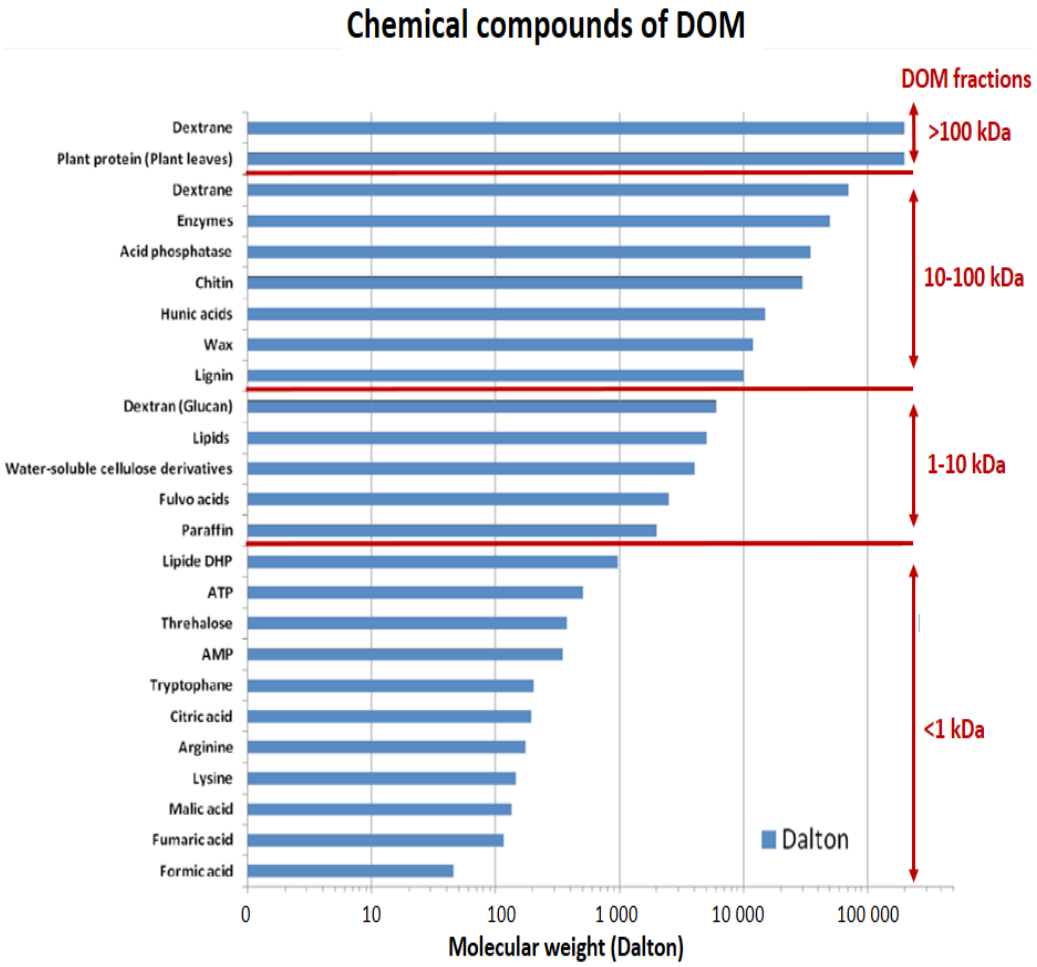


Figure 2: Chemical compounds distribution into four MW fractions (Kiikila et al. 2011) according to their molecular size (Dalton). The MW fractions are highlighted by red arrows (modified from Schomakers et al. 2014).

Spectroscopic analyses may be also used to characterize structural composition of DOM. The most often used parameters are: SUVA 254, SUVA 280, E250/E365, E470/E665 and E210/E254 referring to light absorbance at specific wavelengths. SUVA 254 (specific UV absorbance at 254 nm) provides an estimation of the aromaticity per unit of C (Hur et al. 2006). Value larger than 4 indicates highly hydrophobic and aromatic DOM, while the value < 3 indicates hydrophilic and less

aromatic DOM (Peuravuori and Pihlaja 2007; Volk et al. 2002). Similarly, SUVA 280 is also used for an estimation of DOM aromaticity (Weishaar et al. 2003). The ratios of E250/E365 and E210/E254 are used for an estimation of the average MW of DOM. Their values decrease with increasing MW of DOM due to stronger light absorption of high MW compounds at longer wavelengths. It is expected that lower MW compounds will have lower concentration of activated aromatic rings but they will be enriched by non-aromatic groups (Korshin et al. 1997; Her et al. 2008). Degree of humification is estimated by the parameter E470/E665 (Hur et al. 2006). This ratio referees to the MW of humic substances, where the value for humic acids is usually < 5.0, whereas the value for fulvic acids ranges from 6.0 to 8.5 (Thurman 1985; Peuravuori and Pihlaja 1997). Finally, the slopes of the absorption spectra in the 275–295 nm and 350–400 nm regions (SR index) are related to the MW of the organic molecules (Helms et al. 2008).

1.5.4 DOM processing

DOM is subjected to numerous transformation processes, from which the most important are: DOM production, decomposition, sorption/desorption, complexation/decomplexation and leaching. DOM production, decomposition and transformation are affected by composition and activity of vegetation and of microbial community, pH (McDowell et al. 2006; Schmidt et al. 2011), temperature (Koehler et al. 2009; Preston et al. 2011), soil moisture (Kane et al. 2010), peat SOM quality (Laiho et al. 2003; Wickland et al. 2007) and redox status (Freeman et al. 1993). All these parameters are directly or indirectly related to the water table position. Therefore, the hydrological conditions in peatlands are the key factor affecting DOM production and transformation.

Similarly to the total SOM, the peatland DOM is rather resistant to microbial degradation as compared to other terrestrial ecosystems (Kalbitz 2003a; Tfaily et al. 2013). Moreover, anaerobic conditions reduce DOM decomposition in comparison to aerobic conditions (Freeman et al. 2001; Moore and Dalva 2001; Jungkunst et al. 2008). The retarded DOM decomposition can result in a DOM accumulation and an increase of DOM proportions in the total SOM (Kalbitz et al. 2000 and citations therein).

However, root exudates and leachates from freshly senesced vascular plant litter supplying the DOM during vegetation season and after plant senescence, respectively, contain easily decomposable organic compounds, which can stimulate microbial activity. These easily available compounds are rapidly utilized by microorganisms, partly incorporated into their biomass and partly respired (Brüggemann et al. 2011; Blagodatskaya and Kuzyakov 2008; Fontaine et al. 2003). Generally, the input of labile substrates first stimulates the growth of r-strategists (Kuzyakov 2010). Later, the r-strategists are followed by K-strategists that utilize more complex C compounds including the necromass of r-strategists (Fig. 3). The microbial growth is associated with an enhanced nutrient demand of growing microbial biomass (Chen et al. 2014) and an increase of the extracellular enzymatic activity, which leads to a targeted and/or co-metabolic decomposition of pre-existing, less degradable DOM and SOM (Fontaine et al. 2003; Blagodatskaya et al. 2014). The situation, when the input of labile substrates significantly impacts on the current decomposition rate of pre-existing DOM (SOM) due to changed microbial activity is called "**the priming effect**" (Cheng and Kuzyakov 2005).

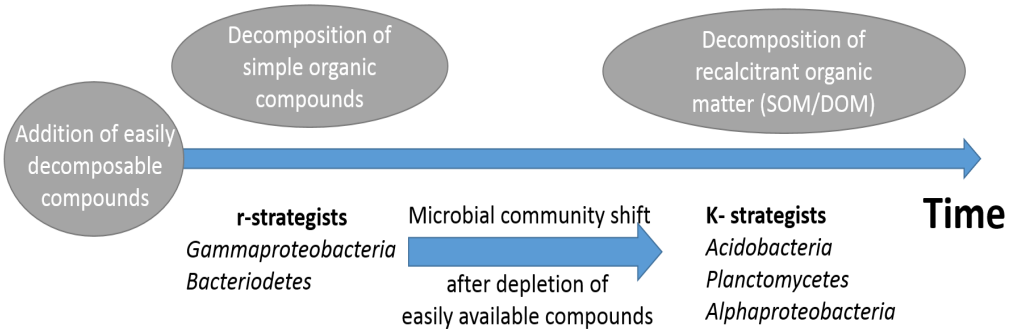


Figure 3: Scheme of the changes in organic matter decomposition after an addition of easily decomposable substrate aka "priming effect". The input of labile substrates stimulates the growth of r-strategists and later of K-strategists that utilize more recalcitrant C. The growing microbes enhance nutrient demand and increase extracellular enzymatic activity, which leads to a decomposition of pre-existing DOM and SOM.

The priming effect on recalcitrant SOM and DOM may be either negative or positive (Fig. 4). The negative priming effect – means that the decomposition of pre-existing DOM (SOM) is reduced compared to common conditions. It usually occurs early after the input of labile substrates (e.g. root exudates, litter leachates). These are preferentially used by present microbes to trigger the metabolism and start the growth (Blagodatskaya et al. 2011). The negative priming usually lasts only a transitional period until the labile substrates are depleted or insufficient to support the enhanced microbial activity. The positive priming effect is the situation when the microbial activity triggered by the input of labile substrates and it leads to a significant enhancement of decomposition rate of pre-existing DOM (SOM) in comparison to common conditions (Wang et al. 2015).

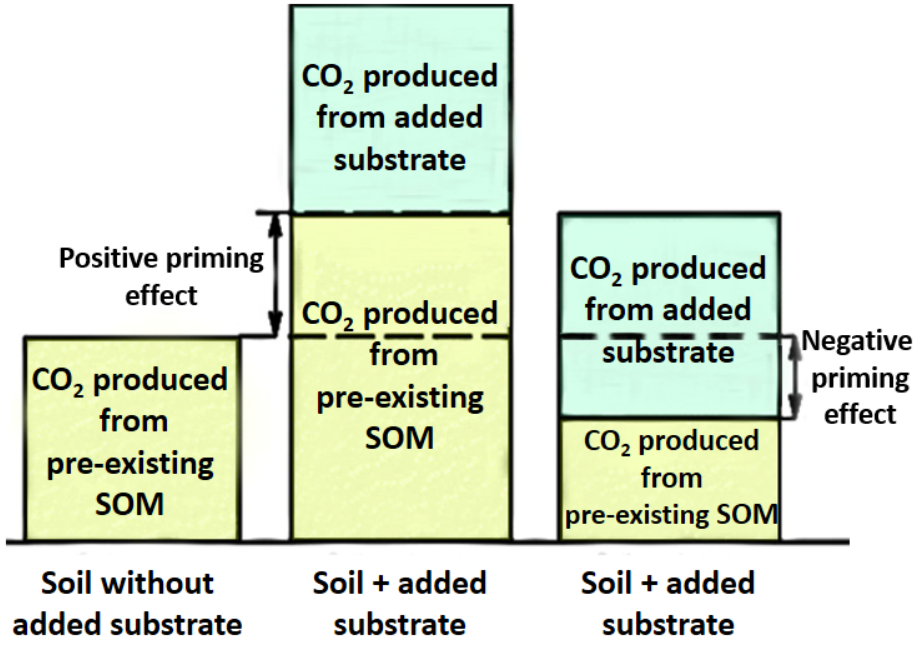


Figure 4: Scheme of positive and negative priming effect (modified from Blagodatskaya and Kuzyakov 2011). The negative priming effect occurs when the decomposition of pre-existing OM is reduced compared to common conditions. The positive priming effect occur when the microbial activity triggered by the input of labile substrates leads to a significant enhancement of decomposition rate of pre-existing OM in comparison to common conditions.

The **DOM sorption and stabilization** are the main processes determining the retention of DOM in soils. DOM sorption to mineral surfaces is one of the most important stabilization processes which reduces decomposability of DOM (Sollins et al. 1996; Kaiser and Guggenberger 2000). Some of the mechanisms responsible for the DOM sorption on mineral surfaces are: anion sorption, ligand exchange, protonation, hydrogen bonding, cation bridging and physical adsorption (Vance and David 1992). However, highly organic soils such as peatland soils contain negligible amounts of mineral particles and thus the DOM sorption to mineral surfaces is not an important stabilization mechanism in peatlands. DOM further forms either soluble DOM–metal(loid) complexes or insoluble OM–metal(loid) complexes. While soluble DOM–metal(loid) freely move in soils, forming of insoluble complexes results in immobilization and stabilization of the SOM in the soil (Guggenberger and Kaiser 2003; Jansen et al. 2005). The DOM sorption can be estimated according to the Fe and Al oxide and hydroxide contents in soil (Moore et al. 1992), because these compounds are the most important sources of variable charge in soils (Jardine et al. 1989; Moore et al. 1992; Kaiser and Zech 1998). The DOM sorption increases with an increasing content of Al and Fe oxides and hydroxides and an increasing content of DOM (especially of hydrophilic DOM) (Jardine et al. 1989; Donald et al. 1993), and it is lower under anaerobic than under aerobic conditions (Kaiser and Zech 1997).

DOM processing and characteristics are also significantly affected by hydrologic regimes, which drive the transport of DOM through soils to aquatic systems. DOM leaching is tightly coupled to water table position (Moore and Dalva 2001; Freeman et al. 1993), when elevated water table can lead to increased DOM leaching due to a release of higher proportion of water-soluble intermediate metabolites (Mulholland et al. 1990). Increased DOM leaching to streams in peatland catchments occurs also during storm events (Clark et al. 2007). In a temperate forest catchment dominated by mineral soils, Inamdar et al. (2011) and Hood et al. (2006) found that the DOM leached during storm events was rich in aromatic structures, which was ascribed to flushing of near-surface soil layers. Increased aromaticity of the leached DOM was also found during snowmelt events in peatland catchment (Agren et al. 2008).

2 Aims and objectives

The aim of this work was to evaluate the effect of three typical peatland plant species – *Sphagnum fallax*, *Eriophorum vaginatum* and *Vaccinium myrtillus*, which represent different plant functional types (peat mosses, graminoids and ericoids, respectively) on soil organic matter (SOM) and dissolved organic matter (DOM) quality and quantity and on the activity of microbial community in peatland ecosystem.

The specific objectives of the thesis were defined:

1/ To evaluate long-term effects of water level drawdown in the peatland on plant community composition, SOM and DOM quantity and quality/recalcitrance.

2/ To determine the *in situ* impact of three common peatland plant dominants on quality and quantity of SOM, DOM and soil microbial community in pristine spruce swamp forests ecosystems.

In more detail, we focused on the role of peatland plant dominants in formation and qualitative changes of DOM, the most mobile fraction of the SOM and the important carbon and energy source for soil microorganisms. We conducted two laboratory experiments with following aims:

3/ To characterize the quality and quantity of DOM leached from the litter of the studied plant dominants during their decomposition.

4/ To evaluate the impact of artificial root exudates of vascular plants (mimicked by a mixture of low molecular weight compounds) on decomposition of recalcitrant peatland DOM (the process named rhizosphere priming effect) and to identify the microorganisms responsible for this process.

3 Results and discussion

3.1 Long-term effects of peatland water level drawdown on vegetation composition, SOM and DOM quality and quantity

The first part of the thesis deals with changes in water regime of spruce swamp forests. In the past, peatlands were often drained for forestry, peat extraction and agricultural purposes. After water level drawdown, an aeration of the upper soil layer led to enhanced peat decomposition and net losses of the accumulated C. We documented that long-term (more than 50 years) water level drawdown caused a retreat of typical peatland plant species such as *Sphagnum* mosses and cotton-grass, which were replaced by forest species e.g. shrubs (ericoids), typical forest mosses and trees (1). As a consequence of a pronounced peat decomposition and changed quality of plant litter input, the peat bulk density increased, its pH decreased and the SOM was enriched in lignin-like compounds, waxes, lipids and other non-hydrolysable compounds, while easily decomposable compounds were depleted as compared to the pristine sites (Fig. 5). Moreover, long-term drainage led to a decrease of soil microbial biomass and CO_2 production rate which indicated possible limitation of microbial community by energy (a lack of easily decomposable C substrates) in the soils of the drained peatlands (1).

In last decades, large-scale restoration efforts were initiated with the aim of revitalizing the peatlands – of bringing back their original functions such as peat formation, water retention and functioning as biodiversity hotspots. Restoration of the original water regime enables a spreading of *Sphagnum* and sedges and the partial retreat of forest mosses and shrubs (Maanavilja et al. 2014). However, 7–11 years after the restoration was not sufficient time to find considerable changes in overall SOM quality (1). We only found a smaller proportion of the hot-water-extractable SOM fraction in rewetted peatland soils as compared to the pristine and drained ones likely because this fraction was quickly metabolized by the activated soil microorganisms (Ghani et al. 2003). The activation of soil microbes and related changes in SOM degradability after spruce swamp forests restoration were further indicated by an increased soil microbial biomass and CO_2 production, and measurable rates of potential methanotrophy and methanogenesis. These were early indicators of a development of rewetted sites towards undisturbed peatland

ecosystems similarly as found in some other studies (Mummey et al. 2002; Huttunen et al. 2003). However, we did not find any considerable change in the SOM quality in the upper soil layer (1). The SOM still contained a large portion of non-hydrolysable compounds with no significant signs of an accumulation of new, more hydrolysable compounds or DOM. It is evident that the restoration of the functions such as peat accumulation connected with an efficient water retention in the previously long-term drained peatlands is dependent on a recovery and full development of the peat-forming vegetation, which needs longer time period than a decade (1).

The obtained results may be used for predictions of the peatland development under ongoing climate change. Similar changes, which we observed at the drained sites, can be expected when water level in peatlands will decrease as a consequence of changed precipitation pattern and increased temperatures. In such case, *Sphagnum* mosses coverage will decrease, the upper soil layer will be more aerated and vascular plants will spread. To understand better potential implications of the changes in plant community composition on peatland functioning, in following studies we focused on the effects of typical peatland plant dominants on peat characteristics, nutrient availability, soil microbial community and its activity, and in detail on the DOM formation and its composition.

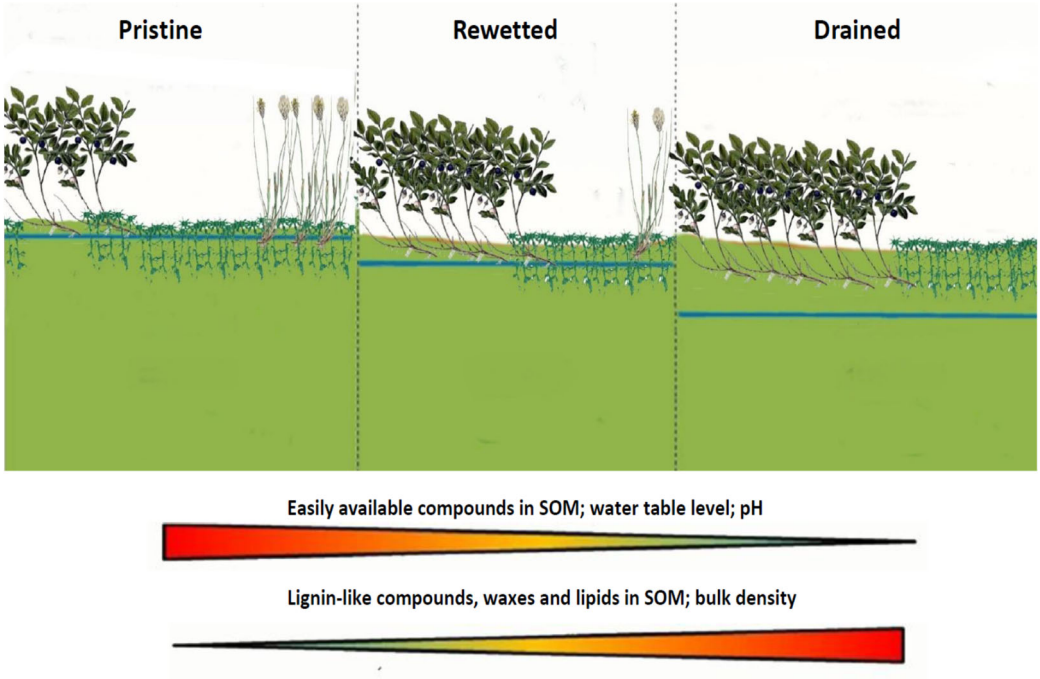


Figure 5: Scheme of the effect of peatland water level on the peatland ecosystem. Different water level conditions cause shifts in the plant community, chemical composition of soil organic matter, peat bulk density and pH.

3.2 Effects of peatland plant dominants on SOM, nutrient transformation, microbial biomass and enzymatic activities

In our second study, we focused on how the long-term presence of blueberry and cotton-grass is reflected in the peat characteristics and soil microbial biomass and what could be possible implications of their spread for nutrient cycling and C storage in these ecosystems. We showed that different biomass composition (chemistry, stoichiometry) of the studied peatland plant dominants – *Sphagnum*, cotton-grass and blueberry was mirrored in the peat formed in the microhabitats dominated by particular species (2).

Sphagnum biomass as well as the peat formed in microhabitats where only *Sphagnum* was present, had the lowest aromaticity and was relatively rich in polysaccharides. Cotton-grass biomass also had a high content of polysaccharides but was further enriched in polypeptides, aliphatic and aromatic compounds (in the

leaves more than in the roots) than *Sphagnum,* which was also typical for the peat formed in the cotton-grass presence. Blueberry biomass and also the peat in blueberry-dominated microhabitats were characterized by the highest content of aromatic compounds and the lowest content of polysaccharides and polypeptides among all the species (Fig. 6).

Biomass of particular species and the formed peat also differed in elemental composition, C/N/P stoichiometry, nutrient availability in the soil solution and in soil microbial activities.

Sphagnum formed the microhabitats with the highest concentrations of ammonia within the nutrient poor spruce swamp forest system (2). The high N availability in the *Sphagnum*-peat is likely connected with biological atmospheric N_2 fixation by cyanobacteria and methanotrophs associated with *Sphagnum* (Larmola et al. 2014), a high cation exchange capacity (Verhoeven and Liefveld 1997) and a low N resorption efficiency of *Sphagnum* (2). The presence of available N in the form of ammonia corresponded to prevailing anaerobic conditions in *Sphagnum*-dominated microhabitats with a high water table. In contrast to relatively high N availability, we found the highest C/P ratio of the *Sphagnum* litter, which corresponded well to the extremely high C/SRP (soluble reactive phosphorus) ratios of the leachate (DOM) produced during *Sphagnum* litter decomposition as compared to that from the litter of vascular plants (2, 3). Therefore, our results indicate possible P limitation of microbial community in *Sphagnum* microhabitats. Microbial biomass and activities were also lower in *Sphagnum*-dominated microhabitats as compared to vascular plants-dominated microhabitats (2), which could be also connected with antimicrobial properties of *Sphagnum* (Painter 1991).

In contrast, the cotton-grass shoots and also DOM produced during their decomposition had the highest N and P concentrations and thus the lowest C/N and C/P ratios among the studied species, whereas its roots had significantly lower nutrient content than shoots (2). The high concentration of nutrients in the cotton-grass biomass was associated with the most efficient nutrient resorption (especially in the case of P) from the senescing biomass. Cotton-grass is also effective in nutrient uptake from soil due to a large root exudation flux, which was first deduced from increased CO_2 and CH_4 effluxes in its presence (Saarnio et al. 2004; Laine et al.

2012; Kuiper et al. 2014; Robroek et al. 2015) and also directly measured (Edwards et al. 2018). The high effectivity of nutrient uptake, immobilization and reuse by cotton-grass causes a depletion of N and P from the soil solution and SOM in the cotton-grass dominated microhabitats (2) (Fig. 6). In accordance with nutrient-poor peat, microbial biomass in cotton-grass dominated microhabitats had the highest C/P and N/P ratio out of the three plant species (Fig. 6). It indicates severe P limitation of microbial growth, which restrained OM decomposition in the cotton-grass microhabitats. Nutrient limitation of microbial activity together with high primary productivity and suppressed root decomposition (2) makes the cotton-grass a typical peat-forming species (Tuittila et al. 1999; Silvan et al. 2004; Kivimaki et al. 2008).

Blueberry was the nutrient poorest plant dominant with the lowest nutrient resorption efficiency (2). The soil solution in the microhabitats dominated by blueberry was characteristic by the highest concentrations of nutrients in mineral forms and the lowest N/P ratio of all plant dominants (2). It indicated that nutrients, and specifically P, were more available for microbes and plants in blueberry microhabitats than in microhabitats dominated by other species. The increased P availability was also reflected in the largest soil microbial biomass including its highest N and P contents among all plant species (Fig. 6). The high P availability in the blueberry microhabitats is likely related to ericoid mycorrhizae with a large capability to mine nutrients from SOM by extracellular enzymes (Read 1996; Read et al. 2004). In result, nutrient cycling under blueberry was more opened and the increased nutrient availability in soil solution could support decomposition of OM. In accord with others (Bragazza et al. 2007, 2013, 2015), our results suggest that a spreading of ericoids in peatlands due to a lowering of water table would lead to enhanced OM decomposition and accelerated nutrient cycling, with negative implications for C sequestration.

The differences in biomass composition and nutrient content among the three studied peatland plant species resulted in various decomposability of their litter and biodegradability of the litter-leached DOM. *Sphagnum* litter is considered as a recalcitrant substrate due to a high content of sphagnan and other enzyme

inhibiting compounds (Painter 1991; Hájek et al. 2011). In accord, we measured significantly slower decomposition rates of *Sphagnum* litter (2) and lower biodegradability of the DOM leached from the dead *Sphagnum* biomass during its decomposition (3) in comparison to the vascular plant litter and their DOM. The decomposition rate of cotton-grass shoot litter and the biodegradability of the DOM released during its decomposition were the highest from the studied species (2, 3). On contrary, cotton-grass root litter decomposed comparably slowly as the *Sphagnum* litter (2) (Fig. 6). Such difference in decomposability of cotton-grass shoots and roots was also found in the study of Straková et al. (2012) and could be partly caused by very low nutrient content in roots. Blueberry leaves were less decomposable than cotton-grass leaves and vice versa for roots (2) (Fig. 6). The decomposability of blueberry leaf litter and biodegradability of its leached DOM was higher as compared to *Sphagnum* (2, 3).

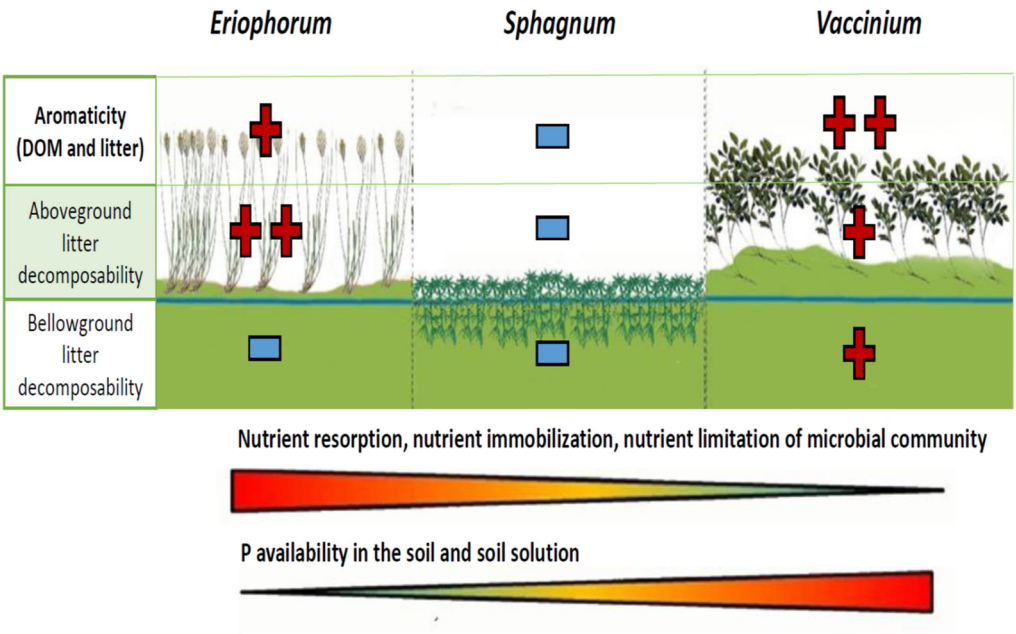


Figure 6: Characteristics of peatland plant dominants (chemistry of litter and DOM, litter decomposability) and their relation to nutrient resorption, immobilization and nutrient status of their microhabitats (2, 3).

3.3 Quality of DOM produced during litter decomposition of peatland plant dominants

In the third study we studied a process of litter decomposition as a potential source of the DOM, including detailed characterization of the leachate DOM composition and biodegradability. We have found that the DOM released from freshly senesced vascular plant litter was easily degradable, containing 38–48 % of C mineralizable within 40 days, while the DOM leached from the litter decomposing for 200 days contained only 12–23 % of the mineralizable C. Such values of leachate-DOM biodegradability were comparable to that of the DOM extracted from peatland *in situ* (3). Similar decrease of DOM biodegradability in time was also found by Pinsonneault et al. (2016), who also worked with peatland plants. Although the C/N/P stoichiometry of the leachates differed among different litter types, it was not directly related to DOM biodegradability (3). Our results suggest that the DOM leached from freshly senesced litter represents an input of easily available substrate and nutrients for the microbial community in the peat, while partially decomposed litter released only low amounts of decomposable DOM regardless on the plant species.

We continued with the chemical compositional characterization of the leached DOM to seek for differences between species and to explain significant temporal decrease in DOM biodegradability. Molecular weight (MW) fractions of DOM did not show significant differences in C and N distribution among DOM fractions were found neither within litter types nor in time (3). Therefore, a DOM distribution among the C and N fractions of various MW was not important factor driving DOM biodegradability (3).

More than 60 % of DOM was equally divided between the smallest <1kDa and the largest >100kDa MW fractions, while the remaining DOM was equally distributed between the 1-100 kDa MW fractions. Thus, the majority of DOM compounds had lower MW than 100 kDa, which is in accord with findings of others, that DOM compounds in soil solution are usually shifted towards lower MW as a result of the degradation of initial complex compounds in litter under oxidised conditions (Schulze 2005; Kothawala et al. 2012; Malik and Gleixner 2013; Kiikkilä et al. 2013; Jokubauskaite et al. 2015, Rouwane et al. 2018). The proportion of C in <1 kDa

fraction remained similar in the litter DOM leachates during the whole 200-day decomposition experiment, while the concentration of simple sugars, amino acids and simple organic acids decreased after 70 days regardless of the litter type (3). It indicated that these compounds were replaced by other simple low MW compounds like phenolics (Li et al. 2004; Richard et al. 2007; Kiikkila et al. 2012), oligopeptides and oligonucleotides. Diminishing concentration of easily degradable compounds corresponded with decreasing DOM biodegradability towards the end of the incubation.

Spectroscopic (UV-VIS) characteristics of DOM MW fractions revealed the differences neither between litter types nor in time, except for the DOM aromaticity index SUVA 254. SUVA 254 reflected the differences in content of lignin-like compounds among litters (2) and increased in order *Sphagnum* < cotton-grass < blueberry (3) (Fig. 6). In this aspect, the SUVA 254 values measured in our experiment agreed with other studies, where the blueberry DOM is generally considered as highly hydrophobic and aromatic (Peuravuori and Pihlaja 2007; Volk et al. 2002), while the *Sphagnum* DOM has more aliphatic character (Castells et al. 2005; Giudice and Lindo 2017). However, we did not find any systematic changes among MW fractions in DOM SUVA 254 values during litter decomposition, neither between litter types nor in time. This contrasts with others, who found increasing aromaticity of the organic compounds at later stages of litter decomposition (e.g. Don and Kalbitz 2005).

In contrast to C and N, the distribution of P among the DOM MW fractions significantly differed for each studied litter (3). Especially *Sphagnum* DOM was very specific: the P in fresh litter was associated with the high MW DOM, while later was redistributed to lower MW fractions and complexed with Al and Fe (3). The P complexation could retard SRP leaching, which was negligible from the *Sphagnum* litter (3). Leachates from cotton-grass shoots contained highest concentration of P from all litter types, which may support its fast decomposition. There was also a difference in P distribution among MW fractions between cotton-grass shoots and roots. Most of the P in the leachates from cotton-grass shoots was in the 1-10 kDa

MW fraction, while cotton-grass roots leachate had most of the P in the larger 10-100 kDa MW fraction.

We concluded that the leaf litter of vascular plants serves as a source of easily degradable compounds and nutrients for microbial community, while P immobilization could limit decomposition of *Sphagnum* litter and contribute to the low biodegradability of its leached DOM (3) (Fig. 6). Our results indicate that environmental changes connected with spreading of vascular plants could partly relieve P limitation of the microbial activity in the *Sphagnum*-dominated peatlands, with potentially negative effects on their ability to act as C sinks.

3.4 Priming effect on peatland DOM decomposition induced by root exudates

Spreading of vascular plants in the peatlands, which is expected due to climate change or due to drainage for extraction, forestry or agriculture, would bring changes in the input and chemical composition of compounds exuded to the peatland DOM by the plants. Due to significantly higher decomposability of vascular plant exudates than *Sphagnum* exudates (Edwards et al. 2018), root exudation of vascular plants could lead to increased microbial activity and consequently to the positive priming effect on DOM and SOM decomposition. In our fourth study, we conducted laboratory experiment to examine whether there is a priming effect after input of easily decomposable compounds (a mixture of sugar, amino acid and organic acid) into the peatland DOM having low biodegradability. In purpose, we set two doses of exudate addition, which we calculated from directly measured exudation fluxes of cotton-grass and blueberry (Edwards et al. 2018). The low dose of exudate addition (exudate C input reaching 2 % of the DOM) corresponded to the real contribution of root exudates into the natural DOM *in situ* in the early vegetation season, while the high dose (exudate C input reaching 5 % of the DOM) represented the exudate contribution to the peatland DOM in the peak of the growing season. Our results showed that any input of the root exudates into the peatland DOM stimulated microbial growth and shifted composition of the present microbial community. However, while the low exudate input had no significant effect on the decomposition of the peatland DOM, the higher dose caused a positive priming effect (4) (Fig. 7). Moreover, increasing C/N ratio of root exudates from 7 to

50, when applied at high dosage, lead to enhanced positive priming effect. This was likely connected with enhanced nutrients demands of growing microbial community, which was not covered by the exudate input with high C/N ratio, and larger exoenzymatic production. It aimed into the targeted „nutrient mining“ and also a non-targeted co-metabolism of the DOM (4). We therefore showed that a positive rhizosphere priming effect could occur *in situ* in our study sites in the peak of vegetation season, when the root exudation of the vascular plants contributes to the peatland DOM ca by 5 % (4).

Fungi/bacteria ratio of the peat microbial community lower than 0.005 indicated that bacteria play the key role in peatland DOM transformation (2). We found that r-strategic bacteria, which growth was directly stimulated by the input of root exudates, could markedly contribute to the observed positive priming effect (4) (Fig. 6). Namely, *Pseudomonas* and *Burkholderia* genera, which exhibited rapid growth after higher input of exudates, have high enzymatic potential (Lladó et al. 2016; Sun et al. 2016). Therefore, we suggest that enzymes produced by these bacteria together with enzymes produced by later emerging K-strategic *Acidobacteria* were mainly responsible for the observed positive priming effect on DOM decomposition (4).

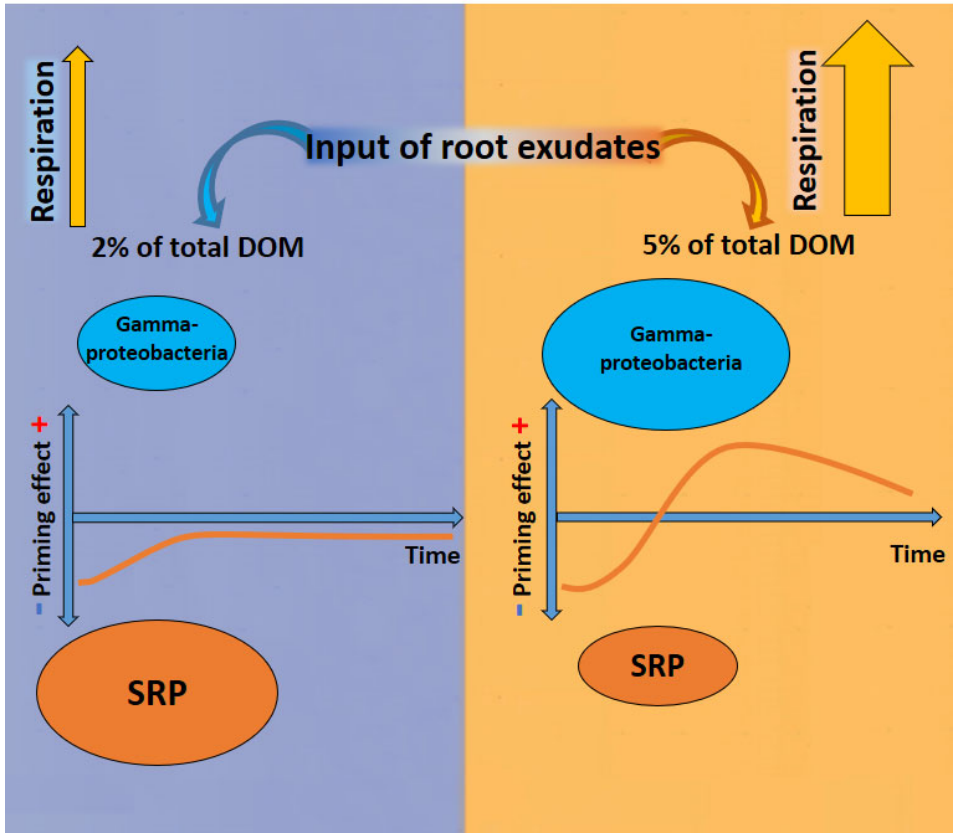


Figure 7: Effect of different level of added artificial root exudates on recalcitrant peatland DOM decomposition. The higher dose of root exudates caused a positive priming effect. It was connected with enhanced nutrients demands (especially soluble reactive phosphorus – SRP) of growing microbial community and larger exoenzymatic production. The r-strategic bacteria were stimulated by the input of root exudates. Gamma-proteobacteria (namely Pseudomonas and Burkholderia) exhibited rapid growth after higher input of exudates.

4 Conclusions

Long-term water level drawdown (more than 50 years) led to changes in vegetation composition and subsequently in SOM quality, because aerobic conditions supported SOM decomposition and enabled the spread of vascular plant species with different biomass composition, being richer in aromatics than Sphagnum. In the long-term, labile fractions of SOM were depleted leaving behind recalcitrant compounds. Due to a lack of easily available compounds in the soil, the present microbial community showed signs of energy limitation as indicated by its low biomass and respiratory and enzymatic activity as compared to undisturbed peatlands (1).

With decreased water levels, vascular plants are expected to spread over Sphagnum dominated areas, which will bring changes in litter, SOM and DOM quality. In our study, we focused on three typical peatland plants which belong to three functional types: Sphagnum (peat moss), cotton-grass (graminoids) and blueberry (ericoids). Sphagnum was characterized by low decomposability of its litter and the DOM leached from Sphagnum litter during its decomposition. This low decomposability could be caused by P limitation, which is indicated by the extremely high C/P ratio of the leachate and P associated with the high MW DOM, while in the later stage of decomposition P was complexed with Al and Fe in the lower MW fractions (3). The leaf litter of the vascular plant species, cotton-grass and blueberry, is in general more decomposable and a source of more degradable DOM for the microbial community than Sphagnum. These leaf litters also released significant amounts of nutrients and simple organic acids, sugars and amino acids (3). Yet, the two vascular species had distinct final effects on OM decomposition.

Cotton-grass provides large amounts of easily degradable leaf litter and DOM to the microbial community, as is shown by our data from both in situ (2) and laboratory experiments (3). Cotton-grass leaf litter contains large amounts of P, which is efficiently acquired from peat, helping to make this litter more easily degradable (2). On the contrary, cotton-grass root litter decomposes comparably as slowly as the Sphagnum litter (2, 3). During cotton-grass leaf litter decomposition (and to a lesser extent during root litter decomposition) more aromatic DOM with higher

biodegradability and more P is released than from Sphagnum litter (3). Moreover, cotton-grass has a high level of exudation (Edwards et al. 2018) and, according to our laboratory study, such root exudate input supports microbial activity leading to a significant positive priming effect and related nutrient mining (4). The positive priming effect was caused mainly by the activity of the r-strategy Gammaproteobacteria and K-strategy Acidobacteria with large enzymatic potentials (4). However, cotton-grass effectively recycles N and P within the plant (especially P immobilization and resorption) (2). Its presence strongly depletes P and N from the peat, with the consequent low nutrient availability reflected in the C/N/P stoichiometry of the microbial biomass (2). The strong nutrient limitation of microbial activity together with the prevailing anoxic conditions thus supports OM accumulation in the peatland ecosystem.

Blueberry occurs in microhabitats with lower water levels and thus more oxidized soil than Sphagnum and cotton-grass. Blueberry leaf litter and its DOM are more decomposable and they contain more nutrients than Sphagnum litter (3). Its presence is associated with ericoid mycorrhiza and exudation of easily decomposable compounds. The blueberry nutrient pool is more opened and thus it facilitates decomposition of OM, increases nutrient availability, enhances microbial biomass and stimulates heterotrophic microbial activities (2). Its massive spread could change the peatland ecosystem from a C sink to a C source.

5 References

Agren A., Buffam I., Berggren M., Bishop K., Jansson M., Laudon H. 2008. Dissolved organic carbon characteristics in boreal streams in a forest-wetland gradient during the transition between winter and summer. The Journal of Geophysical Research, 113, G03031.

Alm J., Sculman L., Walden J., Nykänen H., Martikainen P.J., Silvola J. 1999. Carbon balance of a boreal bog during a year with an exceptionally dry summer. Ecology 80 (1), 161–174.

Amelung W., Cheshire M.V., Guggenberger G. 1996. Determination of neutral and acidic sugars in soil by capillary gas–liquid chromatography after trifluoroacetic acid hydrolysis. Soil Biology and Biochemistry, 28, 1631–1639.

Aurela M., Riutta T., Laurila T., Tuovinen J.-P., Vesala T., Tuittila E.-S., Rinne J., Haapanala S., Laine J. 2007. CO_2 exchange of a sedge fen in southern Finland – the impact of a drought period. Tellus, 59B, 826–837.

Ausec L., Kraigher B., Mandic-Mulec I. 2009. Differences in the activity and bacterial community structure of drained grassland and forest peat soils. Soil Biology and Biochemistry 41: 1874–1881.

Backe´us I. 1985. Above ground production and growth dynamics of vascular bog plants in Central Sweden. Acta Phytogeographica Suecica 74: 1–98.

Baldock J.A., Oades J.M., Waters A.G., Peng X., Vassallo A.M., Wilson M.A. 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state 13C NMR spectroscopy. Biogeochemistry 16, 1–42.

Beare M.H., Hendrix P.F., Coleman D.C. 1994. Water-stable aggregates and organic matter fractions in conventional- and no-tillage soils. Soil Science Society of America Journal, 58, 777–786.

Berg B., McClaugherty C. 2003. Plant Litter. Decomposition, Humus Formation, Carbon Sequestration. Springer, Berlin.

Blagodatskaya E., Kuzyakov Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils, 45, 115–131.

Blagodatskaya E., Yuyukina T., Blagodatsky S., Kuzyakov Y. 2011. Three-source-partitioning of microbial biomass and of CO_2 efflux from soil to evaluate mechanisms of priming effects, Soil Biology and Biochemistry, 43, 778–786.

Blagodatskaya E., Khomyakov N., Myachina O., Bogomolova I., Blagodatsky S., Kuzyakov Y. 2014. Microbial interactions affect sources of priming induced by cellulose. Soil Biology and Biochemistry 74, 39-49.

Bombonato L., Siffi C., Gerdo, R. 2010. Variations in the foliar nutrient content of mire plants: effects of growth-form based grouping and habitat. Plant Ecology, 211, 235–251.

Bosatta E., Ågren G. 1991. Dynamics of carbon and nitrogen in the organic matter of the soil: a general theory. The American Naturalist, 138, 227–245.

Bradford M.A., Berg B., Maynard D.S., Wieder W.R., Wood S.A. 2016. Understanding the dominant controls on litter decomposition. Journal of Ecology, 104, 229–238.

Bragazza L., Siffi C., Iacumin P., Gerdol R. 2007. Mass loss and nutrient release during litter decay in peatland: The role of microbial adaptability to litter chemistry. Soil Biology and Biochemistry, 39, 257–267.

Bragazza L., Parisod J., Buttler A., Bardgett R.D. 2013. Biogeochemical plant-soil microbe feedback in response to climate warming in peatlands. Nature Climate Change, 3, 273–277.

Bragazza L., Bardgett R.D., Mitchell E.A.D., Buttler A. 2015. Linking soil microbial communities to vascular plant abundance along a climate gradient. New Phytologist, 205, 1175–1182.

Brüggemann, N., Gessler A., Kayler Z., Keel S. G., Badeck F., Barthel M., Boeckx P., Buchmann N., Brugnoli E., Esperschutz J., Gavrichkova O., Ghashghaie J., Gomez-Casanovas N., Keitel C., Knohl A., Kuptz D., Palacio S., Salmon Y., Uchida Y., Bahn M. 2011. Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review, Biogeosciences, 8, 3457–3489.

Bubier J., Crill P., Mosedale A., Frolking S., Linder E. 2003. Peatland responses to varying interannual moisture conditions as measured by automatic CO_2 chambers. Global Biogeochemical Cycles, 17, 1-15.

Cairney J.W.G., Meharg A.A. 2003. Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. The European Journal of Soil Science, 54, 735–740.

Carter C. W., Suffet I. H. 1982. Binding of DDT to dissolved humic material. Environmental Science & Technology 16, 735–740.

Castells E., Peñuelas J., Valentine D.W. 2005. Effects of plant leachates from four boreal understory species on soil N mineralization, and white spruce (*Picea glauca*) germination and seedling growth. Annals of Botany, 95, 1247–1252.

Clark J.M., Lane S.N., Chapman P.J., Adamson J.K. 2007. Export of dissolved organic carbon from an upland peatland during storm events: implications for flux estimates. Journal of Hydrology, 347 (3–4), 438–447.

Clark, J.M., Lane, S.N., Chapman, P.J., Adamson, J.K. 2008. Link between DOC in near surface peat and stream water in an upland catchment. The Science of the Total Environment, 404, 308–315.

Clymo, R. S. 1984. The limits to peat bog growth. Philosophical Transactions of the Royal Society, London B 303, 605–54.

Cocozza C., D'Orazio V., Miano T.M., Shotyk W. 2003. Characterization of solid and aqueous phases of a peat bog profile using molecular fluorescence spectroscopy, ESR and FT-IR, and comparison with physical properties, Organic Geochemistry, 34, 49–60.

Conant R.T., Ryan M.G., Ågren G.I., Birge H.E., Davidson E.A., Eliasson P.E., Evans S.E., Frey S.D., Giardina C.P., Hopkins F.M., Hyvönen R., Kirschbaum M.U.F., Lavallee J.M., Leifeld J., Parton W.J., Megan Steinweg J., Wallenstein M.D., Martin Wetterstedt J.Å., Bradford M.A. 2011. Temperature and soil organic matter decomposition rates e synthesis of current knowledge and a way forward. Global Change Biology, 17, 3392-3404.

Crow S.E., Wieder R.K. 2005. Sources of CO_2 emission from a northern peatland: root respiration, exudation, and decomposition. Ecology, 86, 1825–1834.

Crow S.E., Lajtha K., Bowden R.D., Yano Y., Brant J.B., Caldwell B.A., Sulzman E.W. 2009. Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-growth forest. Forest Ecology and Management 258, 2224-2232.

Dai K.H., David M.B., Vance G.F. 1996. Characterization of soil and dissolved carbon in a spruce-fir Spodosol. Biogeochemistry, 35, 339–65.

Damman, A. W. H. 1988. Regulation of nitrogen removal and retention in Sphagnum bogs and other peatlands. Oikos, 51, 291-305.

Davidson E. A., Janssens I. A. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature, 440, 165–173.

Delarue, F., Laggoun-Défarge F., Disnar J.R., Lottier N., Gogo S. 2011. Organic matter sources and decay assessment in a Sphagnum-dominated peatland (Le Forbonnet, Jura Mountains, France): Impact of moisture conditions. Biogeochemistry, 106, 39–52.

Dodla S. K., Wang J. J., DeLaune R. D. 2012. Characterization of labile organic carbon in coastal wetland soils of the Mississippi River deltaic plain: relationships to carbon functionalities. Science of the Total Environment, 435–436, 151–158.

Don A., Kalbitz K. 2005. Amounts and degradability of dissolved organic carbon from foliar litter at different decomposition stages. Soil Biology and Biochemistry 37, 2171–2179.

Donald R.G., Anderson D.W., Steward, J.W.B. 1993. Potential role of dissolved organic carbon in phosphorous transport in forested soils. Soil Science Society of America Journal, 57, 1611-1618.

Dorrepaal E. 2007. Are plant growth-form-based classifications useful in predicting northern ecosystem carbon cycling feedbacks to climate change? Journal of Ecology, 95, 1167-1180.

Dieleman C.M., Branfireun B.A., McLaughlin J.W., Lindo Z. 2015. Climate change drives a shift in peatland ecosystem plant community: implications for ecosystem function and stability. Global Change Biology, 21, 388–395.

Edwards K.R., Kaštovská E., Borovec J., Šantrůčková H., Picek T. 2018. Species effects and seasonal trends on plant efflux quantity and quality in a spruce swamp forest. Plant Soil, 426, 179–96.

Ehrman T. 1996. Determination of acid-soluble lignin in biomass. LAP-004 1-7

Elmendorf S.C., Henry G.H.R., Hollister R.D., Bjork R.G., Boulanger-Lapointe N., Cooper E.J., Cornelissen J.H.C., Day T.A., Dorrepaal E., Elumeeva T.G., Gill M., Gould W.A., Harte J., Hik D.S., Hofgaard A., Johnson D.R., Johnstone J.F., Jonsdottir I.S., Jorgenson J.C., Klanderud K., Klein J.A., Koh S., Kudo G., Lara M., Levesque E., Magnusson B., May J.L., Mercado-Diaz J.A., Michelsen A., Molau U., Myers-Smith I.H., Oberbauer S.F., Onipchenko V.G., Rixen C., Schmidt N.M., Shaver G.R., Spasojevic M.J., Porhallsdottir P.E., Tolvanen A., Troxler T., Tweedie C.E., Villareal S., Wahren C-H., Walker X., Webber P.J., Welker J.M., Wipf S. 2012. Plot-scale evidence of tundra vegetation change and links to recent summer warming. Nature Climate Change, 2, 453-457.

Fenner N., Freeman C. 2011. Drought-induced carbon loss in peatlands. Nature Geoscience, 4, 895-900.

Fontaine S., Mariotti A., Abbadie L. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology and Biochemistry, 35, 837–843.

Freeman C., Lock M., Reynolds B. 1993. Climatic change and the release of immobilized nutrients from Welsh riparian wetland soils, Ecological Engineering, 2(4), 367– 373.

Freeman C., Ostle N., Kang H. 2001. An enzymatic latch on a global carbon store. Nature, 409, 149.

Gregorich E.G., Beare M.H., Stoklas U., St-Georges P. 2003. Biodegradability of soluble organic matter in maize-cropped soils. Geoderma, 113, 237–252.

Guggenberger G., Zech W., Schulten H.-R. 1994a. Formation and mobilization pathways of dissolved organic matter: evidence from structural studies of organic matter fractions in acid forest floor solutions. Organic Geochemistry, 21, 51–66.

Guggenberger G., Christensen B.T., Zech W. 1994b. Land-use effects on the composition of organic matter in particle-size separates of soil. I. Lignin and carbohydrate signature. European Journal of Soil Science, 45, 449–458.

Guggenberge, G., Kaiser K. 2003. Dissolved organic matter in soil: challenging the paradigm of sorptive preservation. Geoderma, 113, 293 – 310.

Giudice R.D., Lindo Z. 2017. Short-term leaching dynamics of three peatland plant species reveals how shifts in plant communities may affect decomposition processes. Geoderma, 285, 110–116.

Gunnarson U. 2005. Global patterns of Sphagnum productivity. Journal of Bryology, 27, 267-277.

Haichar F.Z., Marol C., Berge O., Rangel-Castro J.I., Prosser J.I., Balesdent J., Heulin T., Achouak W. 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME Journal, 2, 1221–1230.

Hájek T., Balance S., Limpens J., Zijlstra M., Verhoeven J.T.A. 2011. Cell-wall polysaccharides play an important role in decay resistance of Sphagnum and actively depressed decomposition in vitro. Biogeochemistry, 103, 45–57.

Hardie A.G., Dynes J.J., Kozak L.M., Huang P.M. 2007. Influence of polyphenols on the integrated polyphenol-maillard reaction humification pathway as catalysed by birnessite. Annals of Environmental Science, 1, 91–110.

Hatakka A. 2001. Biodegradation of lignin. Lignin, Humic Substances and Coal, Vol. 1 (Hofrichter M & Steinbüchel A, eds), pp. 129–179. Wiley-VCH, Weinheim.

Hayward P.M. Clymo R.S. 1983. The growth of *Sphagnum*: experiments on, and simulation of, some effect of light flux and water-table depth. Journal of Ecology, 71, 845–863.

Helms J. R., Stubbins A., Ritchie J. D., Minor E. C., Kieber D. J.,Mopper, K. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnology and Oceanography, 53, 955–969.

Her N., Amy G., Foss D., Cho J.W. 2002. Variations of molecular weight estimation by HP-size exclusion chromatography with UVA versus online DOC detection. Environmental Science & Technology, 36, 3393–3399.

Her N., Amy G., Sohn J., Gunten U. 2008. UV absorbance ratio index with size exclusion chromatography (URI-SEC) as an NOM property indicator. Journal of Water Supply Research and Technology-Aqua, 57, 35-44.

Hobbie S.E. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. Ecological Monographs, 66, 503-522.

Holden J., Chapman P.J., Labadz, J.C. 2004. Artificial drainage of peatlands: hydrological and hydrochemical process and wetland restoration. Progress in Physical Geography, 28, 95–123.

Hood E., Gooseff M. N., Johnson, S. L. 2006. Changes in the character of stream water dissolved organic carbon during 20 flushing in three small watersheds, Oregon, The Journal of Geophysical Research, 111, 1-8.

Hur J., Williams M.A., Schlautman M.A. 2006. Evaluating spectroscopic and chromatographic techniques to resolve dissolved organic matter via end member mixing analysis. Chemosphere, 63, 387–402.

Huttunen J.T., Nykänen H., Turunen J., Martikainen P.J. 2003. Methane emissions from natural peatlands in the northern boreal zone in Finland, Fennoscandia. Atmospheric Environment, 37, 147–151.

Chanton J. P., Whiting G. J. 1996. Methane stable isotopic distributions as indicators of gas transport mechanisms in emergent aquatic plants. Aquatic Botany, 54, 227—236.

Chapin F.S., Van Cleve K., Chapin M.C. 1979. Soil temperature and nutrient cycling in the tussock growth form of *Eriophorum vaginatum*. Journal of Ecology, 67, 169-189.

Chapin F.S., Moilanen L., Kielland K. 1993. Preferential use of organic nitrogen for growth by a nonmycorrhizal Artic sedge. Nature 361, 150–153.

Chapin F.S., Bret-Harte M.S., Hobbie S., Zhong, H. 1996. Plant functional types as predictors of the transient response of arctic vegetation to global change. Journal of Vegetation Science, 7, 347-357.

Chapin F.S., Zavaleta E.S., Eviner V.T., Naylor R.L., Vitousek P.M., Reynolds H.L., Hooper D.U., Lavorel S., Sala O.E., Hobbie S.E., Mack M.C., Diaz S. 2000. Consequences of changing biodiversity. Nature 405, 234–242.

Charman, D., 2002. Peatlands and Environmental Change. John Wiley & Sons Ltd, Chichester.

Chen R., Senbayram M., Blagodatsky S., Myachina O., Dittert K., Lin X., Kuzyakov Y. 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Global Change Biology, 20, 2356–2367.

Cheng W., Kuzyakov Y. 2005. Root effects on soil organic matter decomposition. In: Wright, S., Zobel, R. (Eds.), Roots and Soil Management: Interactions between Roots and the Soil. Agronomy Monograph No. 48. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, USA, 119-143.

Chefetz, B., Salloum, M.J., Deshmukh, A.P., Hatcher, P.G. 2002. Structural amounts of humic acids as determined by chemical modifications and carbon-13 NMR, pyrolysis-, and thermochemoly- sis-gas chromatography/mass spectrometry. Soil Science Society of America Journal, 66, 1159–1171.

Chroňáková A., Bárta J., Kaštovská E., Urbanová Z., Picek T. 2019. Spatial heterogeneity of belowground microbial communities linked to peatland microhabitats with different plant dominants, *FEMS Microbiology Ecology*, 95 (9), fiz13.

Inamdar S., Singh S., Dutta S., Levia D., Mitchell M., Scott D., Bais H., McHale P. 2011. Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed. The Journal of Geophysical Research, 116, G03043.

Jardine P.M., Weber N.L., McCarthy J.F. 1989. Mechanisms of dissolved organic carbon adsorption on soil. Soil Science Society of America Journal, 53, 1378-1385.

Jandl R., Sollins P. 1997. Water extractable carbon in relation to below-ground carbon cycle. Biology and Fertility of Soils, 25, 196–201.

Jansen B., Nierop K.G.J., Verstraten J.M. 2003. Mobility of Fe(II), Fe(III) and Al in acidic forest soils mediated by dissolved organic matter: influence of solution pH and metal/organic carbon ratios. Geoderma 113, 323–340.

Jansen B., Nierop K. G. J., Verstraten J. M. 2005. Mechanisms of Controlling the Mobility of Dissolved Organic Matter, Aluminium and Iron in Podzol B Horizons. European Journal of Soil Science, 56(4), 537-550.

Jokubauskaite I., Amaleviciute K., Lepane V., Slepetiene A., Slepetys J., Liaudanskiene I., Karcauskiene D., Booth C.A. 2015. High-performance liquid chromatography (HPLC)-size exclusion chromatography (SEC) for qualitative detection of humic substances and dissolved organic matter in mineral soils and peats in Lithuania. International Journal of Environmental Analytical Chemistry 95, 508–519.

Jones D.L., Hodge A., Kuzyakov Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytology 163, 459–480.

Jones R.T., Robeson M.S., Lauber C.L., Hamady M., Knight R., Fierer N. 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. ISME Journal, 3, 442–453.

Jones D.L., Willett V.B., Stockdale E.A., Macdonald A.J., Murphy D.V. 2012. Molecular weight of dissolved organic carbon, nitrogen, and phenolics in grassland soils. Soil Science Society of America Journal, 76(1), 142-150.

Jungkunst H.F., Flessa H., Scherber C., Fiedler S. 2008. Groundwater level controls CO_2, N_2O and CH_4 fluxes of three different hydromorphic soil types of a temperate forest ecosystem. Soil Biology and Biochemistry, 40 (8), 2047–2054.

Kaiser K., Zech W. 1997. Competitive sorption of dissolved organic matter fractions to soils and related mineral phases. Soil Science Society of America Journal, 61, 64–69.

Kaiser K., Zech W. 1998. Soil dissolved organic matter sorption as influenced by organic and sesquioxide coatings and sorbed sulphate. Soil Science Society of America Journal, 62, 129–136.

Kaiser K., Guggenberger G. 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Organic Geochemistry, 31, 711–725.

Kaiser K., Guggenberger G., Haumaier L., Zech W. 2001. Seasonal variations in the chemical composition of dissolved organic matter in organic forest floor layer leachates of old-growth Scots pine (*Pinus sylvestris L.*) and European beech (*Fagus*

sylvatica L.) stands in northeastern Bavaria, Germany. Biogeochemistry, 55, 103–143.

Kalbitz K., Solinger S., Park J.H., Michalzik B., Matzner E. 2000. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Science, 165, 277–304.

Kalbitz K., Schmerwitz J., Schwesig D., Matzner E. 2003a. Biodegradation of soil-derived dissolved organic matter as related to its properties, Geoderma, 113(3), 273– 291.

Kalbitz K., Schwesig D., Schmerwitz J., Kaiser K., Haumaier L., Glaser B., Ellerbrock R., Leinweber P. 2003b. Changes in properties of soil-derived dissolved organic matter induced by biodegradation. Soil Biology and Biochemistry, 35, 1129–1142.

Kalbitz K., Meyer A., Yang R., Gerstberger P. 2007. Response of dissolved organic matter in the forest floor to long-term manipulation of litter and throughfall inputs. Biogeochemistry, 86, 301–318.

Kane E.S., Turetsky M.R., Harden J.W., McGuire A.D., Waddington J.M. 2010. Seasonal ice and hydrologic controls on dissolved organic carbon and nitrogen concentrations in a boreal-rich fen. Journal of Geophysical Research – Biogeosciences, 115 (G04).

Kemper W.D., Rosenau R.C. 1986. Aggregate stability and size distribution. In: Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. Agronomy Monograph no. 9. Society of Agronomy/Soil Science Society of America, 425– 442.

Kiikkilä O., Kitunen V., Smolander A. 2012. Chemical and biological characterization of dissolved organic matter derived from Norway spruce litter divided into fractions according to molecular size. European Journal of Soil Biology 50, 109–111.

Kiikkilä O., Smolander A., Kitunen V. 2013. Degradability, molecular weight and adsorption properties of dissolved organic carbon and nitrogen leached from different types of decomposing litter. Plant and Soil 373, 787–798.

Kiikkilä O., Kanerva S., Kitunen V., Smolander A. 2014. Soil microbial activity in relation to dissolved organic matter properties under different tree species. Plant and Soil, 377, 169–177.

Killops S.D., Killops V.J. 2005. 2nd ed. Introduction to Organic Geochemistry, vol. 152 Blackwell Publishing.

Kirk P.M., Cannon P.F., Minter D.W., Stalpers J.A. 2008. Dictionary of the Fungi, 10th edn. CABI, Wallingford.

Kivimaki A., Yli-petays M., Tuittila. E. 2008. Carbon sink function of sedge and *Sphagnum* patches in a restored cut-away peatland: increased functional diversity leads to higher production. Journal of Applied Ecology, 45, 921–929.

Klavins M., Sire J., Purmalis O., Melecis V. 2008. Approaches to estimating humification indicators for peat. Mires Peat, 3, 1–15.

Knief K.C. 2015. Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. Frontiers in Microbiology, 6, 1346.

Koehler A.-K., Murphy K., Kiely G., Sottocornola M. 2009. Seasonal variation of DOC concentration and annual loss of DOC from an Atlantic blanket bog in South Western Ireland. Biogeochemistry 95, 231–242.

Kögel-Knabner I., Hatcher P.G., Tegelaar E.W., Deleeuw J.W. 1992. Aliphatic components of forest soil organic matter as determined by solidstate 13C NMR and analytical pyrolysis. The Science of the Total Environment, 113, 89–106.

Kögel-Knabner I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry, 34, 139–162.

Kool A., Heijmans M.M.P.D. 2009. Dwarf shrubs are stronger competitors than graminoid species at high nutrient supply in peat bogs. Plant Ecology, 204, 125–134.

Korshin G.V., Li C.W., Benjamin M.M. 1997. Monitoring the properties of natural organic matter through UV spectroscopy: A consistent theory. Water Research, 31, 1787-1795.

Kothawala D.N., Roehm C., Blodau C., Moore T.R. 2012. Selective adsorption of dissolved organic matter to mineral soils. Geoderma, 189–190, 334–342.

Kuiper J.J., Mooij W.M., Bragazza L., Robroek B.J. 2014. Plant functional types define magnitude of drought response in peatland CO_2 exchange. Ecology, 95, 123–131.

Kuzyakov Y., Friedel J.K., Stahr K. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry, 32, 1485-1498.

Kuzyakov Y. 2010. Priming effects: interactions between living and dead organic matter. Soil Biology and Biochemistry, 42, 1363–1371.

Lafleur P.M., Roulet N.T., Bubier J.L., Frolking S., Moore T.R. 2003. Interannual variability in the peatland–atmosphere carbon dioxide exchange at an ombrotrophic bog. Global Biogeochemical Cycles, 17, 1036–1049.

Laiho R., Vasander H., Penttilä T., Laine J. 2003. Dynamics of plant-mediated organic matter and nutrient cycling following water-level drawdown in boreal peatlands, Global Biogeochemical Cycles, 17(2), 1053.

Laine A.M., Bubier J.L., Riutta T., Nilsson M.B., Moore T.R., Vasander H., Tuittila E.-S. 2012. Abundance and composition of plant biomass as potential controls for mire net ecosytem CO_2 exchange. Botany, 90, 63–74.

Landry C., Tremblay L. 2012. Compositional differences between size classes of dissolved organic matter from freshwater and seawater revealed by an HPLC-FTIR system. Environmental Science & Technology, 46, 1700–1707.

Larmola T., Leppanen S.M., Tuittila E.S., Aarva M., Merila P., Fritze H., Tiirola M. 2014. Methanotrophy induces nitrogen fixation during peatland development, Proceedings of the National Academy of Sciences of USA, 111, 734–739.

Lavoie C., Marcoux K., Saint-Louis A., Price J.S. 2005. The dynamics of a cotton-grass (*Eriophorum vaginatum L.*) cover expansion in a vacuum-mined peatland, Southern Quebec, Canada. Wetlands, 25, 64-75.

Le Mer J., Roger P. 2001. Production, oxidation, emission and consumption of methane by soils: A review. European Journal of Soil Biology, 37(1), 25–50.

Li L., Zhao Z., Huang W., Peng P., Sheng G., Fu J. 2004. Characterization of humic acids fractionated by ultrafiltration. Ogranic Geochemistry 35, 1025–1037.

Lladó S., Žifčáková L., Větrovský T., Eichlerová I., Baldrian P. 2016. Functional screening of abundant bacteria from acidic forest soil indicates the metabolic potential of *Acidobacteria subdivision 1* for polysaccharide decomposition. Biology and Fertility of Soils, 52, 251–260.

Lynch JM. 1982. Limits to microbial-growth in soil. Journal of general microbiology, 128, 405–410.

Maanavilja L., Aapala K., Haapelehto T., Kotiaho J.S., Tuittila E.-S. 2014. Impact of drainage and hydrological restoration on vegetation structure in boreal spruce swamp forests. Forest ecology and management, 330, 115–125.

Madgwick J., Parish F. 2008. Foreword by Global Environment Centre and Wetlands International. In: Parish F., Sirin A., Charman D., Joosten H., Minayeva T., Silvius M., Stringer L. (Eds.), Assessment on Peatlands, Biodiversity and Climate Change: Main Report. Global Environment Centre, Wetlands International, Kuala Lumpur, Wageningen, p. iv.

Malik A., Gleixner G. 2013. Importance of microbial soil organic matter processing in dissolved organic carbon production. FEMS Microbiology Ecology, 86, 139–148.

Marschner B., Kalbitz K. 2003. Controls of bioavailablity and biodegradability of dissolved organic matter in soils. Geoderma, 113, 211–235.

McDowell W.H., Currie W.S., Aber J.D., Yano Y. 1998. Effects of chronic nitrogen amendments on production of dissolved organic carbon and nitrogen in forest soils. Water Air & Soil Pollution, 105, 17–182.

McDowell W.H. 2003. Dissolved organic matter in soils-future directions and unanswered questions. Geoderma, 113, 179–186.

McDowell W.H., Zsolnay A., Aitkenhead-Peterson J.A., Gregorich E.G., Jones D.L., Jodemann D., Kalbitz K., Marschner B., Schwesig D. 2006. A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. Soil Biology and Biochemistry, 38, 1933-1942.

Miltner A., Kindler R., Knicker H., Richnow H.-H., Kästner M. 2009. Fate of microbial biomass-derived amino acids in soil and their contribution to soil organic matter. Organic Geochemistry, 40, 978–985.

Moore T.R., De Souza W., Koprivnjak J.-F. 1992. Controls on the sorption of dissolved organic carbon by soils. Soil Science, 154: 120-129.

Moore T.R., Dalva M. 1993. The influence of temperature and water table position on carbon dioxide and methane emissions from laboratory columns of peatland soils. Journal of Soil Science, 44, 651–664.

Moore T.R., Dalva M. 2001. Some controls on the release of dissolved organic carbon by plant tissues and soils. Soil Science, 166(1), 38– 47.

Moore T.R., Basiliko N. 2006. Decomposition. In: Wieder R.K., Vitt D.H., Eds. Boreal peatland ecosystems, ecological studies, vol 188. Heidelberg: Springer. pp 126–143.

Moore T.R., Bubier J.L., Bledzki L. 2007. Litter decomposition in temperate peatland ecosystems: The effect of substrate and site. Ecosystems, 10, 949-963.

Mooshammer M., Wanek W., Zechmeister-Boltenstern S., Richter A.A. 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Frontiers in Microbiology, 5, 22.

Mueller M.B., Schmitt D., Frimmel F.H. 2000. Fractionation of natural organic matter by size exclusion chromatography–properties and stability of fractions. Environmental Science & Technology, 34, 4867–4872.

Mulholland P.J., Dahm C.N., David M.B., DiToro D.M., Fisher T.R., Kögel-Knabner I., Meybeck M.H., Meyer J. L., Sedell J.R. 1990. What are the temporal and spatial variations of organic acids at the ecosystem level? In: Organic Acids in Aquatic Ecosystems. Perdue E.M, Gjessing E.T. (eds.). Life Sciences Research Report 48. John Wiley & Sons, Chichester, 315-329.

Müller M., Alewell C., Hagedorn F. 2009. Effective retention of litter-derived dissolved organic carbon in organic layers. Soil Biology and Biochemistry, 41, 1066–1074.

Mummey D.L., Stahl P.D., Buyer J.S. 2002. Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. Applied Soil Ecology, 21, 251–259.

Murray K.J., Tenhunen J.D., Kummerow J. 1989. Limitations on *Sphagnum* growth and net primary production in the foothills of the Philip Smith Mountains, Alaska. Oecologia, 80: 256–262.

Myers B., Webster K.L., Mclaughlin J.W., Basiliko N. 2012. Microbial activity across a boreal peatland nutrient gradient: the role of fungi and bacteria. Wetlands Ecology and Management, 20, 77– 88.

Oades J.M. 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant and Soil, 76, 319–337.

Oliver B.G., Thurman E.M., Malcolm R.L. 1983. The contribution of humic substances to the acidity of colored natural waters, Geochimica et Cosmochimica Acta, 47, 2031-2035.

Olk D.C., Gregorich E.G. 2006. Overview of the symposium proceedings, ‘‘meaningful pools in determining soil carbon and nitrogen dynamics’’. Soil Science Society of America Journal, 70, 967–974.

Painter T.J. 1983. Carbohydrate origin of aquatic humus from peat. Carbohydrate Research, 124, 22-26.

Painter T.J. 1991. Lindow man, Tollund man and other peat-bog bodies—the preservative and antimicrobial action of sphagnan, a reactive glycuronoglycan with tanning and sequestering properties. Carbohydrate Polymers, 15, 123–142.

Pankratov T.A., Ivanova A.O., Dedysh S.N., Liesack W. 2011. Bacterial populations and environmental factors controlling cellulose degradation in an acidic Sphagnum peat. Environmental Microbiology, 13, 1800–14.

Paul E.A., Collins H.P., Leavitt S.W. 2001. Dynamics of resistant soil carbon of Midwestern agricultural soils measured by naturally occurring ^{14}C abundance. Geoderma, 104, 239–56.

Peuravuori J., Pihlaja K., 2007. Characterization of freshwater humic matter. In: Nollet, L.M.L. (Ed.), Handbook of Water Analysis. CRC Press, Boca Raton, London, New York, 435–448.

Philippot L., Andersson S.G.E., Battin T.J., Prosser J.I., Schimel J.P., Whitman W.B., Hallin S. 2010. The ecological coherence of high bacterial taxonomic ranks. Nature Reviews Microbiology, 8, 523–529.

Piccolo A., Conte P., Scheunert I., Paci M. 1998. Atrazine interactions with soil humic substances of different molecular structure. J. Environ. Q. 27, 1324–1333.

Piccolo A. 2002. The supramolecular structure of humic substances: A novel understanding of humus chemistry and implications in soil science. In Advances in Agronomy, Vol. 75. Academic Press, New York, 57–134.

Pinsonneault A.J., Moore T.R., Roulet N.T., Lapierre J.-L., Pinsonneault A.J., Moore T.R., Roulet N.T., Lapierre J.F. 2016. Biodegradability of vegetation-derived dissolved organic carbon in a cool temperate ombrotrophic bog. Ecosystems, 19 (6), 1023–1036.

Plante A.F., Conant R.T., Paul E.A., Paustian K., Six J. 2006. Acid hydrolysis of easily dispersed and microaggregate-derived and silt- and clay-sized fractions to isolate biologically resistant soil organic matter. European Journal of Soil Science, 57, 456–467.

Poirier N., Derenne S., Balesdent J., Mariotti A., Massiot D., Largeau C. 2003. Isolation and analysis of the non-hydrolysable fraction of a forest soil and an arable soil (Lacade´e, south-west France). European Journal of Soil Science, 54, 243–255.

Preston M.D., Eimers M.C., Watmough S.A. 2011. Effect of moisture and temperature variation on DOC release from a peatland: Conflicting results from laboratory, field and historical data analysis, The Science of the Total Environment, 409(7), 1235– 1242.

Priha O., Smolander A. 1999. Nitrogen transformations in soil under Pinus sylvestris, Picea abies and Betula pendula at originally similar forest sites. Soil Biology and Biochemistry, 31, 965–977.

Priha O., Grayston S.J., Hiukka R., Pennanen T., Smolander A. 2001. Microbial community structure and characteristics of the organic matter in soils under *Pinus*

sylvestris, Picea abies and *Betula pendula* at two forest sites. Biology and Fertility of Soils, 33, 17–24.

Qualls R.G., Haines B.L. 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. Soil Science Society of America Journal, 56, 578–586.

K. Quénéa, Derenne S., Gonzalez-Vila J.A., Mariotti A., Largeau C. 2006. Double-shot pyrolysis of the non-hydrolysable organic fraction isolated from a sandy forest soil (Landes de Gascogne, south-west France). Comparison with classical Curie point pyrolysis, Journal of Analytical and Applied Pyrolysis 76, 271-279.

Read D.J. 1996. The structure and function of the ericoid mycorrhizal root. Annales Botanica, 77, 365-391.

Read D.J., Leake J.R., Perez-Moreno J. 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Canadian Journal of Botany, 82, 1243–1263.

Redbo-Torstensson, P. 1994. The demographic consequences of nitrogen fertilization of a population of sundew, *Drosera rotundifolia*. Acta Botanica Neerlandica, 43, 175–188.

Rice A.V., Currah R.S. 2001. Physiological and morphological variation in *Oidiodendron maius*. Mycotaxon, 74, 383–396.

Richard C., Guyot G., Rivaton A., Trubetskaya O., Trubetskoj O., Cavani L., Ciavatta C. 2007. Spectroscopic approach for elucidation of structural peculiarities of Andisol soil humic acid fractionated by SEC-PAGE setup. Geoderma, 142, 210–216.

Robroek B.J.M., Jassey V.E.J., Kox M.A.R., Berendsen R.L., Mills R.T.E., Cécillon L., Puissant J., Meima-Franke M., Bakker P.A.H.M., Bodelier P.L.E. 2015. Peatland vascular plant functional types affect methane dynamics by altering microbial community structure. Journal of Ecology, 103, 925–934.

Robroek, B.J.M., Albrecht, R.J.H., Hamard, S., Pulgarin, A., Bragazza, L., Buttler, A., Jassey, V.E.J. 2016. Peatland vascular plant functional types affect dissolved organic matter chemistry. Plant and Soil, 407 (1–2), 135–143.

Roulet N. 2000. Peatlands, carbon storage, greenhouse gases, and the Kyoto protocol: prospects and significance for Canada. Wetlands, 20 (4), 605–615.

Rouwane A., Grybos M., Bourven I., Rabiet M., Guibaud G. 2018. Waterlogging and soil reduction affect the amount and apparent molecular weight distribution of

dissolved organic matter in wetland soil: a laboratory study. Soil Research, 56(1), 28-38.

Rydin H., Jeglum J.K. 2013. The Biology of Peatlands. Second edition, Oxford University Press, Oxford.

Saarnio S., Wittenmayer L., Merbach W. 2004. Rhizospheric exudation of *Eriophorum vaginatum L.* – potential link to methanogenesis. Plant and Soil, 267, 343–355.

Sanderman J., Baldock J.A., Amundson R. 2008. Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. Biogeochemistry, 89, 181-198.

Sarkanen K.V., Ludwig C.H. 1971. Lignins: Occurrence, Formation, Structure and Reactions. Wiley-Interscience, New York.

Shaver G.R., Fetcher N., Chapin F.S. 1986. Growth and flowering in *Eriophorum vaginatum* - annual and latitudinal variation. Ecology, 67, 1524-1535.

Shurpali N.J., Verma S.B., Kim J., Arkebauer T.J. 1995. Carbon dioxide exchange in a peatland ecosystem. The Journal of Geophysical Research, 100, 14, 319–326.

Scheffer R.A., van Logtestijn R.S.P., Verhoeven J.T.A. 2001. Decomposition of *Carex* and *Sphagnum* litter in two mesotrophic fens differing in dominant plant species. Oikos, 92, 44–54.

Schmidt M.W., Torn M.S., Abiven S., Dittmar T., Guggenberger G., Janssens I.A., Kleber M., Kögel-Knabner I., Lehmann J., Manning D.A.C., Nannipieri P., Rasse D.P., Weiner S., Trumbore S.E. 2011. Persistence of soil organic matter as an ecosystem property. Nature, 478, 49-56.

Schnitzer M., Khan S.U. 1972. Humic Substances in the Environment. Marcel Dekker, New York.

Schomakers J., Mentler A., Mayer H. 2014. Determination of dissolved organic carbon in soils with UV spectroscopy, ultrasonic dispersion pre-treatment and separation with wize exlusion chromatography. Spanish Journal of Soil Science, 4, 127-142.

Schulz E. 2002. Influence of extreme management on decomposable soil organic matter pool. Archiev Acker, Pflanze und Boden, 48, 101-105.

Silvan N., Tuittila E.-S., Vasander H., Laine J. 2004. *Eriophorum vaginatum* plays a major role in nutrient immobilisation in boreal peatlands. Annales Botanici Fennici, 41, 189–199.

Six J., Elliott E.T., Paustian K. 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry, 32, 2099–2103.

Six J., Conant R.T., Paul E.A., Paustian K. 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil, 241, 155–176.

Smith S.E., Read D.J. 1997. Mycorrhizal Symbiosis, second ed. Academic Press, New York, NY.

Sohi S.P., Mahieu N., Arah J.R.M., Powlsen D.S., Madari B., Gaunt J.L. 2001. A procedure for isolating soil organic matter fractions suitable for modeling. Soil Science Society of America Journal, 65, 1121–1128.

Sollins, P., Homann, P., Caldwell, B.A. 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma, 74, 65-105.

Sparling G.P., Vojvodic-Vukovic M., Schipper L.A. 1998. Hot water soluble C as simple measure of labile soil organic matter: the relationship with microbial biomass C. Soil Biology and Biochemistry, 30, 1469-1472.

Straková P., Anttila J., Spetz P., Kitunen V., Tapanila T., Laiho R. 2010. Litter quality and its response to water level drawdown in boreal peatlands at plant species and community level. Plant and Soil, 335, 501-520.

Straková P., Penttilä T., Laine J., Laiho R. 2012. Disentangling direct and indirect effects of water table drawdown on above- and belowground plant litter decomposition: Consequences for accumulation of organic matter in boreal peatlands. Global Change Biology, 18, 322–335.

Sun D., Meng J., Xu E.G., Chen W. 2016. Microbial community structure and predicted bacterial metabolic functions in biochar pellets aged in soil after 34 months. Applied Soil Ecology, 100, 135–143.

Sundh I., Mikkelä C., Nilsson M., Svensson B.H. 1995. Potential aerobic methane oxidation in a Sphagnum-dominated peatland - controlling factors and relation to methane emission. Soil Biology and Biochemistry, 27, 829–837.

Sutton R., Sposito G. 2005. Molecular structure in soil humic substances: the new view. Environmental Science & Technology, 39 (23), 9009–9015.

Svensson B. M. 1995. Competition between *Sphagnum fuscum* and *Drosera rotundifolia*: a case of ecosystem engeneering? – Oikos, 74, 205–212.

Tfaily M.M., Hamdan R., Corbett J.E., Chanton J.P., Glaser P.H., Cooper W. 2013. Investigating dissolved organic matter decomposition in northern peatlands using

complimentary analytical techiques. Geochimica et Cosmochimica Acta, 112, 116–129.

Thacker S.A., Tipping E., Gondar D., Baker A. 2008. Functional properties of DOM in a stream draining peat. The Science of the Total Environment 407, 566–573.

Thormann M.N., Currah R.S., Bayley S.E. 2001. Microfungi isolated from *Sphagnum fuscum* from a southern boreal bog in Alberta, Canada. Bryologist, 104, 548-559.

Thormann,M.N., Currah R.S., Bayley S.E. 2002. The relative ability of fungi from *Sphagnum fuscum* to decompose selected carbon sources. Canadian Journal of Microbiology, 48, 204-211.

Thormann M.N. 2006. Diversity and function of fungi in peatlands: a carbon cycling perspective. Canadian Journal of Soil Science, 86, 281-293.

Thormann M.N., Rice A.V. 2007. Fungi in peatlands. Fungal Diversity, 24, 241–299.

Thurman E.M. 1985. Orangic Geochemistry of Natural Waters. Kluwer Academic Publishers Group.

Tipping E. 1998. Modelling the properties and behavior of dissolved organic matter in soils. Mitteilungsblatt.- Dtsch. Bodenkd. Ges. 87, 237– 252.

Trinder C.J., Johnson D., Artz R.R.E. 2009. Litter type, but not plant cover, regulates initial litter decomposition and fungal community structure in a recolonising cutover peatland. Soil Biology and Biochemistry, 41, 651-655.

Tuittila E.-S., Komulainen V.-M., Vasander H., Laine J. 1999. Restored cut-away peatland as a sink for atmospheric CO_2. Oecologia, 120, 563-574.

Updegraff K., Pastor J., Bridgham S.D., Johnston C.A. 1996. Environmental and substrate controls over carbon and nitrogen mineralization in northern wetlands. Ecological Applications, 5, 151–163.

Urbanová Z., Bárta J. 2014. Microbial community composition and in silico predicted metabolic potential reflect biogeochemical gradients between distinct peatland types. FEMS Microbiology Ecology, 90, 633–646.

van Hees P.A., Jones D.L., Finlay R., Godbold D.L., Lundström U.S. 2005. The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry, 37(1), 1–13.

Vance G.F., David M.B. 1992. Dissolved organic carbon and sulfate sorption by spodosol mineral horizons. Soil Science, 154, 136-144.

Verhoeven J.T.A., Liefveld W.M. 1997. The ecological significance of organochemical compounds in *Sphagnum*. Acta Botanica Neerlandica, 46, 117–130.

Verhoeven J.T.A., Toth E. 1995. Decomposition of *Carex* and *Sphagnum* litter in fens: effect of litter quality and inhibition by living tissue homogenates. Soil Biology and Biochemistry, 27, 271–275.

Volk C., Wood L., Johnson B., Robinson J., Zhu H.W., Kaplan L. 2002. Monitoring dissolved organic carbon in surface and drinking waters. Journal of Environmental Monitoring, 4, 43–47.

von Lützow C., Kögel-Knabner I., Elkscmidt K., Flessa H., Guggenberger G., Matzner E., Marschner B. 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry, 39, 2183-2207.

von Lützow M., Kögel-Knabner I. 2009. Temperature sensitivity of soil organic matter decomposition what do we know? Biology and Fertility of Soils, 46, 1-15.

Wang H., Boutton, Thomas W., Xu W.H., Hu G.Q., Jiang P., Bai E. 2015. Quality of fresh organic matter affects priming of soil organic matter and substrate utilization patterns of microbes. Scientific Reports, 5, 10102.

Wein R.W. 1973. Biological flora of the british Isles: *Eriophorum vaginatum L*. Journal of Ecology, 61, 601–615.

Weishaar, J.L., Aiken, G.R., Bergamaschii, B.A., Fram, M.S., Fugii, R., Mopper, K. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science & Technology 37, 4702–4708.

Weltzin J.F., Pastor J., Harth C., Bridgham S.D., Updegraff K., Chapin C.T. 2000. Response of bog and fen plant communities to warming and water-table manipulations. Ecology, 81, 3464–3478.

Weltzin J.F., Bridgham S.D., Pastor J., Chen, J. Harth C. 2003. Potential effects of warming and drying on peatland plant community composition. Global Change Biology, 9, 141–151.

Wickland K.P., Neff J.C., Aiken G.R. 2007. Dissolved organic carbon in Alaskan boreal forest: Sources, chemical characteristics, and biodegradability, Ecosystems, 10(8), 1323–1340.

Wiedermann M.M., Nordin A., Gunnarsson U., Nilsson M.B., Ericsson L. 2007. Global change shifts vegetation and plant-parasite interactions in a boreal mire. Ecology, 88, 454–464.

Williams R.T., Crawford R.L. 1983. Microbial diversity of Minnesota peatlands. Microbial Ecology, 9, 201-214.

Williams C.J., Shingara E.A., Yavitt J.B. 2000. Phenol oxidase activity in peatlands in New York State: Response to summer drought and peat type. Wetlands, 20, 416–421.

Winsborough C, Basiliko N. 2010. Fungal and bacterial activity in northern peatlands. Geomicrobiology Journal, 27, 315–320.

Worrall F., Reed M., Warburton J., Burt T. 2003. Carbon budget for a British upland peat catchment. The Science of the Total Environment, 312, 133–146.

Yano Y., McDowell W.H., Aber J.D. 2000. Biodegradable dissolved organic carbon in forest soil solution and effects of chronic nitrogen deposition. Soil Biology and Biochemistry, 32, 1743–1751.

Yavitt J.B., Williams C.J., Wieder R.K. 1997. Production of methane and carbon dioxide in peatland ecosystems across North America: Effects of temperature, aeration, and organic chemistry of the peat. Geomicrobiol. J. 14, 299–316.

Yu Z., Loisel J., Brosseau D., Beilman D., Hunt S. 2010. Global peatland dynamics since the Last Glacial Maximum. Geophysical Research Letters, 37(13), 1-5.

Yu Z., Beilman D.W., Frolking, S., MacDonald, G.M., Roulet N.T., Camill P.J., Charman D.J. 2011. Peatlands and their role in the global carbon cycle. Eos, 92 (12), 97–98.

Zaccone C., Miano T.M., Shotyk W. 2007. Qualitative comparison between raw peat and related humic acids in an ombrotrophic bog profile, Organic Geochemistry, 38, 151–160.

6 Attached publications

Paper I

Soil organic matter quality and microbial activities in spruce swamp forests affected by drainage and water regime restoration

Mastný J., Urbanová Z., Kaštovská E., Straková P., Šantrůčková H., Edwards K.R., Picek T.

2016

Soil Use and Management, 32, 200–209

Soil Use and Management

Soil Use and Management, June 2016, **32,** 200–209 doi: 10.1111/sum.12260

Soil organic matter quality and microbial activities in spruce swamp forests affected by drainage and water regime restoration

J. Mastný[1], Z. Urbanová[1], E. Kaštovská[1], P. Straková[2], H. Šantrůčková[1], K. R. Edwards[1] & T. Picek[1]

[1]*Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, Branišovskă 31, CZ-370 05, České Budějovice, Czech Republic, and* [2]*Department of Forest Sciences, Peatland Ecology Group, Latokartanonkaari 7, FI-00014 University of Helsinki, Helsinki, Finland*

Abstract

The effect of spruce swamp forest (SSF) drainage and water regime restoration on soil organic matter (SOM) quality and soil microbial heterotrophic activities was studied in pristine, drained and restored SSF in the Bohemian Forest, Czech Republic. Sequential chemical SOM fractionation using cold and hot water and hot acid was used to separate SOM fractions according to their mobility and potential lability/recalcitrance, and Fourier transform infrared spectra were used for SOM characterization. Soil physicochemical parameters and heterotrophic microbial activities were also determined. Drainage of SSF had significant long-term effects (more than 50 yr) on plant communities and SOM quality. On drained sites, cover of sphagnum moss and sedge was much smaller than on pristine locations. A greater proportion of recalcitrant compounds and a smaller proportion of labile compounds were found in drained SSF as compared to pristine sites, which first led to an energy limitation and was followed by a decrease in microbial biomass and heterotrophic microbial activities (CO_2 production, methanogenesis and methanotrophy). Restoration resulted in slow progressive changes in the vegetation cover, including the spread of sphagnum mosses, retreat of mosses typical of drier conditions and increased sedge cover compared with drained SSF. Moreover, soil physicochemical parameters (pH and bulk density), hot-water-extractable C and methanotrophic activity tended to evolve towards the pristine SSF and seem to be good indicators of the restoration process. No other SOM fractions changed significantly after restoration. Thus, to change significantly overall SOM quality and most microbial heterotrophic activities following restoration, more than 7 yr are required.

Keywords: Spruce swamp forests, soil organic matter quality, restoration, drainage, microbial activities

Introduction

Peatlands are specific ecosystems in which a large amount of organic matter is accumulated in the soil owing to limited decomposition under a high water-table level and prevailing anoxic conditions. Therefore, the peatland C sink function is sensitive to water regime changes.

In Central Europe, the area and functioning of peatlands (including bogs, fens and spruce swamp forests) have been strongly affected by drainage (Chivers *et al*., 2009). Peatlands have been artificially drained for centuries, mostly for forestry, peat extraction and agricultural purposes (Joosten & Clarke, 2002). Drainage affects peatland functioning directly through a drawdown of the water level, which causes the aerobic zone to extend deeper into the soil profile (e.g. Jaatinen *et al*., 2008). The first years after drainage bring changes in pore water and soil chemistry (Holden *et al*., 2004) and enhanced soil organic matter (SOM) decomposition, which is supported by easily decomposable compounds accumulated under the previous anoxic conditions (Straková *et al*., 2011). A temporary period of increased microbial activity, connected with enhanced CO_2 production and leaching of nutrients and DOC to surface waters, terminates after the depletion of the labile SOM fraction (Gao *et al*., 2014), while more

Correspondence: J. Mastný. E-mail: Mastny.jiri@seznam.cz
Received September 2015; accepted after revision February 2016

recalcitrant compounds are left behind (Kalbitz, 2001). Peat shrinkage and subsidence caused by a combination of water level drawdown and intensive decomposition lead to increased peat bulk density and altered hydraulic properties over the longer term (Minkkinen & Laine, 1998). Within decades, however, direct effects of drainage on SOM quality are overtaken by indirect effects through changes in vegetation composition (Laiho, 2006). The effects of changes in litter quality on SOM quality and decomposition can be even more important than the direct effects of falling water level (Straková *et al*., 2010).

Efforts over the last few decades to restore the carbon storage function of peatland provide a unique opportunity to study their recovery process (Vasander *et al*., 2003). Restoration of the original water regime is usually established by ditch blocking, which raises the water-table level. In the short-term, rewetting causes water-table fluctuations (Holden *et al*., 2011) and affects peat physicochemical parameters (Tuittila *et al*., 1999; Urbanová *et al*., 2011). Changes in plant community composition are documented over the longer term (Maanavilja *et al*., 2014). However, it is not clear how quickly changes in water regime, soil pH, bulk density and vegetation composition are reflected in SOM quality, nor how fast and successful is the process of recovery of the peat properties and microbial processes characteristic of pristine peatlands. In this context, DOC quality and leaching have mostly been studied in restored peatlands, but studies on the quality of the total SOM are scarce (Gao *et al*., 2014). Further, there is little information about how microbial parameters, such as biomass, CO_2 production and methanogenesis, which are responsive to peatland disturbance (Jaatinen *et al*., 2008), might sensitively reflect changes in the SOM quality initiated by peatland restoration.

The main aim of our study was to evaluate the effect of drainage and restoration of spruce swamp forest (SSF) systems on (i) SOM quality with respect to its availability for the microbial community and its stability (resistance to decomposition) and (ii) microbial heterotrophic activities connected with SOM decomposition. We hypothesized that SSF drainage results in depletion of the available SOM fraction, proportional accumulation of recalcitrant SOM and a decrease in microbial activity, while restoration reverses the trend and shifts the SOM characteristics towards the pristine sites. We expect more pronounced recovery of SOM and microbial characteristics in the upper soil layer directly affected by plant litter and rhizodeposition.

Methods

Study sites

Six SSF sites with different management were chosen for soil sampling, two pristine control sites (P1 and P2), two sites drained before the 1960s (D1 and D2) and two restored (R1 and R2) sites (Table 1). R1 was rewetted in 2008 and R2 in 2004–2005 by blocking ditches with timber dams. All study sites are located in the Bohemian Forest National Park in the southern part of the Czech Republic at an altitude of 1100 m a.s.l. The climate is cold and humid with a mean annual temperature of 4 °C and mean annual precipitation ca. 1200 mm. Peat depth at our study sites is in the range of 0.5–1.5 m with high spatial heterogeneity, and the bedrock is paragneiss.

Total soil C, N and P concentrations and their stoichiometric ratios in the 0–30 soil layer did not vary among the sites (Table 1). At all sites, Norway spruce (*Picea abies*) was the dominant tree. Pristine sites had the highest water-table level, soil pH and sphagnum moss coverage and the smallest soil bulk density. Drainage brought about a decrease in soil pH, an increase in soil bulk density and also changes in understorey vegetation (Table 1). Restoration was followed by increased water-table and pH levels and recovery of sphagnum mosses (Table 1). See Maanavilja *et al*. (2014) for a more detailed site description.

Soil sampling

Soil sampling was performed in July 2011. Six cores were collected using a corer to the depth of 30 cm at each site. In the case of the drained and restored sites, two cores were collected close to the drainage ditch (1 m from the ditch), two cores at a distance of 10 m from the ditch and two cores 20 m from the ditch. The same sampling design (distances between the samples) was followed on the pristine sites. The soil cores were divided into two layers (0–10 and 10–30 cm), based on the assumption that each was at a different stage of degradation/regeneration, and analysed separately.

Soil analyses

The sampled soil was homogenized by passing through a 5-mm sieve. Part of the soil was freeze-dried and used to measure total C and N contents (elemental analyser Micro Cube; Elementar, Germany) and for chemical fractionation and SOM characterization by infrared spectroscopy. The rest of the fresh sample was used for measuring biological parameters (microbial biomass, CO_2 production, enzymatic activities, potential CH_4 production and consumption).

Chemical fractionation was applied to separate SOM pools according to their mobility and availability/recalcitrance. Briefly, subsamples of 0.4 g were extracted with 30 mL of cold water (CW) for 1 h, followed by vacuum filtration through a glass-fibre filter GF/F (0.45 μm). Extracts were then frozen. The samples were further extracted with hot water (HW) (80 °C) for 18 h (Sparling *et al*., 1998). The HW extracts were treated similarly to the

Table 1 Basic soil characteristics of the 0–30 cm layer and vegetation coverage on pristine (P), restored (R) and drained (D) spruce swamp forest sites (mean ± 1 standard deviation, $n = 24$)

Study site	P1	P2	R1	R2	D1	D2
Water table (cm)	−8.0 ± 5.7	−9.9 ± 6.1	−13.1 ± 5.8	−14.6 ± 10.3	−29.6 ± 15.9	−38.4 ± 10.3
pH	4.12 ± 0.16	4.25 ± 0.06	3.86 ± 0.08	4.16 ± 0.45	3.66 ± 0.33	3.75 ± 0.15
Bulk density (g/cm^3)	0.059 ± 0.021	0.056 ± 0.010	0.126 ± 0.021	0.097 ± 0.038	0.127 ± 0.037	0.171 ± 0.029
Total C (%)	46.4 ± 3.7	45.7 ± 8.7	46.8 ± 3.11	45.1 ± 3.5	45.6 ± 2.6	42.1 ± 8.6
Total N (%)	1.64 ± 0.15	1.25 ± 0.30	1.51 ± 0.14	1.64 ± 0.29	1.72 ± 0.28	1.11 ± 0.29
Total P (%)	0.059 ± 0.014	0.080 ± 0.012	0.067 ± 0.012	0.074 ± 0.007	0.080 ± 0.012	0.084 ± 0.008
C/N ratio	33.3 ± 4.0	43.9 ± 9.2	36.5 ± 5.8	32.8 ± 4.5	31.8 ± 6.0	40.8 ± 16.0
C/P ratio	2149 ± 556	1486 ± 316	1855 ± 326	1582 ± 165	1499 ± 262	1375 ± 277
N/P ratio	64.3 ± 13.5	34.4 ± 6.7	51.2 ± 8.2	49.2 ± 8.1	48.5 ± 10.5	30.7 ± 7.9
Sphagnum (%)	82.2 ± 18.6	90.0 ± 0	53.3 ± 27.9	46.2 ± 32.6	33.5 ± 27.9	49.2 ± 24.6
Other mosses (%)	13.3 ± 15.7	4.3 ± 1.5	28.3 ± 15.7	24.2 ± 16.7	37.5 ± 18.4	30.8 ± 14.8
Sedges (%)	15.0 ± 14.1	42.5 ± 10.7	6.7 ± 6.9	9.2 ± 10.5	0 ± 0	1.6 ± 3.7
Shrubs (%)	31.2 ± 28.9	29.2 ± 14.3	55.8 ± 22.8	35 ± 27.1	50.0 ± 30.7	51.7 ± 24.1

CW extracts. CW extracts were analysed for inorganic N forms ($N–NO_3^-$, $N–NH_4^+$) and soluble reactive phosphorus (SRP) using a flow injection analyser (FIA Lachat QC8500; Lachat Instruments, USA), and both hot and CW extracts were analysed for total C and N contents by a LiquiTOCII (Elementar). Acid hydrolysis was then applied on the dried and milled soil samples (1:100, soil to acid, 6 N HCl, at 110 °C for 18 h) according to Leavitt *et al.* (1996). All extractions of the soil samples were carried out in duplicate.

The quality of total SOM was assessed using infrared spectroscopy. Infrared spectra were obtained with a Bruker VERTEX 70 series FTIR (Fourier transform infrared) spectrometer (Bruker Optics, Germany) equipped with a horizontal attenuated total reflectance (ATR) sampling accessory. Freeze-dried and powdered soil samples were inserted directly on the ATR crystal, and a MIRacle high-pressure digital clamp was used to achieve even distribution and contact between the sample and crystal. Each spectrum consisted of 65 averaged absorbance measurements between 4000 and 650 cm^{-1}, with a 4 cm^{-1} resolution. Differences in the amplitude and baseline between different runs (samples) were corrected by standard normal variate transformation and detrending (Barnes *et al.*, 1989) using the Unscrambler software (CAMO, Norway).

Microbial biomass C and N were measured using the chloroform fumigation-extraction method (Vance *et al.*, 1987), and conversion factors of 0.38 (Vance *et al.*, 1987) and 0.54 (Brookes *et al.*, 1985) were used to correct the microbial biomass C and N, respectively. Concentrations of C and N in the soil extracts were analysed on a LiquiTOCII (Elementar).

Potential activities of cellulolytic and lignolytic enzymes were measured at room temperature on a microplate reader using fluorogenic (Methylumbelliferyl – MUB) and chromogenic (ABTS) substrates (Eggert *et al.*, 1996; Marx *et al.*, 2001; Bárta *et al.*, 2013). Lignolytic potential was calculated by summing the laccase, oxidase and peroxidase enzymes.

Potential aerobic and anaerobic CO_2 and CH_4 production was measured under laboratory conditions (Urbanová *et al.*, 2013). The concentrations of CO_2 and CH_4 in the headspace of the incubation flasks were measured at 1-week intervals during the experiment using gas chromatographs HP 6850 and HP 6890 (Agilent, USA).

Potential CH_4 consumption was measured under laboratory conditions at 12 °C using Labco Exetainers (Labco Limited, UK). CH_4 was added to the headspace of the vial at optimal concentration. Then, the concentration was measured after 24 and 72 h using gas chromatography and the rate of CH_4 consumption calculated.

Statistics

Multivariate analysis was conducted to distinguish the effects of management or soil layer (depth) on the measured soil characteristics (Canoco 5). The data were tested by redundancy analysis (RDA) using all measured chemical characteristics (log-transformed, centred and standardized) or the infrared absorbance data as the response variables and either management or soil layer as the explanatory variable. The chemical characteristics of the two soil layers of differently managed SSF sites were compared by two-way ANOVA (STATISTICA 10 for Windows) followed by post hoc comparison (unequal N HSD test). When the characteristics were not significantly different between the upper and lower soil layer, the weighted mean for the 0–30 cm soil layer was calculated and management effect was tested by one-way ANOVA.

Results

Effect of management on SOM fractions

Soil organic matter quality, based on the proportional contribution of different SOM fractions, was comparable among the study sites, causing the 61% overlap of the samples from differently treated peatlands in the ordination diagram (Figure 1a). The samples from the restored sites were most widely distributed over the ordination diagram (Figure 1a), indicating the highest spatial variability in SOM quality. The RDA analysis showed neither a significant effect of management ($P < 0.1$; pseudo-$F = 11.6$) nor of the soil layer (0–10 vs. 10–30 cm) ($P < 0.1$; pseudo-$F = 7.3$) on the proportional contribution of different fractions of SOM. Management explained 25.2% of data variability (Figure 1b), while the effect of the soil layer accounted for 9.4%, with no interactions between the two explanatory variables except for the CW fraction. Therefore, weighted means of the proportional contributions of the different fractions within the SOM for the whole 0–30 cm soil layer were calculated and further displayed.

Generally, the CW fraction formed the smallest part of SOM (about 0.8% for C and 1.0% for N), followed by the HW fraction, which contained <15% of C and N bound in SOM (Figure 2a,b). The proportions of cold- and hot-water-extractable C and N within the total SOM were significantly greater on pristine sites than on the restored and drained sites ($P < 0.05$ in all cases, Figure 2a,b) with the exception of CW-N, where the values were larger for the pristine and restored sites than for the drained sites (Figure 2b). The mean molar C/N ratio of the HW fraction was slightly greater in comparison with the CW fraction, with larger values for the drained sites than for the pristine and restored sites ($P < 0.05$, Figure 2c). The HAE fraction contained ca. 30–45% of C and up to 80% of N bound in the SOM. While HAE-N was similar among the differently managed sites (Figure 2b), HAE-C was larger in pristine than in other sites, with a significant difference only between the pristine and restored sites (Figure 2a). The HAE fraction, containing mainly proteinaceous and partly carbohydrate material, was characterized by the lowest C/N ratio among all the SOM fractions, ranging from 14 to 19, and was not affected by management (Figure 2c). The residual NON fraction, representing the most recalcitrant part of SOM, formed ca half of C (Figure 2a) but <20% of N bound in SOM (Figure 2b). Therefore, it had the highest C/N ratio of all the fractions, exceeding 100 (Figure 2c). The proportion of the NON fraction within the SOM was significantly less on the pristine sites than on the restored and drained sites ($P < 0.05$; Figure 2a,b). The C/N ratio of the NON fraction decreased from the pristine to the drained sites (Figure 2c).

Detailed characterization of CW fraction

The CW-extractable SOM fraction, representing the potentially mobile fraction available for soil microorganisms, was characterized by its macronutrient contents and stoichiometry. The C, N and P concentrations in the CW fraction were significantly affected by site management and mostly also differed between the upper and lower soil layers

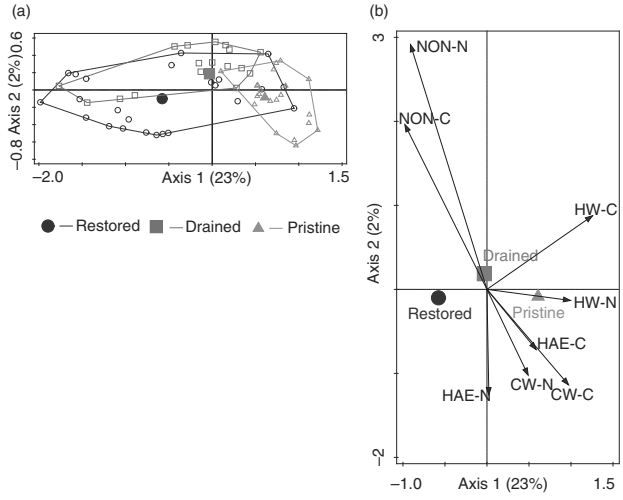


Figure 1 The effect of drainage and restoration on the proportional contribution of chemical fractions within soil organic matter (SOM) shown in (a) a classified sample diagram and (b) a species and environmental variables biplot; CW-C, CW-N – cold-water-extractable carbon and nitrogen; HW-C, HW-N – hot-water-extractable carbon and nitrogen; HAE-C, HAE-N – hot-acid-extractable carbon and nitrogen; NON-C, NON-N – nonhydrolysable carbon and nitrogen ($n = 72$; six study sites).

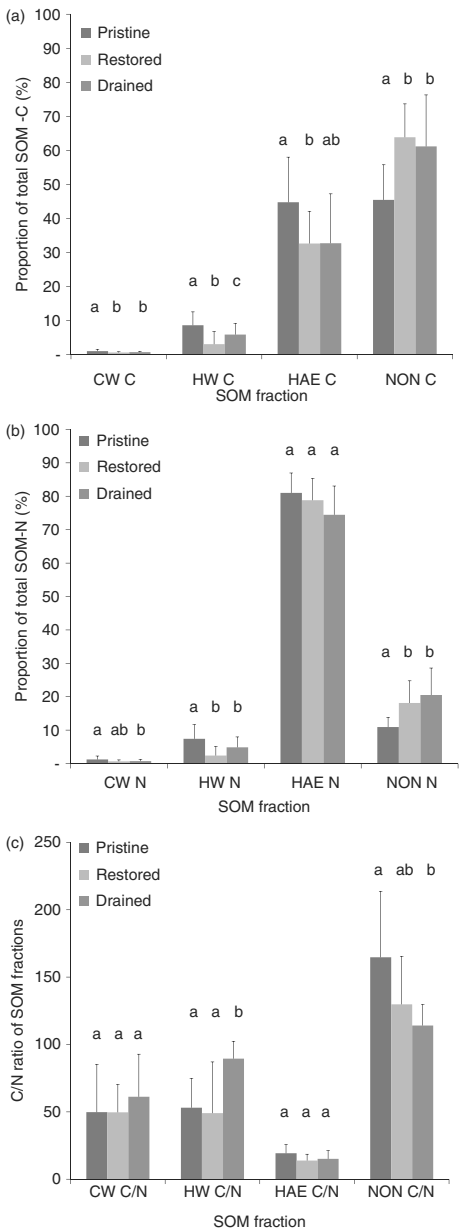


Figure 2 Proportions of different (a) C and (b) N fractions within total soil organic matter (SOM) in the 0–30 cm soil layer on pristine, restored and drained sites and (c) molar C/N ratios of particular SOM fractions; CW, cold water fraction; HW, hot water fraction; HAE, hot acid fraction; NON, nonhydrolysable fraction. Mean values ± standard deviation ($n = 24$) are shown. Significant differences ($P < 0.05$) in characteristics within each particular SOM fraction are marked by lowercase letters.

(Table 2). CW-C was significantly greater on the pristine than on the restored and drained sites, but the difference was significant only in the upper soil layer. The differences in the lower soil layers, containing less CW-C concentrations compared to the upper layers, were not significantly affected by management. This further explains why the CW-C concentration differed significantly between the upper and lower soil layers in the pristine sites but not in the other sites (Table 2). A similar situation was found for CW-N, which significantly decreased from the pristine to the drained sites, but only in the upper soil layer. Again, the lower soil layers contained less CW-N and were not influenced by site management (Table 2). The CW-N concentration differed between the upper and lower soil layers in the pristine and restored but not the drained sites (Table 2). The C/N ratio of the CW SOM fraction ranged from 23 to 42 but with no significant effect of management, with larger values in the lower soil layers (significant only on the restored sites, Table 2).

Management further affected the composition of the CW-N pool. In general, the CW-N pool was dominated by organic N, which formed ca. 80% on the pristine sites but only ca. 60–67% on the drained and restored sites. On the drained and restored sites, the proportion of mineral N, namely ammonium N, increased within the CW-N relative to that of the pristine sites. In addition to ammonium N, the proportion of nitrate N increased on drained sites, especially in the lower layer, where it reached up to 17% of CW-N (Table 2).

The concentration of SRP was greater on the pristine sites than on the restored and drained ones, with significant differences occurring only in the lower soil layer (Table 2). This difference brought about a marked increase in the C/SRP ratio of the CW fraction in the lower layers of the drained and restored sites in comparison with the pristine ones. However, it did not affect the N/SRP ratio of the CW fraction, which was similar for all sites (Table 2).

Characterization of SOM by infrared spectroscopy

Results of the SOM analysis by infrared spectroscopy are in keeping with the results obtained by chemical SOM fractionation. The RDA analysis showed a similar portion of

Table 2 Concentrations of organic C, all N forms and soluble reactive P and their molar ratios in the cold water (CW) soil organic matter fraction from the upper (0–10 cm) and lower (10–30 cm) soil layers of pristine, restored and drained sites (mean ± 1 standard deviation, $n = 12$)

Parameter	Depth (cm)	Pristine	Restored	Drained	Management (M)	Layer (L)	M*L
CW-C (μg/g)	0–10	6334 ± 1862^{b}	3325 ± 1070^{a}	3381 ± 729^{a}	$F = 24.33$	$F = 43.83$	$F = 7.07$
	10–30	3204 ± 1059^{a}	2166 ± 512^{a}	2453 ± 546^{a}	***	***	**
CW-N (μg/g)	0–10	248.1 ± 151.6^{c}	179.4 ± 70.3^{bc}	146.7 ± 42.8^{ab}	$F = 5.97$	$F = 30.34$	$F = 0.76$
	10–30	121.8 ± 54.1^{ab}	69.1 ± 30.0^{a}	75.1 ± 29.0^{a}	**	***	ns
Org N (%CW-N)	0–10	82.4 ± 7.1^{c}	67.7 ± 12.2^{abc}	65.0 ± 19.1^{ab}	$F = 5.45$	$F = 3.59$	$F = 0.39$
	10–30	78.9 ± 9.3^{bc}	63.1 ± 8.3^{ab}	59.0 ± 14.9^{a}	**	(*)	ns
NH_4-N (%CW-N)	0–10	14.3 ± 5.2^{a}	29.5 ± 13.2^{b}	27.3 ± 18.9^{ab}	$F = 8.23$	$F = 0.07$	$F = 0.27$
	10–30	15.6 ± 6.3^{ab}	29.1 ± 8.9^{ab}	23.7 ± 13.7^{ab}	**	ns	ns
NO_3-N (%CW-N)	0–10	3.4 ± 2.4^{a}	2.8 ± 1.3^{a}	7.6 ± 9.0^{a}	$F = 9.82$	$F = 9.43$	$F = 1.87$
	10–30	4.9 ± 3.3^{a}	7.2 ± 4.3^{a}	16.9 ± 12.2^{b}	***	**	ns
SRP (μg/g)	0–10	85.5 ± 41.2^{c}	74.9 ± 32.8^{c}	59.6 ± 15.4^{bc}	$F = 6.30$	$F = 33.16$	$F = 1.57$
	10–30	56.3 ± 26.8^{bc}	20.2 ± 10.4^{a}	29.0 ± 20.4^{ab}	**	***	ns
CW-C/CW-N	0–10	32.7 ± 9.5^{ab}	23.2 ± 6.5^{a}	28.2 ± 6.3^{ab}	$F = 0.34$	$F = 15.31$	$F = 2.08$
	10–30	36.0 ± 18.0^{ab}	41.0 ± 12.1^{b}	41.7 ± 14.1^{b}	ns	***	ns
CW-C/CW-SRP	0–10	220 ± 91^{abc}	127 ± 51^{a}	153 ± 36^{a}	$F = 0.53$	$F = 18.93$	$F = 9.41$
	10–30	180 ± 93^{ab}	300 ± 141^{c}	325 ± 114^{c}	ns	***	***
CW-N/CW-SRP	0–10	6.9 ± 4.0^{a}	5.8 ± 2.8^{a}	5.6 ± 1.5^{a}	$F = 0.83$	$F = 1.28$	$F = 3.32$
	10–30	5.1 ± 2.3^{a}	8.5 ± 3.9^{a}	7.2 ± 2.9^{a}	ns	ns	*

SRP, soluble reactive phosphorus. Results of factorial ANOVA with management (M) and soil layer (L) as categorical predictors are also shown, (*)$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Significant differences found by *post hoc* test for the management and soil layers interaction are indicated by superscript lowercase letters.

variability in the spectral data explained by site management (28%) as in the SOM fractionation data and separated the pristine sites from the managed sites. Soil layer accounted for only 3% of the total variation in the spectral data (Figure 3a). Compared to the pristine sites, SOM from the drained and restored sites was enriched in recalcitrant compounds, represented by lignin-like structures and aliphatic compounds like fats, waxes and lipids, but depleted in the main polysaccharide markers (Figure 3b).

Effect of management on soil microbial biomass and activity

Analyses of microbial biomass and biological activity were carried out for the whole soil profile (0–30 cm). Microbial biomass C and N decreased from the pristine through the restored to the drained sites, with significant differences only between the pristine and drained sites (Table 3). The pristine sites were further characterized by a significantly greater CO_2 production in aerobic and anaerobic conditions and larger methanogenesis in comparison with both the restored and drained sites (Table 3). Methanotrophy was similar on the pristine and restored sites being larger than on the drained sites. The drained sites were characterized by the absence of both CH_4 production and consumption (Table 3). Enzymatic activities were very variable, with no significant effect of management (Table 3).

Discussion

In the last few decades, there has been a growing effort to restore peatland systems and bring back their carbon storage function. We aimed to document whether the restoration process, lasting less than a decade, really affects SOM quality and microbial activity in soils of long-term drained SSFs and whether these characteristics tend to resemble those of the pristine sites. Our data show that the soil microbial biomass and some of the measured microbial activities have already responded to SSF restoration, while overall SOM quality as well as the concentrations and characteristics of the labile SOM fractions remained unaffected so far. Although SOM quality characterized by chemical fractionation and IR spectral characteristics clearly documented the negative effects of long-term drainage of SSF, with few exceptions, this measure was not sensitive enough to detect minor shifts in the properties of the restored systems.

The long-term drained conditions (about 50-yr) of the SSF drained sites significantly shifted SOM quality from that of the pristine sites. The changes in soil physicochemical properties were interconnected with a substitution of plant species adapted to wet conditions by typical forest species – trees, shrubs and mosses. The vegetation changes affected the amount and composition of plant-derived material entering the soil due to increased woody litter input, with a

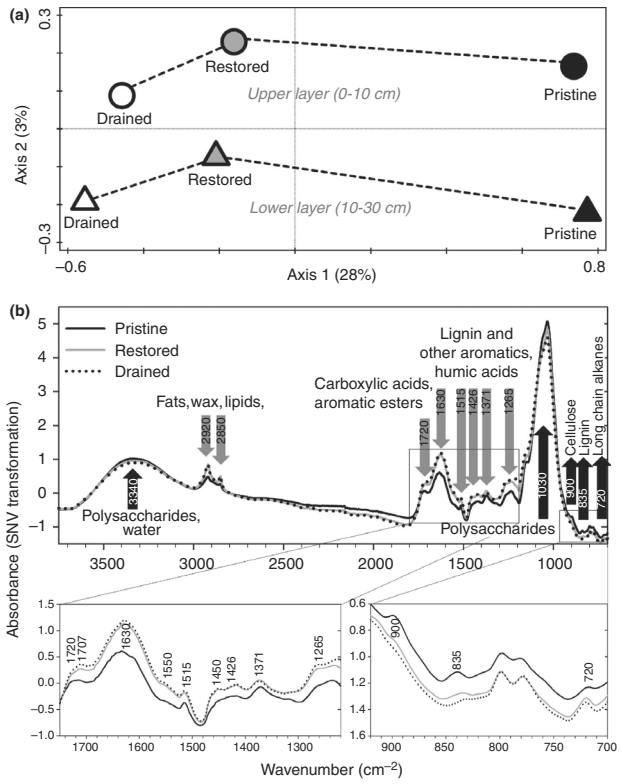


Figure 3 (a) Redundancy analysis of the infrared spectroscopy absorbance data showing variation in soil organic matter (SOM) quality among differently managed sites (variation along the first axis) and between the upper (0–10 cm) and lower (10–30 cm) soil layers (variation along the second axis). (b) Infrared spectra of the soil samples from differently managed sites. Black arrows point to wavenumbers that showed a positive correlation with the first axis (associated chemical compounds were of higher concentration in the soil of pristine sites). The grey arrows point to wavenumbers negatively related with the first axis (associated chemical compounds were of higher concentration in the soil of drained and restored sites). See Artz *et al*. (2008) for the assignments of the bands.

negative effect on SOM quality and its decomposition rate (Straková *et al*., 2010, 2012). As a result, long-term drainage increased SOM recalcitrance, which was documented by the greater proportion of the nonhydrolysable fraction within the SOM, this fraction being rich in lignin-like structures, humic and carboxylic acids and aliphatic compounds, such as fats, waxes and lipids. This was connected to a significant decrease in soil microbial biomass and microbial CO_2 production as a consequence of substrate and energy limitations. Methanogenesis and methanotrophy were totally absent in the long-term drained sites, indicating an absence of suitable substrate in DOM, a change in the composition and functioning of the soil microbial community (Urbanová *et al*., 2013) and significantly different C cycling in comparison with the pristine sites. Similar drainage affects were documented by Francez *et al*. (2000) and Edwards *et al*. (1998), who showed that a decreased water table pushed methanogens and methanotrophs deeper into the soil profile. SSF drainage further diminished the differences in concentrations of the mobile SOM fraction and nutrient availability between the upper most (0–10 cm) and deeper layers (10–30 cm) resulting in a more homogeneous soil profile. The existing differences within the soil profile on the pristine sites point to larger heterogeneity of peat physicochemical properties, SOM characteristics and decomposability (Lieffers, 1988) related to the different composition and activity of microbial communities (Jaatinen *et al*., 2007). Therefore, except for the marked changes in ecosystem functioning, peatland drainage further diminishes the heterogeneity of the system down the soil profile.

After SSF restoration, the water table increased to an intermediate level between the drained and pristine sites, with a larger variation in comparison with the pristine ones. The greater water level fluctuations on the restored sites correspond to the results of other authors (Holden *et al*., 2011). Restoration also led to changes in the vegetation composition, such as spreading of sphagnum mosses and sedges and the partial retreat of other mosses and shrubs

Table 3 Microbial biomass C and N, enzymatic activities (beta-glucosidase and lignolytic potential), CO_2 production, CH_4 production (methanogenesis) and CH_4 consumption (methanotrophy) in the 0–30 cm soil layer on pristine, restored and drained sites of spruce swamp forest (average, ± 1 standard deviation, $n = 12$). Significant differences found by post hoc test for the management are indicated by superscript lowercase letters

Parameter	Pristine	Restored	Drained	One-way ANOVA
Microbial C (μg/g soil)	2806 ± 1177^{a}	2028 ± 568^{ab}	1456 ± 351^{b}	$F = 7.6329$; $P < 0.05$
Microbial N (μg/g soil)	522 ± 223^{a}	380 ± 188^{ab}	274 ± 72^{b}	$F = 5.2203$; $P < 0.05$
Beta-glucosidase (nmol/h/g)	3668 ± 1481^{a}	3647 ± 1482^{a}	3031 ± 1128^{a}	n.s.
Lignolytic potential (nmol/h/g)	11.5 ± 9.5^{a}	13.4 ± 13.6^{a}	4.5 ± 5.1^{a}	n.s.
CO_4 aerobic (μl CO_2/h/g)	11.7 ± 2.9^{a}	7.4 ± 3.5^{b}	4.4 ± 2.3^{b}	$F = 15.840$; $P < 0.05$
CH_4 anaerobic (μl CH_4/h/g)	1.8 ± 0.3^{a}	1.3 ± 0.5^{b}	1.4 ± 0.3^{ab}	$F = 3.7159$; $P < 0.05$
Methanogenesis (μl CH_4/h/g)	0.89 ± 0.58^{a}	0.12 ± 0.19^{b}	0 ± 0^{b}	$F = 20.464$; $P < 0.05$
Methanotrophy (μl CH_4/h/g)	21.4 ± 11.8^{a}	13.6 ± 11.7^{a}	0 ± 0^{b}	$F = 14.248$; $P < 0.05$

typical of drier conditions, especially along flooded ditches (Maanavilja *et al*., 2014). This enhanced the large spatial variability of basic soil and vegetation characteristics within and among our study sites, which is typical for SSF (Økland *et al*., 2008). The two restored study sites varied to a greater extent in nutrient concentrations (total N and P), stoichiometric C:N:P ratios, pH and bulk density as well as in vegetation cover than the pristine and drained sites. However, no significant shifts in overall SOM quality, which would differentiate the restored from the drained sites, were detected. Therefore, more than 7 yr after restoration, which would be associated with a more significant shift in vegetation composition, are needed to significantly change the overall SOM quality of previously drained SSF towards that of pristine sites.

In spite of the absence of changes in overall SOM quality, shifts in microbial biomass and activity indicated finer changes in SOM degradability after SSF restoration. Microbial biomass increased, bringing an enhancement of microbial respiration in the restored systems. Measurable rates of potential methanotrophy and methanogenesis indicate re-establishment of microbial community structure and development of the restored systems towards well-functioning peatlands (Mummey *et al*., 2002; Huttunen *et al*., 2003). The sensitivity of soil microbial biomass, respiration and processes of methane cycling to environmental changes support the concept of using microbial characteristics as sensitive predictors of changes in total SOM caused by different ecosystem management/disturbance (Uhlířová *et al*., 2005).

The small proportion of the hot-water-extractable SOM fraction was the only difference in SOM quality which differentiated the restored sites from the others. We suggest that this can be connected with the restoration of microbial biomass and activity in the rewetted systems. The hot-water-extractable soil fraction has high carbohydrate content and can be quickly metabolized by active soil microorganisms (Ghani *et al*., 2003). In the restored SSF, it can serve as a suitable substrate source supporting greater activity of a larger soil microbial biomass, which could keep its contribution to SOM smaller in comparison with the drained sites. Similarly, Andersen *et al*. (2006) found that subsurface soil layers of restored peatlands were poor in available organic compounds, while Wallage *et al*. (2006) and Höll *et al*. (2009) observed reduced DOC concentration 3–20 yr after drain blocking of peatlands.

Our study did not prove that restoration would lead to faster recovery of SOM quality in the upper soil layer, which directly affected the spread of plant species typical for pristine SSF. Neither the chemical SOM fractionation nor the IR spectra showed any difference in SOM quality between the upper and lower soil layers in the restored SSF. We thus suggest that more pronounced change in the vegetation composition towards pristine sites is needed to occur on restored sites to induce the depth heterogeneity in SOM characteristics.

Conclusions

Overall SOM quality, soil microbial biomass and its activity were significantly affected by SSF drainage. A large proportion of recalcitrant compounds and small contribution of labile fractions within SOM on SSF drained for more than 50 yr suggest that long-term drainage could lead to energy limitation in these ecosystems. This limitation is related to a reduction in soil microbial biomass, microbial respiration and the absence of methanogenesis and methanotrophy. SSF drainage further diminished the heterogeneity of the system down the soil profile.

The restored sites tended to evolve towards the pristine SSF. Concentrations of available N and P increased on restored sites in comparison with the drained ones. The occurrence of methanogenesis and methanotrophy on the restored sites pointed to the presence of suitable substrates for methanogenesis in the SOM and indicated a shift of

microbial community functioning towards that on the pristine SSF within a relatively short period of restoration (4–7 yr). However, the fine shifts in vegetation structure, soil physicochemical properties and microbial functioning were still not related to detectable changes in overall SOM quality. A longer time period is needed for the full restoration of previously drained SSF ecosystems.

Acknowledgements

We thank Terezia Říhová and Daniel Vaněk for helping with the chemical analyses. The study was supported by grants from the Grant Agency of the Czech Republic (No. 13-17398S and 14-17403P), the Academy of Finland (No. 1259190, for PS) and the Grant Agency of the University of South Bohemia (No. 04-146/2013/P).

References

Andersen, R., Francez, A.-J. & Rochefort, L. 2006. The physicochemical and microbial status of a restored bog in Quebec: identification of relevant criteria to monitor success. *Soil Biology & Biochemistry*, **38**, 1375–1387.

Artz, R.R.E., Chapman, S.J., Robertson, A.H.J., Potts, J.M., Laggoun-Défarge, F., Gogo, S., Comont, L., Disnar, J.-R. & Francez, A.-J. 2008. FTIR spectroscopy can predict organic matter quality in regenerating cutover peatlands. *Soil Biology & Biochemistry*, **40**, 515–527.

Barnes, R.J., Dhanoa, M.S. & Lister, S.J. 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Applied Spectroscopy*, **43**, 772–777.

Bárta, J., Šlajsová, P., Tahovská, K., Picek, T. & Šantrůčková, H. 2013. Different temperature sensitivity and kinetics of soil enzymes indicate seasonal shifts in C, N and P nutrient stoichiometry in acid forest soil. *Biogeochemistry*, **114**, 525–537.

Brookes, P.C., Landman, A., Pruden, G. & Jenkinson, D.S. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology & Biochemistry*, **17**, 837–842.

Chivers, M.R., Turetsky, M.R., Waddington, J.M., Harden, J.W. & McGuire, A.D. 2009. Effects of experimental water table and temperature manipulations on ecosystem CO_2 fluxes in an Alaskan Rich Fen. *Ecosystems*, **12**, 1329–1342.

Edwards, C., Hales, B.A., Hall, G.H., McDonald, I.R., Murrell, J.C., Pickup, R., Ritchie, D.A., Saunders, J.R., Simon, B.M. & Upton, M. 1998. Microbiological processes in the terrestrial carbon cycle: methane cycling in peat. *Atmospheric Environment*, **32**, 3247–3255.

Eggert, C., Temp, U. & Eriksson, K.E.L. 1996. The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus*: purification and characterization of the laccase. *Applied Environmental Microbiology*, **62**, 1151–1158.

Francez, A.-J., Gogo, S. & Josselin, N. 2000. Distribution of potential CO_2 and CH_4 productions, denitrification and microbial biomass C and N in the profile of a restored peatland in Brittany (France). *European Journal of Soil Biology*, **36**, 161–168.

Gao, J., Zhang, X., Lei, G. & Wang, G. 2014. Soil organic carbon and its fractions in relation to degradation and restoration of wetlands on the Zoigê Plateau, China. *Wetlands*, **34**, 235–241.

Ghani, A., Dexter, M. & Perrott, K.W. 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology & Biochemistry*, **35**, 1231–1243.

Holden, J., Chapman, P.J. & Labadz, J.C. 2004. Artificial drainage of peatlands: hydrological and hydrochemical process and wetland restoration. *Progress in Physical Geography*, **28**, 95–123.

Holden, J., Wallage, Z.E., Lane, S.N. & McDonald, A.T. 2011. Water table dynamics in undisturbed, drained and restored blanket peat. *Journal of Hydrology*, **402**, 103–114.

Höll, B.S., Fiedler, S., Jungkunst, H.F., Kalbitz, K., Freibauer, A., Drösler, M. & Stahr, K. 2009. Characteristics of dissolved organic matter following 20 years of peatland restoration. *Science of the Total Environment*, **408**, 78–83.

Huttunen, J.T., Nykänen, H., Turunen, J. & Martikainen, P.J. 2003. Methane emissions from natural peatlands in the northern boreal zone in Finland, Fennoscandia. *Atmospheric Environment*, **37**, 147–151.

Jaatinen, K., Fritze, H., Laine, J. & Laiho, R. 2007. Effects of short- and long-term water-level drawdown on the populations and activity of aerobic decomposers in a boreal peatland. *Global Change Biology*, **13**, 491–510.

Jaatinen, K., Laiho, R., Vuorenmaa, A., del Castillo, U., Minkkinen, K., Pennanen, T., Penttila, T. & Fritze, H. 2008. Responses of aerobic microbial communities and soil respiration to water-level drawdown in a northern boreal fen. *Environmental Microbiology*, **10**, 339–353.

Joosten, H. & Clarke, D.H. 2002. *Wise use of mires and peatlands*. International Mire Conservation Group and International Peat Society, Saarijärvi, Finland.

Kalbitz, K. 2001. Properties of organic matter in soil solution in a German Fen area as dependent on land use and depth. *Geoderma*, **104**, 203–214.

Laiho, R. 2006. Decomposition in peatlands: reconciling seemingly contrasting results on the impacts of lowered water levels. *Soil Biology & Biochemistry*, **38**, 2011–2024.

Leavitt, S.W., Follett, R.F. & Paul, E.A. 1996. Estimation of slow- and fast-cycling soil organic carbon pools from 6 N HCl hydrolysis. *Radiocarbon*, **38**, 231–239.

Lieffers, V.J. 1988. Sphagnum and cellulose decomposition in drained and natural areas of an Alberta peatland. *Canadian Journal of Soil Science*, **68**, 755–761.

Maanavilja, L., Aapala, K., Haapelehto, T., Kotiaho, J.S. & Tuittila, E.-S. 2014. Impact of drainage and hydrological restoration on vegetation structure in boreal spruce swamp forests. *Forest ecology and management*, **330**, 115–125.

Marx, M.C., Wood, M. & Jarvis, S.C. 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology & Biochemistry*, **33**, 1633–1640.

Minkkinen, K. & Laine, J. 1998. Effect of forest drainage on the peat bulk density of pine mires in Finland. *Canadian Journal of Forest Research*, **28**, 178–186.

Mummey, D.L., Stahl, P.D. & Buyer, J.S. 2002. Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. *Applied Soil Ecology*, **21**, 251–259.

Økland, R.H., Rydgren, K. & Økland, T. 2008. Species richness in boreal swamp forests of SE Norway: the role of surface microtopography. *Journal of Vegetation Science*, **19**, 67–74.

Sparling, G., Vojvodic-Vukovic, M. & Schipper, L.A. 1998. Hot water-soluble C as a simple measure of labile soil organic matter: the relationship with microbial biomass C. *Soil Biology & Biochemistry*., **30**, 1469–1472.

Straková, P., Anttila, J., Spetz, P., Kitunen, V., Tapanila, T. & Laiho, R. 2010. Litter quality and its response to water level drawdown in boreal peatlands at plant species and community level. *Plant and Soil*, **335**, 501–520.

Straková, P., Niemi, R.M., Freeman, C., Peltoniemi, K., Toberman, H., Heiskanene, I., Fritze, H. & Laiho, R. 2011. Litter type affects the activity of aerobic decomposers in a boreal peatland more than site nutrient and water table regimes. *Biogeosciences*, **8**, 2741–2755.

Straková, P., Penttilä, T., Laine, J. & Laiho, R. 2012. Disentangling direct and indirect effects of water table drawdown on above and belowground plant litter decomposition: consequences for accumulation of organic matter in boreal peatlands. *Global Change Biology*, **18**, 322–335.

Tuittila, E.-S., Komulainen, V.-M., Vasander, H. & Laine, J. 1999. Restored cut-away peatland as a sink for atmospheric CO_2. *Oecologia*, **120**, 563–574.

Uhlířová, E., Šimek, M. & Šantrůčková, H. 2005. Microbial transformation of organic matter in soils of montane grasslands under different management. *Applied Soil Ecology*, **28**, 225–235.

Urbanová, Z., Picek, T. & Barta, J. 2011. Effect of peat re-wetting on carbon and nutrient fluxes, greenhouse gas production and diversity of methanogenic archaeal community. *Ecological Engineering*, **37**, 1017–1026.

Urbanová, Z., Bárta, J. & Picek, T. 2013. Methane emissions and methanogenic Archaea on pristine, drained and restored mountain peatlands, Central Europe. *Ecosystems*, **16**, 664–677.

Vance, E.D., Brookes, P.C. & Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass. *Soil Biology & Biochemistry*, **19**, 703–707.

Vasander, H., Tuittila, E.-S., Lode, E., Lundin, L., Ilomets, M., Sallantaus, T., Heikkila, R., Pitkanen, M.-L. & Laine, J. 2003. Status and restoration of peatlands in Northern Europe. *Wetlands Ecology and Management*, **11**, 51–63.

Wallage, Z.E., Holden, J. & McDonald, A.T. 2006. Drain blocking: an effective treatment for reducing dissolved organic carbon loss and water discolouration in a drained peatland. *Science of the Total Environment*, **367**, 811–821.

Paper II

Cotton-Grass and Blueberery have opposite effect on peat characteristics and nutrient transformation in peatland

Kaštovská E., Straková P, Edwards K.R., Urbanová Z., Bárta J., Mastný J., Šantrůčková H., Picek T.

2018

Ecosystems, 21, 443-458

.

Ecosystems (2018) 21: 443–458
DOI: 10.1007/s10021-017-0159-3

ECOSYSTEMS

Cotton-Grass and Blueberry have Opposite Effect on Peat Characteristics and Nutrient Transformation in Peatland

Eva Kaštovská,[1]* Petra Straková,[2] Keith Edwards,[1] Zuzana Urbanová,[1] Jiří Bárta,[1] Jiří Mastný,[1] Hana Šantrůčková,[1] and Tomáš Picek[1]

[1]*Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, Branišovská 1760, České Budějovice 37005, Czech Republic;* [2]*Department of Forest Sciences, Peatland Ecology Group, University of Helsinki, Latokartanonkaari 7, 00014 Helsinki, Finland*

Abstract

Peatlands are large repositories of carbon (C). *Sphagnum* mosses play a key role in C sequestration, whereas the presence of vascular plants is generally thought to stimulate peat decomposition. Recent studies stress the importance of plant species for peat quality and soil microbial activity. Thus, learning about specific plant–microbe–soil relations and their potential feedbacks for C and nutrient cycling are important for a correct understanding of C sequestration in peatlands and its potential shift associated with vegetation change. We studied how the long-term presence of blueberry and cotton-grass, the main vascular dominants of spruce swamp forests, is reflected in the peat characteristics, soil microbial biomass and activities, and the possible implications of their spread for nutrient cycling and C storage in these systems. We showed that the potential effect of vascular plants on ecosystem functioning is species specific and need not necessarily result in increased organic matter decomposition. Although the presence of blueberry enhanced phosphorus availability, soil microbial biomass and the activities of C-acquiring enzymes, cotton-grass strongly depleted phosphorus and nitrogen from the peat. The harsh conditions and prevailing anoxia retarded the decomposition of cotton-grass litter and caused no significant enhancement in microbial biomass and exoenzymatic activity. Therefore, the spread of blueberry in peatlands may stimulate organic matter decomposition and negatively affect the C sequestration process, whereas the potential spread of cotton-grass would not likely change the functioning of peatlands as C sinks.

Key words: peatlands; C/N/P stoichiometry; vascular plants; Sphagnum; nutrient; availability; decomposition; enzymatic activity.

Received 19 August 2016; accepted 22 May 2017;
published online 6 June 2017

Electronic supplementary material: The online version of this article (doi:10.1007/s10021-017-0159-3) contains supplementary material, which is available to authorized users.

Authors' Contributions: EK, HŠ and TP conceived and designed the study as well as the field methods, KE sampled plant material and measured decomposition rates in the field and performed language corrections, PS analyzed plant material using IR, JB analyzed enzymatic activities, ZU and JM performed the field and laboratory portion of the research, and EK wrote the paper with editorial help of other co-authors.

**Corresponding author; e-mail:* ekastovska@prf.jcu.cz

Introduction

Peatlands are nutrient-deficient systems that can accumulate large amounts of carbon (C) due to slow peat decomposition. This slow rate of decomposition is regulated by the prevailing anaerobic soil conditions and low mean temperatures associated with the high altitudes or latitudes where peatlands are predominantly found (Limpens and others 2008). *Sphagnum* mosses play a key role in this C sequestration due to the production of decay-resistant litter (Hájek and others 2011). Peat accumulation changes the local hydrology and pore water biogeochemistry, which generally positively feeds back to support *Sphagnum* (van Breemen 1995). Therefore, *Sphagnum*-dominated vegetation is considered to be fundamental to many ecosystem functions, including C storage (Limpens and others 2008; Bragazza and others 2013; Kuiper and others 2014), providing the stability and resilience of peatland ecosystems (Turetsky and others 2012).

Besides *Sphagnum* mosses, vascular plants are typical inhabitants of peatlands, with water table, nutrient status and temperature being the dominant factors controlling species composition and biomass (Bragazza 2006; Breeuwer and others 2009; Laine and others 2012; Dieleman and others 2015). Peatland vascular plants are more efficient photosynthetizers under all light levels than *Sphagnum* mosses (Leppala and others 2008), enhancing net ecosystem primary production and net CO_2 exchange (Tuittila and others 1999; Riutta and others 2007; Laine and others 2012). Through the labile C released in root exudates, vascular plants shape soil microbial community structure (Bragazza and others 2015) and their presence can be associated with greater microbial biomass and decomposition activity (Bragazza and others 2013; Jassey and others 2013; Bragazza and others 2015). Moreover, peatland vascular plants contain more N and P in their living tissues in comparison with peat mosses (Wang and Moore 2014), with a positive effect on the decomposability of their tissues (Hobbie 1996; Dorrepaal 2007) and nutrient cycling in peatlands (Jassey and others 2013; Bragazza and others 2015).

Most peatland vascular plants belong to two plant functional types, graminoids (sedges) and ericoid shrubs. Deeply rooting graminoids with aerenchyma generally occur in wetter habitats with a mean water table ranging from −10 to −20 cm and are strong competitors in such habitats, whereas ericoid shrubs are abundant further above the water table, avoiding soil anaerobiosis (Bragazza 2006; Laine and others 2012). The differing life strategies of the two functional types are connected with differences in their tissue and litter chemistry (Moore and others 2007; Wang and Moore 2014). Graminoids have higher photosynthetic capacities and respiration rates (Bubier and others 2003; Riutta and others 2007; Leppala and others 2008) and lower contents of phenolics and lignin in the litter than ericoids (Hobbie 1996; Dorrepaal 2007), suggesting their higher tissue decomposability. Moreover, the plant functional types are associated with soil microbial communities of different composition (Haichar and others 2008; Bragazza and others 2015; Robroek and others 2015b). Therefore, the presence of graminoids or ericoids differentially impacts net ecosystem CO_2 exchange, its drought response (Riutta and others 2007; Laine and others 2012; Kuiper and others 2014) and CH_4 dynamics (Robroek and others 2015b; Strom and others 2015), and very likely also peat composition, soil microbial biomass and nutrient cycling.

It is expected that changing climate conditions will shift the functional composition of peatland vegetation toward a graminoid-dominated system under elevated temperatures or increased precipitation (Wahren and others 2005; Dieleman and others 2015) and toward a shrub-dominated community under a combination of drier and warmer seasons with decreased water levels (Bragazza and others 2013; Heijmans and others 2013). Therefore, learning about specific plant–microbe–soil relations and their potential feedbacks for peat quality and nutrient cycling are important for correctly understanding peatland ecosystem functioning as a C sink and its potential shift associated with vegetation change (Heijmans and others 2013; Bragazza and others 2015). We found only a few papers about peatlands that investigated the cascade effects of different plant species or plant functional types on the characteristics of soil organic matter and microbial biomass. Bragazza and others (2013) linked increasing ericoid shrub abundance in peatlands to the structure, C/N stoichiometry and activity of the soil microbial community and dissolved organic matter chemistry, whereas Robroek and others (2015b) demonstrated a direct effect of the removal of different plant functional types on microbial community composition. Jassey and others (2013) related changes in the peatland vascular plant community resulting from warming effects to the structure of microbial food webs. Recently, Robroek and others (2015a) and Pinsonneault and others (2016) stressed the impor-

tance of plant species (or more generally plant functional types) in dissolved organic matter chemistry and its biodegradability, with a possible influence on microbial respiration and dissolved organic matter export from peatlands.

We selected spruce swamp forests (SSF) as a representative type of peatland for the purpose of linking the specific effect of plant species presence on the characteristics of peat and the soil microbial community. SSF are widespread peatland systems, characterized by a patchy distribution of understory vegetation of different plant functional types reflecting site microtopography. In Central Europe and parts of Western Europe, SSF are considered nutrient-poorer types of peatlands because they are located in mountain areas formed by very old, nutrient-poor, high-grade metamorphic rocks (the so-called Moldanubicum zone). This is reflected in their vegetation composition, characterized by a high presence of cotton-grass (*Eriophorum vaginatum*), a species with a highly developed tolerance to low resources and a large capacity for nutrient immobilization (Cholewa and Griffith 2004; Silvan and others 2004). The wettest places of these SSF are covered only by *Sphagnum* mosses, followed by cotton-grass codominating less wet areas, whereas ericoids—namely blueberry (*Vaccinium myrtillus*)—are codominant on drier hummocks. We wanted to determine how the presence of particular vascular plant species is reflected in peat and soil microbial characteristics and thus to determine the possible implications of their spread for nutrient cycling and C storage in the SSF system. Our particular hypotheses were as follows: (1) The non-mycorrhizal cotton-grass and ericoid blueberry will differ in their tissue chemistry from *Sphagnum* mosses, causing faster decomposition of their litter. Their presence will influence peat chemistry, enhance nutrient availability and stimulate microbial activity in comparison with *Sphagnum* peat. (2) There will also be differences between the vascular plant species. Cotton-grass biomass will contain more nutrients and less polyphenolic and lignin compounds than that of blueberry, which will be reflected in its lower litter C/N/P stoichiometry and higher decomposition rate. Therefore, the stimulating effect on the nutrient cycling rate, microbial biomass and its activity will be more pronounced in the presence of cotton-grass than of blueberry. To attain this, we measured and compared C, N and P contents and their stoichiometric ratios of live and senescent aboveground and belowground tissues of the studied plant species—*Sphagnum*, cotton-grass and blueberry, and in the peat, dissolved organic matter and microbial biomass taken in the patches covered only by peat moss or dominated by one of the particular plant species. The plant and peat samples were also characterized by their organic compound composition using infrared spectroscopy. The activities of extracellular enzymes gaining C, N and P, and microbial respiration measured in peat samples were used to assess the possible changes in peat transformation under different vegetation.

Materials and Methods

Study Sites

The study sites are located in the Šumava Mountains, southwest Czech Republic (48°59′N, 13°28′E). Three spruce swamp forest (SSF) sites are located in the catchments of three different small brooks, situated on an upland plateau at an altitude of approximately 1100 m a.s.l, with a cold and humid climate. The mean annual temperature is 4.0°C with mean annual precipitation of 1100 mm (years 1961–1990, statistics by the Czech Hydro-Meteorological Institute). The SSF are covered by a continuous layer of *Sphagnum* mosses (dominated by *S. fallax* with the rare presence of *S. flexuosum* and *S. girgensohnii*) with wet open patches occupied by *Eriophorum vaginatum* L. (with a coverage of 25–50%) and drier microhabitats with shrubs of *Vaccinium myrtillus* L (with a coverage of 45–75%). Other plant species like *Vaccinium vitis-idaea* L., *Vaccinium oxycoccos L.*, sedges and grasses are also rarely present. The patchy distribution of the three dominants in the understory reflects variations in terrain microtopography and water level in the SSF. The tree canopy cover (*Picea abies*) varies from 0% to 80% with tree height ranging from 8 to 15 m. Total N deposition is 0.5–1 g N m^{-2} y^{-1} (2011, statistics by the Czech Hydro-Meteorological Institute).

Aboveground and Belowground Plant Tissue Sampling and Analyses

Plant samples for the purpose of this study were collected from only one of the studied sites, Tetrevska. This site was chosen due to its easy accessibility and the fact that temperature and water level dataloggers were already located there. Preliminary vegetation biomass sampling (both above- and belowground) found no significant differences between the sites (Edwards, unpublished data). Because the sites are protected areas, the aim was to minimize disturbance to the sites as much as possible. The *Sphagnum* capitula, and the fully expanded sun-exposed mature leaves from the top canopy of blueberry and cotton-grass were

randomly sampled (n = 10) in May (beginning of the growing season), July (top season) and September (end of vegetation season) in 2013 and 2014. The senescent leaves were obtained as follows. In the case of cotton-grass, senescent leaves still attached to plants were sampled in September 2013 and in May, July and September 2014. For blueberry, recently senesced, but still attached, reddish brown leaves were sampled in September 2013 and 2014. For *Sphagnum*, the part of the stem 2–3 cm below the capitulum was considered to represent recently senescent tissue.

Belowground biomass of blueberry and cotton-grass was sampled just below the sampled plant using a soil corer (6.5 × 5.5 cm inner dimension; n = 4) at the same sampling times as the aboveground plant biomass in the patches where particular plants dominated. The roots were carefully separated from the peat by hand, washed and assigned to the studied plant species. Roots from other species were discarded. The belowground samples were further separated to living and senescent tissues according to their color, structure and strength. The above- and belowground plant materials were dried at 60°C for 72 h.

To address the likely differences in the quality of living and senescent biomass between *Sphagnum*, cotton-grass and blueberry, their total C and N concentrations were determined by dry combustion on an elemental analyzer (ThermoQuest, Italy). Total P was measured colorimetrically using the ammonium molybdate–ascorbic acid method on a flow injection analyzer (FIA, Lachat QC8500, Lachat Instruments, USA) after perchloric acid digestion (Kopáček and Hejzlar 1995). Differences in N and P concentrations between live and senescent tissues of the particular plant species were used to estimate nutrient resorption efficiencies; these were calculated separately for the above- and belowground plant tissues.

The chemical composition of the plant material sampled in 2013 was assessed using infrared spectroscopy. Infrared spectra were obtained with a Bruker VERTEX 70 series FTIR (Fourier Transform InfraRed) spectrometer (Bruker Optics, Germany) equipped with a horizontal attenuated total reflectance (ATR) sampling accessory. Dried and powdered samples were inserted directly on the ATR crystal, and a MIRacle high-pressure digital clamp was used to achieve even distribution and contact of the sample and crystal. Each spectrum consisted of 65 averaged absorbance measurements between 4000 and 650 cm^{-1}, with a 4 cm^{-1} resolution. Offsets in baseline and slope between the different runs (samples) were removed by standard normal variate transformation and the second derivative using the Unscrambler software (CAMO, Norway). The individual bands were assigned according to Artz and others (2008). Summed absorbance values of the following bands were used as representative of the different organic compounds in the ordination diagrams of Figure 1: 2920 and 2850 cm^{-1} (fats, wax, lipids); 1515, 1454 and 1265 cm^{-1} (phenolics, lignin); 1550 and 1650 cm^{-1} (polypeptides); 1153, 1030 and 900 cm^{-1} (polysaccharides).

Field Decomposition Study

A decomposition study was conducted at the Tetrevska study site to determine whether the observed changes in the litter quality were reflected in the rate of mass loss under field conditions. Senesced leaves of cotton-grass and blueberry were collected at the end of the 2014 growing season (October) as well as roots of the two species and *Sphagnum* thalli. Subsamples of the litter material were dried at 60°C for 48 h and weighed to determine initial litter dry weight, whereas fresh litter material (1.5 g) was placed into separate litter bags (8 × 8 cm; mesh size = 1 mm). These bags were placed in the field in November 2014 with litter bags containing the leaf litter or *Sphagnum* lain on the ground within clumps of the respective species. Bags containing roots were installed in the top 15 cm of the peat within clumps of the respective species using a shovel to produce a slit into which the litter bags were carefully slid so to ensure contact with the peat. Four replicate bags per each species and litter type were collected after 175 and 345 days of exposure (May and October 2015, respectively). The remaining litter was carefully removed from the mesh bags, gently washed, dried at 60°C for 48 h and weighed.

Peat and Soil Solution Sampling and Analyses

Peat was sampled in all three SSF sites in May, July and September of 2013 and 2014 using a soil corer (6.5 × 5.5 cm inner dimension; n = 4) to a depth of 30 cm in randomly selected places covered only by *Sphagnum* and in patches of cotton-grass or blueberry. The samples were homogenized by hand, and roots and other woody material were removed. A portion of the soil was dried at 60°C to constant weight, milled and analyzed for total C, N and P contents and their organic compounds composition by infrared spectroscopy as described above for plant material.

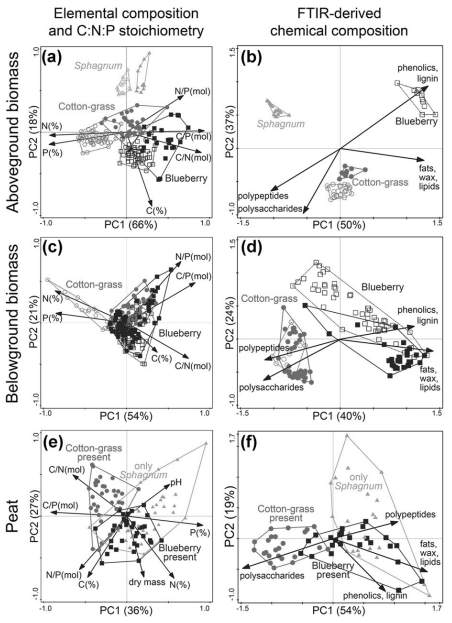


Figure 1. Plant tissue and peat chemistry: ordination diagrams from principal component analysis (PCA) showing the relations between tissue elemental concentrations and the stoichiometry of *Sphagnum*, cotton-grass and blueberry, and FTIR-derived chemical composition for their (**A**, **B**) aboveground and (**C**, **D**) belowground biomass, divided into living (*open symbols*) and senescent (*full symbols*) tissue, and for (**E**, **F**) peat formed in their presence. Graphs **A–D** are based on plant data from the Tetrevska site only; graphs **E–F** show peat data from all three studied sites.

The soil solution was extracted by centrifugal drainage at 4000 g for 1 h at 4°C (Giesler and Lundstrom 1993) from undisturbed peat cores, sampled from a depth of 5–15 cm from the above-defined patches with different vegetation. The extraction was done within 24 h after peat sampling. After filtration through a low-protein-binding Express PLUS polyethersulfone membrane (MILLGPWP) with a 0.22-µm pore size (Merck Millipore Ltd., Ireland), the soil solution was analyzed for organic C (DOC) and N (DN) concentrations on a LiquiTOC II (Elementar, Germany), whereas soluble reactive P (SRP), ammonium and nitrate N were analyzed colorimetrically on a flow injection analyzer (FIA Lachat QC8500, Lachat Instruments, USA). Total soluble P was measured colorimetrically as orthophosphates on a flow injection analyzer after perchloric acid digestion (Kopáček and Hejzlar 1995). The same solution was used to measure pH by a glass pH electrode.

Microbial Biomass and Activity

In fresh peat samples, microbial biomass carbon (MB-C), nitrogen (MB-N) and phosphorus (MB-P) were determined using the chloroform fumigation extraction method (Brookes and others 1982, 1985; Vance and others 1987) within 48 h after sampling. Samples were extracted by 0.5 M potassium sulfate (1:4 w/v) in the case of MB-C and N and by 0.5 M sodium bicarbonate with a pH of 8.5 (1:15 w/v) in the case of MB-P before and after chloroform fumigation for 24 h. The dissolved organic C and dissolved N concentrations in the soil extracts were measured with a TOC/TN analyzer (LiquiTOC II, Elementar, Germany). The P content was measured spectrophotometrically at 886 nm wavelength by the ammonium molybdate–ascorbic acid method. MB-C, MB-N and MB-P were calculated by subtracting the C, N and P concentrations in extracts from the fumigated and nonfumigated samples using correction factors of 0.3, 0.54 and 0.4, respectively.

Microbial respiration was measured as the increase in CO_2 concentration over 48 h during incubation of fresh peat at 10°C in bottles sealed with rubber covers. Anaerobic microbial respiration was assessed similarly, but the headspace of the bottles was flushed with nitrogen. CO_2 concentrations were measured on a gas chromatograph (Agilent 6850 Series, Agilent, USA).

Potential extracellular enzyme activities were determined by microplate fluorometric assays under standardized laboratory conditions. For determination of hydrolytic enzyme activities, 0.5 g of soil was suspended in 50 ml distilled water and sonicated for 4 min to disrupt the soil particles. 200 µl of the soil suspension was added to 50 µl methylumbelliferyl substrate solution for *β*-glucosidase (BG), phosphatase (AP) or *N*-acetylglucosaminidase (NAG) determination or to 50 µl 7-aminomethyl-4-coumarin substrate solution for leucine aminopeptidase (LAP) determination (Marx and others 2001). Plates were incubated at 20°C for 2 h. Fluorescence was quantified at an excitation wavelength of 365 nm and an emission wavelength of 450 nm using the INFINITE F200 microplate reader (TECAN, Germany). All the enzymatic activities were summed.

The activity of BG represented investments into C acquisition, the sum of LAP and NAG showed N acquisition and AP was a measure of P acquisition (Sinsabaugh and others 2009).

Statistics

The molar C/N, C/P and N/P ratios were calculated for the living and dead tissues from each of the three plant dominants, and the peat, soil microbial biomass and soil solution occurring under these dominants (Sterner and Elser 2002). For the peat and microbial characteristics, mean values from all three sites are presented in ''Results,'' whereas data from individual sites are in Supplement.

Variations in plant biomass, peat, MB and soil solution characteristics among patches covered only by *Sphagnum* or with the presence of cotton-grass or blueberry were explored by principal component analysis (PCA, Canoco 5). The measured C, N and P concentrations and their stoichiometric ratios in the samples, or infrared absorbance data, were used as response variables, while variables describing plant species and the live/dead status of the plant tissue (only for analyses of plant tissues) were passive explanatory variables. A constrained analysis (RDA) was then used to determine the proportion of data variability connected with the explanatory variables, plant species, live/dead status, site, time of sampling within the growing season (May, July, September) and sampling year (2013, 2014), from which the best predictors were then selected by interactive forward selection, with a false discovery rate used to adjust the significance of the multiple tests.

The effects of plant species on plant biomass, peat and microbial biomass characteristics were further assessed using a general linear model (with site, vegetation season and sampling year used as covariates), followed by post hoc testing with the Tukey HSD test when the effect was significant (Statistica 10, USA). When necessary, the data were log-transformed to meet the requirements for normality and variance homogeneity.

Between-species differences in decomposition rate were determined by running repeated measures ANOVAs on the relative remaining dry mass data (%) separately for each litter type. The data had normal distributions and homogeneous variances; thus, no data transformations were necessary. The rate of relative mass loss for each species and litter type was determined as the slope of a linear regression (Statistica 10, USA).

Results

Elemental and Stoichiometric Characteristics of the Aboveground and Belowground Plant Tissues

Carbon content of the above- and belowground plant tissues increased in the order *Sphagnum* < cotton-grass < blueberry. The cotton-grass shoots had the highest N and P concentrations and thus the lowest C/N and C/P ratios among the studied plants (Table 1). The two other species, the living *Sphagnum* and blueberry leaves, had lower, but similar, nutrient concentrations. However, the significantly lower C concentration in *Sphagnum* resulted in tissue C/N and C/P ratios markedly lower in comparison with those of blueberry leaves. The N/P tissue ratio ranged from 19 to 23 with no significant difference among the species.

The living belowground parts, although being generally nutrient poorer than the aboveground tissues, mirrored the stoichiometric differences between both vascular plants. Cotton-grass roots were nutrient richer, with lower C/N, C/P and N/P ratios, than the belowground parts of blueberry (Table 1).

The PCA clearly separated all three plants according to the nutrient and stoichiometric characteristics of their living aboveground (pseudo-F = 90.5, $p = 0.002$, Figure 1A) and belowground tissues (pseudo-F = 17.9, $p = 0.002$, Figure 1C). In the first case, the differences among plant species explained 48% of the data variability ($p = 0.005$), whereas this factor explained only 12% of the belowground data variability ($p = 0.005$, results of interactive forward selection). The nutrient and stoichiometric characteristics of the plant tissues for all species changed during the season. In both years, the higher N and P concentrations in May decreased toward autumn leading to higher tissue C/N and C/P ratios in September. These temporal changes explained an additional 3–6% of the data variability ($p < 0.01$ for both above- and belowground biomass).

The ordination diagrams further showed significant nutrient, especially P, depletion of the above- and belowground tissues after senescence, whereas their C concentration was not changed (Figure 1A, C). Therefore, senescent plant parts had markedly higher C/N and C/P, and also N/P ratios than their living tissues (Table 1). The live/dead status of the tissue explained 23% of the aboveground data variability ($p = 0.004$) and 7.3% of the belowground data variability ($p = 0.005$). Because the sampled senescent plant material was still attached

Table 1. Plant Tissue Chemistry

Plant biomass		*Sphagnum*	Cotton-grass	Blueberry
Aboveground biomass				
Live	C (%)	$41.2^{aA} \pm 0.1$	$46.5^{bA} \pm 0.1$	$48.7^{cA} \pm 0.1$
	N (%)	$1.00^{bA} \pm 0.03$	$2.15^{cA} \pm 0.05$	$0.77^{aA} \pm 0.03$
	P (%)	$0.09^{aA} \pm 0.00$	$0.26^{bA} \pm 0.01$	$0.08^{aA} \pm 0.00$
	C/N	$48.7^{bA} \pm 1.3$	$26.3^{aA} \pm 0.8$	$78.6^{cA} \pm 2.9$
	C/P	$1157^{bA} \pm 36$	$501^{aA} \pm 20$	$1641^{cA} \pm 65$
	N/P	$23.4^{bA} \pm 0.8$	$19.0^{aA} \pm 0.4$	$21.1^{aA} \pm 0.6$
Senescent	C (%)	$42.0^{aA} \pm 0.3$	$46.7^{bA} \pm 0.1$	$49.2^{cA} \pm 0.1$
	N (%)	$0.76^{aB} \pm 0.03$	$1.09^{bB} \pm 0.04$	$0.64^{aB} \pm 0.04$
	P (%)	$0.06^{bB} \pm 0.00$	$0.09^{cB} \pm 0.01$	$0.04^{aB} \pm 0.00$
	C/N	$64.2^{bB} \pm 2.1$	$52.6^{aB} \pm 2.0$	$96.2^{cB} \pm 5.2$
	C/P	$1925^{bB} \pm 75$	$1440^{aB} \pm 86$	$3225^{cB} \pm 202$
	N/P	$32.5^{bB} \pm 1.2$	$26.9^{aB} \pm 0.7$	$34.3^{bB} \pm 1.8$
Belowground biomass				
Live	C (%)		$45.7^{aA} \pm 0.7$	$49.6^{bA} \pm 0.2$
	N (%)		$1.46^{bA} \pm 0.17$	$0.56^{aA} \pm 0.02$
	P (%)		$0.27^{bA} \pm 0.03$	$0.05^{aA} \pm 0.00$
	C/N		$51.2^{aA} \pm 10.1$	$114.2^{bA} \pm 4.2$
	C/P		$580^{aA} \pm 81$	$3324^{bA} \pm 209$
	N/P		$13.7^{aA} \pm 1.0$	$30.2^{bA} \pm 1.6$
Senescent	C (%)		$47.4^{aB} \pm 0.1$	$49.4^{bA} \pm 0.1$
	N (%)		$0.67^{aB} \pm 0.02$	$0.71^{aB} \pm 0.03$
	P (%)		$0.03^{aB} \pm 0.00$	$0.05^{aA} \pm 0.00$
	C/N		$88.2^{aB} \pm 2.8$	$93.5^{aB} \pm 4.0$
	C/P		$4784^{bB} \pm 271$	$4227^{aA} \pm 380$
	N/P		$52.8^{aB} \pm 2.2$	$43.0^{aB} \pm 2.6$

Average concentrations of C, N and P (%), and their molar stoichiometric ratios in the aboveground and belowground plant tissues of Sphagnum, cotton-grass and blueberry, either living or senescent (still attached to plant). Plant material was sampled in May, June and September 2013 and 2014 in the site Tetrevska (mean, standard error of the mean SEM, n = 60). Capital letters refer to significant differences in particular characteristics between live and senescent tissues, whereas lowercase letters show differences among plant species (results of one-way ANOVAs and post hoc comparisons, $p < 0.05$).

to plants, its nutrient depletion was mainly ascribed to nutrient resorption into living tissues. Generally, cotton-grass displayed the most efficient nutrient resorption with P resorption being greater than that of N. The aboveground P resorption was 65, 52 and 41%, whereas N resorption was 50, 17 and 25% for cotton-grass, blueberry and *Sphagnum*, respectively. Below ground, cotton-grass resorbed even more, 89% of P and 55% of N, whereas no significant nutrient resorption was found from senescent roots of blueberry. In summary, the characteristics of the aboveground senescent tissues still reflected the stoichiometric differences found among the living tissues, with cotton-grass being nutrient-richer litter than blueberry. Below ground, the initially differing nutrient and stoichiometric characteristics converged in the senescent material of both vascular plants (Figure 1C), making the senescent *Sphagnum* tissue relatively the nutrient richest and senescent cotton-grass roots the most P-depleted sources for microbial decomposition (Table 1).

Infrared Spectra of the Aboveground and Belowground Plant Tissues

All three plant dominants differed in both their aboveground (pseudo-F = 109, $p = 0.002$, Figure 1B) and belowground (pseudo-F = 33.8, $p = 0.002$, Figure 1D) tissue chemical composition. The differences among plant species explained 74% of the aboveground FTIR data variability ($p = 0.002$), whereas it explained only 37% of the variability for the belowground FTIR data ($p = 0.002$). *Sphagnum* biomass had a high relative content of polysaccharides (Figure 2A, bands at 3340, 1153, 1030 and 900 cm^{-1}) and the lowest content of aliphatic and aromatic compounds: fats, waxes, lipids (bands at 2920 and 2850 cm^{-1}), phenolic and lignin-like compounds (bands in the region 1735–1265 cm^{-1}). Cotton-grass had a similarly high content of polysaccharides as *Sphagnum*, but differed (leaves more than roots) by having a somewhat higher content of aliphatic and aromatic

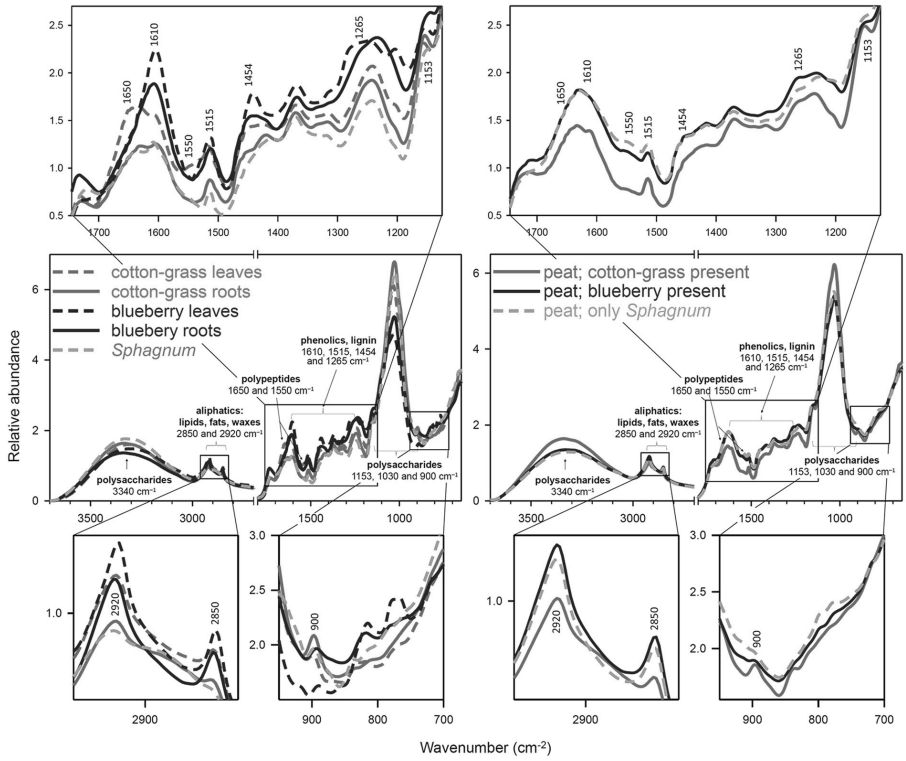


Figure 2. Infrared spectra of (**A**) leaves and roots of cotton-grass and blueberry, and *Sphagnum* capitula and (**B**) peat formed in the prevalence of particular plants, with detailed views of the parts of the spectra showing significant differences among plant tissues. Band assignments as according to Artz and others (2008).

compounds, and polypeptides (bands at 1650 and 1550 cm^{-1}). Blueberry biomass had the highest content of aliphatic and aromatic compounds (again, leaves more than roots) and the lowest content of polysaccharides and polypeptides.

As the plant material used for IR analysis did not contain living aboveground biomass of *Sphagnum* and blueberry, we cannot document any shifts in the contents of the chemical compounds caused by senescence of these species. In the case of cotton-grass, living shoots contained more polypeptides (Figure 2A, bands at 1650 and 1550 cm^{-1}) and a larger proportion of lignin to polysaccharides than dead ones (Figure 1B).

The FTIR-derived characteristics of plant tissues of all species also changed during the season. However, these temporal changes explained only 1–2% of data variability. The contents of phenolic and lignin-like structures tended to increase from May to September. Polysaccharides showed the highest abundance in July and the lowest in September. Polypeptides had an opposite seasonal pattern than that of polysaccharides.

Litter Decomposition of Different Plant Species

The field data on relative mass loss were fitted by linear regression, which is relevant for the early stage of litter decomposition. Different slopes for the fitted lines pointed to significantly different litter decomposition rates of the studied species. The mass loss of aboveground litter was fastest for cotton-grass leaves, which lost 68.9 ± 14.3% of

Table 2. Peat Chemistry

	Sphagnum	Cotton-grass	Blueberry	Plant	Site	Time
Ctot	$43.8^{a} \pm 0.9$	$45.1^{a} \pm 0.2$	$46.8^{a} \pm 0.3$	ns	***	ns
Ntot	$1.63^{b} \pm 0.05$	$1.12^{a} \pm 0.05$	$1.59^{b} \pm 0.03$	***	**	ns
Ptot	$0.09^{b} \pm 0.00$	$0.05^{a} \pm 0.00$	$0.08^{b} \pm 0.00$	***	ns	ns
C/N	$25.0^{a} \pm 0.6$	$44.3^{b} \pm 1.8$	$29.2^{a} \pm 0.8$	***	ns	ns
C/P	$1457^{a} \pm 132$	$2226^{b} \pm 71$	$1609^{a} \pm 69$	***	**	ns
N/P	$44.0^{a} \pm 2.6$	$46.0^{a} \pm 1.5$	$46.1^{a} \pm 1.5$	ns	***	ns
pH	$4.31^{a} \pm 0.04$	$4.01^{a} \pm 0.03$	$3.94^{b} \pm 0.02$	***	***	**

Average concentrations of C, N and P (%), their molar stoichiometric ratios and pH of the peat formed in patches covered only by Sphagnum or affected by the presence of cotton-grass or blueberry. Peat cores were sampled in May, June and September 2013 and 2014 in three spruce swamp forest sites (mean, standard error of the mean SEM, n = 72). Results of GLM on the effect of plant dominants are shown, with site and sampling time as covariates. Lowercase letters show differences among peat characteristics formed in the presence of different plant dominants ($p < 0.05$).
*ns, nonsignificant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

their weight during the first year of incubation in the field, whereas the decomposition rates for blueberry leaves ($37.9 \pm 3.7\%\ y^{-1}$) and *Sphagnum* ($24.2 \pm 8.7\%\ y^{-1}$) were significantly lower ($p < 0.001$). Between-species differences for the roots showed the opposite result, with blueberry roots decomposing at a significantly faster rate ($42.2 \pm 7.6\%\ y^{-1}$) than those of cotton-grass ($18.1 \pm 13.4\%\ y^{-1}$, $p < 0.001$). As a result, cotton-grass roots decomposed much more slowly than its leaves, and, in fact, as slowly as the decomposition-resistant *Sphagnum* litter. The decomposition rates of blueberry leaves and roots were only slightly different.

Peat and Soil Solution Characteristics and C/N/P Stoichiometry

The presence of cotton-grass significantly lowered the N and P contents in peat (Table 2), enhancing its C/N and C/P ratios (Figure 1E). The nutrient-depriving effect of cotton-grass was consistent at all three sites (Table S1). The cotton-grass further had a potentially acidifying effect on peat (Table 2), which occurred on two of the three sites (Table S1). The blueberry, located on drier patches in the SSF sites (relation with soil dry mass in Figure 1E), did not affect peat nutrient content but significantly acidified it (Table 1) at all three sites (Table S1). Generally, the total N and P contents were correlated ($r = 0.418$, $p < 0.05$) across all peat types. Accordingly, the peat N/P ratio remained relatively constant (Table 2). Using RDA, we were able to explain 41% of the variability in the peat characteristics, of which 28% can be ascribed to the presence of vascular plants (namely cotton-grass, 21%), 9% to the site and 4% to seasonal variations, mainly related to changes in soil water content (results of interactive forward selection).

The PCA ordination diagram for the FTIR-derived peat chemical composition separated the peats formed under particular plant species relatively well (Figure 1F). The chemical composition of the peat formed in the presence of the two vascular plant dominants differed in a similar manner as found for their biomass. Compared to *Sphagnum* peat, the peat formed under the polysaccharide-rich cotton-grass was enriched in polysaccharides, whereas the peat with the contribution of blueberry was enriched in aliphatic and aromatic compounds (fats, waxes, lipids, lignin-like and phenolic compounds) (Figure 2B). Using RDA, we were able to explain 43% of FTIR data variability, of which 38% can be ascribed to the presence of vascular plants, namely cotton-grass, and 5% to the site. Generally, our results on peat elemental and organic compound composition show that differences in peat characteristics attributed to plant dominants are significant and larger than those caused by site differences.

In comparison with bulk peat, the soil solution contained more P, but less N, relative to C, as shown by the higher C/N but lower C/P and N/P ratios (Table 3). Similarly to the situation in the peat, each plant dominant had a specific effect also on the soil solution chemistry. The presence of blueberry in the understory enhanced the concentration of DOC (Table 3) at two of the three sites (Table S2), which indicated higher mobility and lability of soil C. Because the soluble N concentration did not change under blueberry, this soil solution had a higher C/N ratio in comparison with other plants. Overall, the content of mineral N forms in the soil solution was intermediate for blueberry peat, but the increased content of nitrates as compared to *Sphagnum* and cotton-grass peat indicated more oxic conditions (Table 3).

Table 3. Soil Solution Chemistry

	Sphagnum	Cotton-grass	Blueberry	Plant	Site	Time
DOC	$66.5^{a} \pm 5.5$	$72.9^{a} \pm 5.2$	$92.3^{b} \pm 6.8$	**	**	***
SN	$1.92^{a} \pm 0.15$	$1.59^{a} \pm 0.10$	$1.78^{a} \pm 0.14$	ns	***	ns
SP	$0.28^{a} \pm 0.04$	$0.23^{a} \pm 0.02$	$0.51^{b} \pm 0.12$	*	***	ns
C/N	$51.3^{a} \pm 4.6$	$67.4^{ab} \pm 6.2$	$77.8^{b} \pm 7.8$	**	ns	***
C/P	$889^{a} \pm 72$	$1124^{a} \pm 106$	$927^{a} \pm 76$	*	***	***
N/P	$21.5^{b} \pm 1.5$	$20.6^{b} \pm 1.7$	$14.9^{a} \pm 1.1$	**	*	***
NH_4	$0.59^{c} \pm 0.06$	$0.15^{a} \pm 0.01$	$0.26^{b} \pm 0.03$	***	***	***
NO_3	$0.09^{a} \pm 0.01$	$0.10^{a} \pm 0.01$	$0.17^{b} \pm 0.03$	**	ns	*
SRP	$0.17^{a} \pm 0.03$	$0.11^{a} \pm 0.01$	$0.27^{a} \pm 0.09$	ns	***	ns

Average concentrations of dissolved organic C (DOC; mg l^{-1}), soluble N (SN; mg l^{-1}) and P (SP; μg l^{-1}), their molar stoichiometric ratios, concentrations of mineral N forms (N-NH4, N-NO3; mg l^{-1}) and soluble reactive P (SRP, μg l^{-1}) in the soil solution extracted from peat cores from patches covered by Sphagnum and affected by the presence of cotton-grass or blueberry. Peat cores were sampled in May, June and September 2013 and 2014 in the three spruce swamp forest sites (mean, standard error of the mean SEM, n = 72). Results of GLM on the effect of plant dominants are shown, with site and sampling time as covariates. Lowercase letters show differences among solution characteristics in the presence of different plant dominants ($p < 0.05$).
*ns, nonsignificant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

Blueberry presence further had the potential to mobilize P, evidenced by an enhanced concentration of soluble P found at two of the three sites, and also by a higher concentration of soluble reactive P at the P richest Tetrevska site (Table S2). This ability of blueberry resulted in the systematically lowest soil solution N/P ratio from all plant dominants (Table 3). The effect of cotton-grass on the soil solution was not significant, but was coincident with its nutrient-depleting effect on the bulk peat. Overall, the soil solution in the cotton-grass peat had the lowest concentration of mineral N at all three sites (Table 3 and S2). The concentrations of soluble P and soluble reactive P were lowered by cotton-grass presence only at the Tetrevska site, characterized by the highest concentrations of soluble P from the three sites (Table S2). The soil solution in patches covered only by *Sphagnum* always had the highest ammonium-N concentration indicating the highest N availability but lack of oxic conditions. In total, however, only 7% of the variability in the soil solution data can be ascribed to the dominant vegetation (RDA analysis and interactive forward selection, $p = 0.003$), with site explaining 9%, and changes within vegetation season, mainly the increase in DOC concentration in summer ($p = 0.004$), and inter-annual differences in the nitrate concentration in soil solution ($p = 0.004$) (detailed data not shown) explaining 12% of data variability.

MB, Its Elemental Stoichiometry and Activity

Microbial biomass C, N and P were higher in the patches with blueberry in comparison with the other two dominants (Table 4). This blueberry effect was consistent in all three sites, although the increase in microbial C and N was not always statistically significant (Table S3). In two of the three sites, microbial P, but no other element bound in the microbial biomass, was also enhanced under cotton-grass (Table S3). The changes in microbial biomass did not significantly affect its elemental stoichiometry, although the microbial biomass under blueberry appeared to have the lowest C/P ratio, whereas that under cotton-grass the highest C/N ratio (Table 4). In comparison with *Sphagnum* peat, the portion of peat N and P bound in microbial biomass was higher, exceeding 2% for N and 20% for P in the presence of both vascular plants, making microbial biomass an important nutrient pool in those types of peat. The interactive forward selection of explanatory variables showed that only 5% of the variability in the microbial biomass and its elemental stoichiometry could be ascribed to the effect of vegetation of the sampled patch, with a similar portion of variability explained by site (5%) and temporal changes in MB. A decrease in the microbial C/P and N/P ratios from May to the end of the growing season explained 5% of data variability and the year-to-year decrease in MB-N content from 2013 to 2014 explained an additional 3% ($p < 0.05$ in all cases, detailed data not shown).

Aerobic and anaerobic microbial respiration and total enzymatic activity were comparable among the patches with different vegetation (Table 4), with the exception of the Kvilda site, where microbial respiration was higher under cotton-grass than in the other types of vegetation (Table S4). A majority of enzymatic activity (≥75%) was directed

Table 4. Microbial Biomass

	Sphagnum	Cotton-grass	Blueberry	Plant	Site	Time
MB-C	$3217^{a} \pm 321$	$3613^{ab} \pm 240$	$4354^{b} \pm 336$	*	***	ns
MB-N	$276.2^{a} \pm 25.1$	$301.3^{a} \pm 31.3$	$459.0^{b} \pm 39.4$	***	**	ns
MB-P	$123.3^{a} \pm 9.7$	$150.1^{a} \pm 12.4$	$201.6^{b} \pm 14.4$	***	ns	**
C/N	$17.35^{a} \pm 1.77$	$24.24^{b} \pm 4.67$	$15.34^{a} \pm 1.58$	*	*	**
C/P	$90.15^{a} \pm 15.34$	$97.82^{a} \pm 19.07$	$68.4^{a} \pm 6.39$	ns	**	**
N/P	$9.23^{a} \pm 2.36$	$6.41^{a} \pm 0.95$	$6.23^{a} \pm 0.63$	ns	**	**
MB-C/Ctot	$0.75^{a} \pm 0.08$	$0.82^{a} \pm 0.05$	$0.94^{a} \pm 0.07$	ns	***	*
MB-N/Ntot	$1.42^{a} \pm 0.15$	$2.12^{b} \pm 0.22$	$2.26^{b} \pm 0.19$	***	***	ns
MB-P/Ptot	$15.07^{a} \pm 1.63$	$27.62^{b} \pm 2.37$	$26.51^{b} \pm 2.05$	***	ns	***
Microbial respiration aerobic	$7.78^{b} \pm 0.48$	$8.31^{b} \pm 0.44$	$6.02^{a} \pm 0.32$	**	***	*
Microbial resp. anaerobic	$1.32^{a} \pm 0.13$	$1.35^{a} \pm 0.13$	$1.17^{a} \pm 0.09$	ns	ns	***
Sum of enzymatic activity	933.1 ± 83.6	754.1 ± 49.9	866.5 ± 72.0	ns	**	*
% C-gaining enzymes	$14.5^{a} \pm 1.0$	$12.8^{a} \pm 0.7$	$18.1^{b} \pm 1.4$	**	ns	ns
% N-gaining enzymes	$2.5^{a} \pm 0.3$	$3.6^{a} \pm 0.3$	$3.5^{a} \pm 0.4$	*	***	ns
% P-gaining enzymes	$83.0^{b} \pm 1.2$	$83.6^{b} \pm 0.9$	$78.5^{a} \pm 1.5$	**	*	ns

C, N and P (MB-C, MB-N, MB-P; $\mu g\ g^{-1}$) and their molar stoichiometric ratios, proportions of peat C, N and P bound in the microbial biomass (%), microbial respiration in aerobic and anaerobic conditions ($\mu l\ CO_2\ g^{-1}\ h^{-1}$) and the sum of hydrolytic enzymatic activity and proportions of C-, N- and P-gaining enzymatic activities in the peat formed in patches covered only by Sphagnum or affected by the presence of cotton-grass or blueberry. Peat cores were sampled in May, June and September 2013 and 2014 in the three spruce swamp forest sites (mean, standard error of the mean SEM, n = 72). Results of GLM on the effect of plant dominants are shown, with site and sampling time as covariates. Lowercase letters show differences among peat characteristics formed in the presence of different plant dominants.
*ns, nonsignificant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

to P acquisition under all types of vegetation. However, in two of the sites, the portion of P-gaining enzymes was lower, whereas the portion of enzymes mining C was higher, in the peat formed in the presence of blueberry in comparison with both other peat types (Table S4).

Discussion

Shifts in the peatland plant community toward vascular plant dominance, driven by climate change, are predicted to have negative consequences for peatland C sink functioning (Bragazza and others 2013; Jassey and others 2013; Buttler and others 2015; Dieleman and others 2015). However, we demonstrated here that the implications on ecosystem behavior are species specific and markedly differ between blueberry and cotton-grass, the most common vascular plants codominating spruce swamp forests together with *Sphagnum* spp.

Sphagnum Mosses Formed the N Richest Environment within the Generally Nutrient-poor Spruce Swamp Forest System

Against the general assumption that *Sphagnum* is a low-quality substrate (Hájek and others 2011; Turetsky and others 2012), we found that *Sphagnum* biomass is rather rich in polysaccharides and nutrients. *Sphagnum* peat, formed in the absence of any vascular plants, was also relatively nutrient rich and its soil solution contained the highest concentration of mineral (ammonium) N from the three peat types studied (Figure 3). We suggest that the relatively high N availability in the *Sphagnum* peat could be related to several specific characteristics of *Sphagnum* mosses: biological atmospheric N_2 fixation by cyanobacteria and methanotrophs associated with *Sphagnum* (Larmola and others 2014), high cation exchange capacity (Verhoeven and Liefveld 1997) and low N resorption efficiency from the senescing *Sphagnum* (only approximately 25% of N was resorbed, calculated on a mass basis). Despite the highest N availability in the *Sphagnum* peat within the studied peatland, the *Sphagnum* litter decomposed very slowly in comparison with the other types of litters, and microbial biomass and activity were also low. Therefore, these microsites undoubtedly acted as C sinks. Besides the possible suppression of microbial activity by polyphenolics (e.g., Verhoeven and Liefveld) and the prevailing anaerobic conditions, another reason is that the system is also P limited. This limitation is indicated by the high peat C/P and N/P ratios in comparison with common soils (Cleveland and Liptzin 2007) and also by the large investments in P acquisition, which exceeded 80% of the measured hydrolytic enzyme activity. These findings agree with the

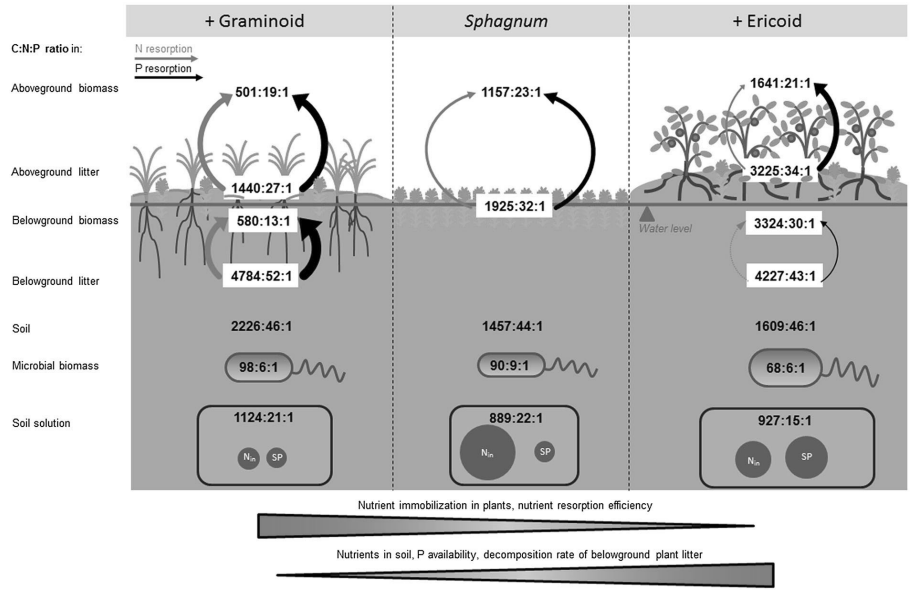


Figure 3. Scheme of the functioning of the spruce swamp forest system in the presence of the studied plant dominants; with *Sphagnum* only (*in the center*), in the presence of cotton-grass (*on the left*) and blueberry (*on the right*). The C/N/P stoichiometry of the living and senescent biomass of particular plant dominant is shown; their nutrient resorption efficiencies are indicated by the width of the *arrows*. Belowground, the C/N/P stoichiometry of the peat, microbial biomass and soil solution formed in the presence of particular plant dominant is shown, based on average data from the three study sites. Sizes of *ovals* and *circles* show the relative differences in the microbial biomass, soil organic C pool and concentration of mineral N and soluble phosphorus among the areas affected by different plant dominants. The figure demonstrates the decreasing concentration of nutrients in the living plant tissues but also decreasing nutrient efficiencies from cotton-grass over *Sphagnum* to blueberry. At the same time, P availability increased in the soil, which was connected with enhanced microbial biomass and the decomposition rate of belowground plant litter in the same direction.

suggestion that primary production and microbial growth are P limited in peatlands (Hill and others 2014). Therefore, environmental changes affecting P cycling and relieving P limitation of the microbial activity in the *Sphagnum*-dominated peatland should be expected to impact its ability to act as a C sink.

Cotton-Grass Depleted Nutrients from the Peat and Its Presence did not Stimulate Peat Decomposition in Spruce Swamp Forests

Both above- and belowground living cotton-grass biomass was rich in polysaccharides and formed significant nutrient pools within the spruce swamp forest system. Such biomass characteristics suggest that cotton-grass presence should be connected with fast litter decomposition, the enhancement of nutrient concentrations in soil solution and acceleration of peat decomposition (Chapin and others 2003). However, our results did not support any of these expectations and showed that the system with cotton-grass functioned differently.

Cotton-grass appeared to be the center of a closed nutrient pool, with only small losses into the soil solution and bulk peat. The high nutrient resorption of cotton-grass, reaching 50% for N and 60–80% of P, secured effective internal N and P recycling within the plant (Figure 3). Its large nutrient immobilization capacity can be ascribed to effective biomass production (Tuittila and others 1999), significant allocation of nutrients in the slowly decomposing storage organs (Silvan and others

2004), unusual vascular system of cotton-grass enabling efficient internal nutrient recycling (Cholewa and Griffith 2004) and the long life span of individual tussocks (Shaver and others 1986). The deeply rooting system of cotton-grass facilitated nutrient uptake by the plant. The mechanisms of efficient nutrient uptake and immobilization within the plant resulted in N and P depletion from the soil solution and the formation of the nutrient poorest peat within the spruce swamp forest system (Figure 3).

Cotton-grass is further thought to have large root exudation, which is deduced from increased CO_2 and CH_4 effluxes in its presence (Saarnio and others 2004; Laine and others 2012; Kuiper and others 2014; Robroek and others 2015b). The exudation of low molecular weight compounds commonly enhances microbial activity in the vicinity of roots and accelerates soil organic matter decomposition by the rhizosphere priming effect (Kuzyakov 2002). We found that cotton-grass presence enhanced the proportions of total peat N and P bound in the soil microbial biomass in comparison with the *Sphagnum* peat, which documented the high efficiency of plant–microbe relations in mining of nutrients from the peat and subsequent immobilization. However, we did not find any significant increase in either soil microbial biomass or exoenzymatic activity, which could be a sign of increased peat decomposition in the presence of cotton-grass. Still, the fact that cotton-grass enriched the peat with easily decomposable compounds can be deduced from enhanced microbial respiration under aerobic conditions, as found in one of the three studied sites. In anaerobic conditions, however, this cotton-grass effect disappeared. Lack of the expected stimulation effect of cotton-grass might be explained by strong nutrient limitation of microbial activity, because of the nutrient depletion by cotton-grass, as well as the prevailing anoxic conditions, which disable the functioning of oxidative enzymes and lower the energetic gain of microbial metabolism.

The decomposition rate of the polysaccharide-rich but nutrient-poor cotton-grass root litter was very low under such conditions, similar to that of the decay-resistant *Sphagnum* litter (Hájek and others 2011). This seems to be a general phenomenon because decomposition of cotton-grass root litter was markedly slower in comparison with other root litter types (*Carex* sp., *Betula nana*, fine roots of *Pinus sylvestris*) as found in a field study in boreal peatlands (Straková and others 2012). Well recognizable residues of its dead organs can be found even in peat layers formed thousands of years ago (Kalnina and others 2015). As a result, a combination of high primary productivity and slow tissue decomposition makes cotton-grass a typical peat-forming species with C sink function (Tuittila and others 1999; Silvan and others 2004; Kivimaki and others 2008).

Based on these results, we suggest that cotton-grass presence does not relieve the microbial community from nutrient limitation and supports the peatland C sink function. Supportive data about its efficient C and nutrient economics suggest that cotton-grass will have a similar effect on peat chemistry over a wide range of peatlands in which it can typically be found, including nutrient-poor open bogs and pine bogs, boreal peatlands, cutaway and also restored peatlands.

Blueberry Promoted Soil Organic Matter Decomposition by Significantly Enhancing P Availability

As hypothesized, blueberry biomass and attributes varied from that of the two other plant species. Noteworthy, blueberry was the nutrient poorest plant dominant but still it was not efficient in the internal recycling of nutrients within its biomass (Figure 3). It resorbed only 17% of N and approximately 52% of P from senescing leaves and almost no nutrients from its dying belowground biomass. The lower need for closed nutrient cycling within blueberry biomass could be due to nutrient income via ericoid mycorrhizal symbionts, which are able to mobilize N and P complexed in recalcitrant organic matter and facilitate plant uptake (Cairney and Meharg 2003; Read and others 2004).

The more open nutrient cycling within blueberry biomass increased P availability, resulting in the highest concentrations of P in the soil solution among all three plant species (Figure 3). In relation to this, microorganisms decreased their costs expenditure for gaining P but invested more into enzymes connected to C acquisition. Ericoid mycorrhizae associated with blueberry roots can play an important role in extracellular enzyme production and increased P availability (Read 1996; Read and others 2004). Enhanced concentrations of nitrate–N in the soil solution and its decreasing pH indicated more oxic conditions in the peat under blueberry. The blueberry litter, although rather nutrient poor and rich in aliphatic and aromatic compounds, thus decomposed relatively faster. The increasing amount of dissolved organic C in the soil solution supported the assumption about faster decomposition.

The higher nutrient availability and presence of ericoid mycorrhizae were mirrored in the microbial biomass, which was larger than in the other peat types and had lower C/N/P stoichiometry (Figure 3). Therefore, the microbial biomass under blueberry represented an important sink/source of nutrients with relatively fast turnover in the range of days to weeks (Schmidt and others 2007). This made nutrient cycling under blueberry more dynamic with the nutrients released during microbial turnover more accessible to plant uptake.

In summary, the blueberry tissues represented a less concentrated but, thanks to the large biomass, still significant nutrient stock within the spruce swamp forest, similar to the cotton-grass biomass. However, contrary to cotton-grass, the blueberry nutrient pool seemed to be more open, with larger losses of N and P to the peat, which could reduce nutrient limitation of the microbial decomposers and potentially stimulate peat decomposition. Our results thus support the projection of others (Bragazza and others 2013, 2015) that spreading of ericoid shrubs in peatlands would enhance organic matter decomposition and increase nutrient cycling, with negative implications for C sequestration.

Conclusions

Vascular plant cover in peatlands will likely increase with climate change (Elmendorf and others 2012). Previous studies demonstrated that this spreading can feed back to change microbial activity in the peat (Bragazza and others 2015; Robroek and others 2015b). Our data showed that the potential effect of vascular plants on peat properties, and soil microbial biomass and activity is species specific and need not necessarily result in increased organic matter decomposition.

The presence of blueberry enhanced the availability of the limiting nutrient P, connected with an increase in soil microbial biomass and the activities of C-acquiring enzymes. Its spread in peatlands, which could occur with lower water levels and the formation of an oxic upper peat layer, thus may result in increased organic matter decomposition, which would negatively affect the capacity of peatlands to act as C sinks, as proposed by others (Bragazza and others 2013, 2015).

Differently, the potential spread of cotton-grass can occur in nutrient-poorer peatlands with increasing temperatures but stable water levels (Wahren and others 2005; Salmon and others 2016). Cotton-grass is thought to provide large amounts of easily degradable compounds to the soil microbial community. However, at the same time, it has a high capacity to immobilize nutrients and strongly depletes P and N from the peat, which is further reflected in the C/N/P stoichiometry of the microbial biomass. The harsh conditions and prevailing anoxia in the upper peat layer retard decomposition of cotton-grass litter and result in no significant enhancement in microbial biomass and exoenzymatic activity. Therefore, the spread of cotton-grass would not change or may even enhance the functioning of peatlands as C sinks.

Acknowledgements

This study was supported by the Grant Agency of Czech Republic (13-17398S), the Ministry of Youth Sports and Education of Czech Republic (LM2015075) and the Academy of Finland (No. 1259190, for PS). We thank Terezia Říhová for analyses on FIA, Ville Narhi for elemental analyses, Dan Vaněk for analyses on LiquiTOC and Ondřej Žampach for his help with the field work and FTIR measurements.

References

Artz RRE, Chapman SJ, Robertson AHJ, Potts JM, Laggoun-Defarge F, Gogo S, Comont L, Disnar J-R, Francez A-J. 2008. FTIR spectroscopy can be used as a screening tool for organic matter quality in regenerating cutover peatlands. Soil Biol Biochem 40:515–27.

Bragazza L. 2006. A decade of plant species changes on a mire in the Italian Alps: vegetation-controlled or climate-driven mechanisms? Clim Change 77:415–29.

Bragazza L, Bardgett RD, Mitchell EAD, Buttler A. 2015. Linking soil microbial communities to vascular plant abundance along a climate gradient. New Phytol 205:1175–82.

Bragazza L, Parisod J, Buttler A, Bardgett RD. 2013. Biogeochemical plant-soil microbe feedback in response to climate warming in peatlands. Nat Clim Change 3:273–7.

Breeuwer A, Robroek BJM, Limpens J, Heijmans M, Schouten MGC, Berendse F. 2009. Decreased summer water table depth affects peatland vegetation. Basic Appl Ecol 10:330–9.

Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen—a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–42.

Brookes PC, Powlson DS, Jenkinson DS. 1982. Measurement of microbial phosphorus in soil. Soil Biol Biochem 14:319–29.

Bubier J, Crill P, Mosedale A, Frolking S, Linder E. 2003. Peatland responses to varying interannual moisture conditions as measured by automatic CO_2 chambers. Global Biogeochem Cy 17:1–15.

Cairney JWG, Meharg AA. 2003. Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. Eur J Soil Sci 54:735–40.

Chapin FSIII. 2003. Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. Ann Bot 91:455–63.

Cholewa E, Griffith M. 2004. The unusual vascular structure of the corm of *Eriophorum vaginatum*: implications for efficient retranslocation of nutrients. J Exp Bot 55:731–41.

Cleveland CC, Liptzin D. 2007. C:N:P stoichiometry in soil: is there a ''Redfield ratio'' for the microbial biomass? Biogeochemistry 85:235–52.

Dieleman CM, Branfireun BA, McLaughlin JW, Lindo Z. 2015. Climate change drives a shift in peatland ecosystem plant community: implications for ecosystem function and stability. Glob Change Biol 21:388–95.

Dorrepaal E. 2007. Are plant growth-form-based classifications useful in predicting northern ecosystem carbon cycling feedbacks to climate change? J Ecol 95:1167–80.

Elmendorf SC, Henry GHR, Hollister RD, Bjork RG, Boulanger-Lapointe N, Cooper EJ, Cornelissen JHC, Day TA, Dorrepaal E, Elumeeva TG, Gill M, Gould WA, Harte J, Hik DS, Hofgaard A, Johnson DR, Johnstone JF, Jonsdottir IS, Jorgenson JC, Klanderud K, Klein JA, Koh S, Kudo G, Lara M, Levesque E, Magnusson B, May JL, Mercado-Diaz JA, Michelsen A, Molau U, Myers-Smith IH, Oberbauer SF, Onipchenko VG, Rixen C, Schmidt NM, Shaver GR, Spasojevic MJ, Porhallsdottir PE, Tolvanen A, Troxler T, Tweedie CE, Villareal S, Wahren C-H, Walker X, Webber PJ, Welker JM, Wipf S. 2012. Plot-scale evidence of tundra vegetation change and links to recent summer warming. Nat Clim Change 2:453–7.

Giesler R, Lundstrom U. 1993. Soil solution chemistry—effects of bulking soil samples. Soil Sci Soc Am J 57:1283–8.

Haichar FEZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, Heulin T, Achouak W. 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME J 2:221–1230.

Hájek T, Ballance S, Limpens J, Zijlstra M, Verhoeven JTA. 2011. Cell-wall polysaccharides play an important role in decay resistance of *Sphagnum* and actively depressed decomposition in vitro. Biogeochemistry 103:45–57.

Heijmans MMPD, van der Knaap YAM, Holmgren M, Limpens J. 2013. Persistent versus transient tree encroachment of temperate peat bogs: effects of climate warming and drought events. Glob Change Biol 19:2240–50.

Hill BH, Elonen CM, Jicha TM, Kolka RK, Lehto LLP, Sebestyen SD, Seifert-Monson LR. 2014. Ecoenzymatic stoichiometry and microbial processing of organic matter in northern bogs and fens reveals a common P-limitation between peatland types. Biogeochemistry 120:203–24.

Hobbie SE. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. Ecol Monogr 66:503–22.

Jassey VEJ, Chiapusio G, Binet P, Buttler A, Laggoun-Defarge F, Delarue F, Bernard N, Mitchell EAD, Toussaint M-L, Francez A-J, Gilbert D. 2013. Above—and belowground linkages in *Sphagnum* peatland: climate warming affects plant-microbial interactions. Glob Change Biol 19:811–23.

Kalnina L, Stivrins N, Kuske E, Ozola I, Pujate A, Zeimule S, Grudzinska I, Ratniece V. 2015. Peat stratigraphy and changes in peat formation during the Holocene in Latvia. Quatern Int 383:186–95.

Kivimaki SK, Yli-Petays M, Tuittila ES. 2008. Carbon sink function of sedge and *Sphagnum* patches in a restored cutaway peatland: increased functional diversity leads to higher production. J Appl Ecol 45:921–9.

Kopáček J, Hejzlar J. 1995. Semi-micro determination of total phosphorus in soils, sediments and organic materials—a simplified perchloric-acid digestion procedure. Commun Soil Sci Plant Anal 26:1935–46.

Kuiper JJ, Mooij WM, Bragazza L, Robroek BJM. 2014. Plant functional types define magnitude of drought response in peatland CO_2 exchange. Ecology 95:123–31.

Kuzyakov Y. 2002. Review: factors affecting rhizosphere priming effects. J Plant Nutr Soil Sci 165:382–96.

Laine AM, Bubier J, Riutta T, Nilsson MB, Moore TR, Vasander H, Tuittila E-S. 2012. Abundance and composition of plant biomass as potential controls for mire net ecosytem CO_2 exchange. Botany 90:63–74.

Larmola T, Leppanen SM, Tuittila E-S, Aarva M, Merila P, Fritze H, Tiirola M. 2014. Methanotrophy induces nitrogen fixation during peatland development. Proc Natl Acad Sci USA 111(2):734–9.

Leppala M, Kukko-Oja K, Laine J, Tuittila E-S. 2008. Seasonal dynamics of CO_2 exchange during primary succession of boreal mires as controlled by phenology of plants. Ecoscience 15:460–71.

Limpens J, Berendse F, Blodau C, Canadell JG, Freeman C, Holden J, Roulet N, Rydin H, Schaepman-Strub G. 2008. Peatlands and the carbon cycle: from local processes to global implications a synthesis. Biogeosciences 5:1739.

Moore TR, Bubier JL, Bledzki L. 2007. Litter decomposition in temperate peatland ecosystems: the effect of substrate and site. Ecosystems 10:949–63.

Pinsonneault AJ, Moore TR, Roulet NT, Lapierre J-F. 2016. Biodegradability of vegetation-derived dissolved organic carbon in a cool temperate ombrotrophic bog. Ecosystems 19:1023–36.

Read DJ. 1996. The structure and function of the ericoid mycorrhizal root. Ann Bot 77:365–74.

Read DJ, Leake JR, Perez-Moreno J. 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Can J Bot 82:1243–63.

Riutta T, Laine J, Tuittila ES. 2007. Sensitivity of CO_2 exchange of fen ecosystem components to water level variation. Ecosystems 10:718–33.

Robroek BJM, Albrecht RJH, Hamard S, Pulgarin A, Bragazza L, Buttler A, Jassey VE. 2015a. Peatland vascular plant functional types affect dissolved organic matter chemistry. Plant Soil 407:135–43.

Robroek BJM, Jassey VEJ, Kox MAR, Berendsen RL, Mills RTE, Cecillon L, Puissant J, Meima-Franke M, Bakker P, Bodelier PLE. 2015b. Peatland vascular plant functional types affect methane dynamics by altering microbial community structure. J Ecol 103:925–34.

Saarnio S, Wittenmayer L, Merbach W. 2004. Rhizospheric exudation of *Eriophorum vaginatum* L.—Potential link to methanogenesis. Plant Soil 267:343–55.

Salmon VG, Soucy P, Mauritz M, Celis G, Natali SM, Mack MC, Schuur EAG. 2016. Nitrogen availability increases in a tundra ecosystem during five years of experimental permafrost thaw. Glob Change Biol 22:1927–41.

Schmidt SK, Costello EK, Nemergut DR, Cleveland CC, Reed SC, Weintraub MN, Meyer AF, Martin AM. 2007. Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. Ecology 88:1379–85.

Shaver GR, Fetcher N, Chapin FS. 1986. Growth and flowering in *Eriophorum vaginatum*—annual and latitudinal variation. Ecology 67:1524–35.

Silvan N, Tuittila ES, Vasander H, Laine J. 2004. *Eriophorum vaginatum* plays a major role in nutrient immobilisation in boreal peatlands. Ann Bot Fenn 41:189–99.

Sinsabaugh RL, Hill BH, Shah JJF. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–8.

Sterner RW, Elser JJ. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton: Princeton University Press. p 441.

Straková P, Penttila T, Laine J, Laiho R. 2012. Disentangling direct and indirect effects of water table drawdown on above- and belowground plant litter decomposition: consequences for accumulation of organic matter in boreal peatlands. Glob Change Biol 18:322–35.

Strom L, Falk JM, Skov K, Jackowicz-Korczynski M, Mastepanov M, Christensen TR, Lund M, Schmidt NM. 2015. Controls of spatial and temporal variability in CH_4 flux in a high arctic fen over three years. Biogeochemistry 125:21–35.

Tuittila ES, Komulainen VM, Vasander H, Laine J. 1999. Restored cut-away peatland as a sink for atmospheric CO_2. Oecologia 120:563–74.

Turetsky MR, Bond-Lamberty B, Euskirchen E, Talbot J, Frolking S, McGuire AD, Tuittila ES. 2012. The resilience and functional role of moss in boreal and arctic ecosystems. New Phytol 196:49–67.

van Breemen N. 1995. How *Sphagnum* bogs down other plants. Trends Ecol Evol 10:270–5.

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass-C. Soil Biol Biochem 19:703–7.

Verhoeven JTA, Liefveld WM. 1997. The ecological significance of organochemical compounds in *Sphagnum*. Acta Bot Neerl 46:117–30.

Wahren Ch, Walker MD, Bret-Harte MS. 2005. Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. Glob Change Biol 11:537–52.

Wang M, Moore TR. 2014. Carbon, nitrogen, phosphorus, and potassium stoichiometry in an ombrotrophic peatland reflects plant functional type. Ecosystems 17:673–84.

Paper III

Quality of DOC produced during litter decomposition of peatland plant dominants

Mastný J., Kaštovská E., Bárta J., Chroňáková A., Borovec J., Šantrůčková H., Urbanová Z., Edwards K.R., Picek T.

2018

Soil Biology and Biochemistry, 121, 221–230

.

Soil Biology and Biochemistry 121 (2018) 221–230

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Quality of DOC produced during litter decomposition of peatland plant dominants

J. Mastný[a,*], E. Kaštovská[a], J. Bárta[a], A. Chroňáková[b], J. Borovec[c], H. Šantrůčková[a,c], Z. Urbanová[a], K.R. Edwards[a], T. Picek[a]

[a] Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, Branišovská 1760, České Budějovice 37005, Czech Republic
[b] Biology Centre CAS, Institute of Soil Biology, Na Sádkách 7, České Budějovice 37005, Czech Republic
[c] Biology Centre CAS, SoWa Research Infrastructure, Na Sádkách 7, České Budějovice 37005, Czech Republic

ARTICLE INFO

Keywords:
Litter decomposition
DOC
Molecular weight fractions
Biodegradability
Peatland

ABSTRACT

Litter decomposition is an important source of dissolved organic matter (DOC). In peatlands with hardly degradable soil DOC, the input of DOC from vascular plant litter can represent an important source of nutrients and decomposable substrates for soil microorganisms. We established a laboratory incubation with the litters of three peatland plant dominants (*Sphagnum fallax, Vaccinium myrtillus and Eriophorum vaginatum*) for 200 days, aiming to study DOC production and its quality. The quality of the DOC leached from the litters was characterized by a distribution of C, N and P among molecular weight (MW) fractions (< 1, 1-10, 10-100 and > 100 kDa), their aromaticity, composition of low molecular weight compounds (organic acids, sugars and amino acids) and DOC biodegradability.

The leaves of vascular plants decomposed the fastest, releasing larger amounts of nutrients and easily degradable organic acids, sugars and amino acids to the leachate, when compared to their roots and *Sphagnum*. DOC distribution to the MW fractions did not differ among the litters. Neither the DOC distribution to the respective fractions nor leachate C/N/P stoichiometry were factors driving DOC biodegradability. Total dissolved P distribution to the MW fractions significantly differed among the litters, with *Sphagnum* being very specific: P was initially associated only with high molecular weight DOC, while later it was redistributed to the lower MW fractions and complexed with Al and Fe. The complexation may retard soluble reactive P leaching especially from *Sphagnum* litter. DOC biodegradability was higher for the vascular plant leaf litter than for the *Sphagnum* litter in the early stages of decomposition (20 days) but later decreased and became more uniform for all litters. These temporal differences (by decomposition stage) were more pronounced than those caused by litter origin. Our results indicate that mainly leaf litter of vascular plants can release significant amounts of DOC during the early stage of decomposition. This DOC is more aromatic with higher biodegradability and more nutrients (especially P) as compared to *Sphagnum* and can thus temporarily stimulate microbial activity in habitats dominated by the vascular plants.

1. Introduction

Peatland ecosystems represent a large pool of the terrestrial organic C (Gorham, 1991) and they are a substantial source of dissolved organic carbon (DOC) to surface water (Clark et al., 2008; Thacker et al., 2008). DOC fluxes from peatlands contribute up to approximately 35% of the overall peatland carbon budget (Worrall et al., 2003). DOC is composed of compounds with various molecular weight (MW): humic and fulvic acids have the highest MW, whereas oligopeptides, organic acids and sugars have the lowest (Leenheer and Croue, 2003). Peatland DOC is generally of low biodegradability (Tfaily et al., 2013), which is attributed to compounds resistant to decomposition like phenolic and uronic acids (Verhoeven and Toth, 1995; Verhoeven and Liefveld, 1997) and sphagnan released from *Sphagnum* mosses (Painter, 1991). Moreover, these compounds acidify the environment and inhibit microbial growth (Stalheim et al., 2009).

Beside peat mosses, vascular plants, mainly ericoids and graminoids, commonly inhabit peatlands. As in other terrestrial ecosystems, fresh plant litter is an important source of DOC which differs from peat by its quality and biodegradability (Moore and Dalva, 2001; Wickland

* Corresponding author. Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, Branišovská 1716/31c, České Budějovice 37005, Czech Republic.
E-mail addresses: mastnj00@prf.jcu.cz (J. Mastný), ekastovska@prf.jcu.cz (E. Kaštovská), jiri.barta@prf.jcu.cz (J. Bárta), alicach@upb.cas.cz (A. Chroňáková), jakub.borovec@bc.cas.cz (J. Borovec), hasan@prf.jcu.cz (H. Šantrůčková), urbanz00@prf.jcu.cz (Z. Urbanová), edwards@prf.jcu.cz (K.R. Edwards), picek@prf.jcu.cz (T. Picek).

https://doi.org/10.1016/j.soilbio.2018.03.018
Received 25 August 2017; Received in revised form 7 February 2018; Accepted 11 March 2018
Available online 28 March 2018
0038-0717/

et al., 2007). Robroek et al. (2016) recently found that vascular plants significantly influence the quality of peatland DOC by enriching it with low molecular weight (LMW) compounds, which increased heterotrophic microbial activity in the peat under the plants.

In our recent study (Kaštovská et al., 2017), we described the different effects of characteristic peatland species, the peatland moss *Sphagnum fallax,* an ericoid shrub *Vaccinium myrtillus* and the graminoid *Eriophorum vaginatum*, on the elemental and chemical composition of the peat formed under these three functional types. Nutrient and DOC concentrations in the soil solution also significantly differed. *Sphagnum* formed polysaccharide- and ammonium-rich peat. *Vaccinium* biomass was enriched in aromatic lignin-like compounds. The peat formed under *Vaccinium* contained more DOC and was characterized by having higher P availability. Differently, *Eriophorum* biomass was composed mainly of polysaccharides and represented a significant nutrient pool, while the peat under the plant was very nutrient poor. The differences result from a combination of several abiotic and biotic factors including the direct effect of the input of litter with different quality, different plant exudation quality and quantity (Edwards et al. under review) and various microbial communities associated with these species. The effects of particular factors are, however, difficult to separate in the field.

In this study we asked what is the direct effect of litter quality on the quality of the DOC produced during the decomposition process. To this end, we incubated the litters of three peatland plant dominants (*Sphagnum fallax, Vaccinium myrtillus* and *Eriophorum vaginatum*) under laboratory conditions for 200 days in order to study DOC production and the quality of produced DOC characterized by molecular weight fractionation, the elemental composition and aromaticity of the fractions, composition of easily degradable low MW (LMW) compounds – amino acids, organic acids and sugars, and DOC biodegradability. Based on past studies and our recent study, we hypothesized that: (i) Because of having the highest content of LMW organic compounds in their biomass and lower C/N/P stoichiometry, the decomposition of fresh vascular plant leaf litter will be faster compared to *Sphagnum* and root litter. (ii) Litter C/N/P stoichiometry and the C distribution among the molecular weight fractions will be the main driving factors affecting DOC quality and biodegradability. (iii) The leaves of vascular plants will produce DOC with a higher content of LMW compounds and lower C/N/P stoichiometry, leading to its higher biodegradability as compared to roots and *Sphagnum*.

2. Methods

2.1. Plant litter characteristics

Shoots of *Sphagnum fallax* and aboveground and belowground litter of *Vaccinium myrtillus* and *Eriophorum vaginatum* were used in the experiment. Leaf litter of *Vaccinium* (VA) and *Eriophorum* (EA) was collected in autumn 2014 as freshly senesced leaves still attached to the plant. Belowground litter (VB) and (EB) was collected by removing several *Vaccinium* and *Eriophorum* plants and cutting off the roots, which were then cleaned of any adhering soil by washing in water. *Sphagnum* shoots were divided into the upper green capitulum (ca 2 cm, SA) and the lower senescent part below the capitulum (SB). At the beginning of the incubation, the litter samples were analysed for basic chemical properties. Total C and N contents in litter samples were measured on an elemental analyser (Micro-cube, Elementar, Germany). Total P was measured by the ammonium molybdate-ascorbic acid method on a flow injection analyser (FIA, Lachat QC8500, Lachat Instruments, USA) after perchoric acid digestion (Kopáček and Hejzlar, 1995).

2.2. Experimental design of incubation

Glass bottles (620 ml) with gas-tight lids and septa for litter incubation and gas sampling were used. Pure sand (180 g) was put on the bottom of each flask, moistened by 40 ml of deionized water of pH 4 (treated with hydrochloric acid), which is the average pH of peat on the study sites (Kaštovská et al., 2017). Before being placement into the bottles, the litter samples were inoculated by spraying with a peat suspension and carefully homogenized. This suspension was prepared from a mixture of the three peat samples (1:1:1), which were taken from the upper 10 cm layer under each studied plant dominant on our study sites. The peat suspension was generated by shaking the peat mixture with deionized water (1:10 ratio; w:v) on an end-over-end shaking machine for 1 h and then centrifugation at 1000*g* for 5 min. Then intact fresh litter (10 g) was put into a bottle on a nylon net, which was placed on the sand surface. In total, 16 replicate bottles per each litter type were prepared. An additional four bottles filled with moist sand were used as blanks. All bottles were incubated in the dark at 15 °C for 200 days.

2.3. Respiratory C losses from litter as a proxy for decomposition

The CO_2 concentration in the headspace of each flask was regularly measured at 3-4 week intervals using a HP 6850 gas chromatograph (Agilent, USA). After each measurement, the flasks were opened, ventilated and closed again. The CO_2 data were used to calculate respiration rates and cumulative respiratory losses for each litter type, which were used as a proxy for decomposition losses. Moisture of the litter remained stable during the whole incubation.

2.4. Litter extraction and leachate characterization

Destructive litter samplings were done at the beginning of the incubation and then after 20, 70 and 200 days (Fig S1). On each sampling occasion, 4 g of fresh litter from each of four bottles were extracted with 40 ml of cold deionized water by shaking at 4 °C for 1 h – this litter extract is hereafter referred to as the "leachate". We used a relatively short time period for litter – water contact to simulate DOC and nutrient leaching from the litter during a rain event, but also to obtain sufficient C concentration in the leachate for the subsequent biodegradation assay and qualitative analysis of selected groups of LMW organic compounds (Soong et al., 2014). A low temperature was used to slow down degradation of these compounds in the leachate before analysis (Rousk and Jones, 2010). The leachate was filtered through a 0.2 µm express plus PES (polyethersulfone) membrane filter (GPWP14250, Merck Milipore Ltd. Ireland) using vacuum filtration. Filtrates were stored at 4 °C and, within one day, were analysed for concentrations of inorganic N forms ($N–NO_3^-$, $N–NH_4^+$) and soluble reactive phosphorus (SRP) by flow-injection analyser (FIA Lachat QC8500, Lachat Instruments, USA) and for dissolved organic carbon (DOC) and dissolved nitrogen (DN) contents using a LiquiTOCII (Elementar, Germany). Leachate pH was measured by a glass electrode. The DOC, DN and nutrients concentrations were calculated per gram of initial litter dry weight.

2.5. Sugars, amino acids and organic acids (identified LMW) in the DOC

Capillary ion-chromatography was used to separate organic acids, amino acids and sugars in the filtrates. Organic acids were separated using an AS11-HC column (Thermo Scientific) and determined by conductivity detection. Their concentrations were calculated in reference to standards. Carbohydrates and amino acids were separated using an Aminopack PA10 column (ThermoScientific) and detected amperometrically (Thermo ICS 5000, USA). Based on this, four groups of these compounds were determined: sugar alcohols, neutral sugars, amino acids and oligosaccharides. Their concentrations were evaluated semi-quantitatively using a 0-10 scale based on their peak shape and area. Identified LMW compounds were calculated per gram of litter dry weight. Normalization of identified LMW compound contents per gram of DOC was used to show the differences in DOC quality among the litters.

2.6. DOC molecular weight fractionation and characterization of the fractions

Size exclusion chromatography with an isocratic pump (Dionex DX 320, USA) was used to separate DOC into four fractions differing in their molecular weights: > 100 kDa, 10–100 kDa, 1–10 kDa, < 1 kDa. The concentrations of Al, P and Fe within each fraction were analysed by ICP-QQQ (Agilent 8800, Japan). The working conditions were optimized for each measuring mode by using a standard 1 μg l^{-1} solution of 7Li+, 89Y+ and 209Bi+. All operational parameters are summarized in Table S1. Total dissolved C and N were measured using a LiquiTOCII (Elementar, Germany). The UV–VIS spectra of each fraction, except the < 1kDa one, were measured using a Photodiode Array Detector (Dionex PDA, USA) in the range from 190 to 800 nm. We excluded the < 1kDa fraction from the results due to the possible effect of dissolved nitrate and iron on the measured absorbance (Weishaar et al., 2003). Specific UV absorbance (SUVA 254), defined as the UV absorbance at 254 nm normalized for DOC concentration (L mg C^{-1} m^{-1}), was used for estimating the aromaticity of each DOC fraction (aromaticity index, Weishaar et al., 2003).

2.7. DOC biodegradability

DOC biodegradability was estimated from the dynamics of CO_2 loss from leachates incubated at 15 °C under aerobic conditions for 42 days (McDowell et al., 2006). Before incubation, the filtered leachates were diluted to DOC concentration of 20 mg l^{-1} and the pH was adjusted to 4.0 by hydrochloric acid to simulate field conditions. Then 2 ml of leachate were pipetted to 6 ml Labco Exetainer vials (Labco, UK) and inoculated with 50 μl of peat suspension prepared in the same way as described above for the litter inoculation. One replicate was prepared for each sample and incubated under aerobic conditions (ambient air). The CO_2 production from DOC mineralization was measured after 0, 3, 7, 14 and 42 days using a HP 6850 gas chromatograph (Agilent, USA). The samples were regularly ventilated. To characterize short-term DOC biodegradability, the relative data on cumulative DOC loss (in % initial DOC) were fitted using a one-phase decay model to obtain the decay rate constant (% of present C day^{-1}) and the proportion of relatively easily biodegradable compounds within the DOC (% DOC decomposed in the short-term, plateau of the fitting), hereafter called DOC biodegradability.

2.8. Statistical analyses

Multivariate analysis (Canoco 5) was used to evaluate the effect of plant litter type on the (i) DOC characteristics (C and nutrient contents and their C/N/P stoichiometric ratios), (ii) C, N, P, Fe and Al contents in the DOC molecular weight fractions, (iii) content and portion of organic acids in DOC, and (iv) semi-quantitative contents of sugars and amino acids. The chemistry data were log-transformed, centered and analysed by redundancy analysis (RDA). Plant litter type was used as an explanatory variable with time as a supplementary variable and vice versa. Further, the effect of litter type and time on each parameter was tested by repeated–measures ANOVA (STATISTICA 10, USA) followed by post hoc comparison (unequal N HSD test).

3. Results

3.1. Basic characteristics of fresh litters and their decomposition rates

The fresh litters were significantly different in their chemical characteristics (Table 1). Their C content differed among plant species, increasing in the order: SA = SB < EB = EA < VB < VA. The leaf litter of the vascular plants (EA, VA) had the lowest C/N and C/P ratios while SB had the highest. The N/P ratio decreased in the order: *Vaccinium* > *Sphagnum* > *Eriophorum* being similar for both aboveground and belowground parts of particular plant species.

During 200 days of litter decomposition, between 8.8 and 21.3% of the initial C were lost as CO_2, with the fastest decomposition and accordingly largest losses for EA, followed by VA (Fig. 1A). The lowest respiratory C losses were measured for SA, EB and VB. While the respiration rates rather decreased with ongoing decomposition in the case of the vascular plant litters, those from *Sphagnum* remained similar or even increased (for SB) over time (Fig. 1).

3.2. DOC production and leachate chemistry

The fresh litters leached relatively small amounts of DOC (Fig. 2). Then the *Sphagnum* litter produced relatively low and stable amounts of DOC during the whole decomposition period, while the DOC production from vascular plant litters significantly increased during the first stages of their decomposition. Both *Eriophorum* litters leached significant DOC amounts already during the first 20 days of decomposition, while this occurred between days 20–70 in the *Vaccinium* litter. In both cases, leaf litter leached significantly more DOC than root litter of a particular species and *Eriophorum* litters leached markedly more DOC than *Vaccinium* litters, with the exception of similarly low EB and VB DOC production after 200 days of incubation (Fig. 2).

Leachate pH, DOC, N and SRP (per gram of litter) and C/N/P stoichiometry differed among litter types and incubation times (Fig. 3A, Table S2). According to the RDA (with time as a covariate), 50.1% of adjusted variability in these leachate characteristics could be ascribed to differences among litter types ($p < 0.01$, pseudo-F = 19.5). *Sphagnum* leachates were acidic (pH 4.06–5.51), while those of vascular plants were neutral (*Vaccinium,* pH 5.09–6.59) or slightly alkaline (*Eriophorum,* pH 6.43–7.53). Moreover, both *Sphagnum* leachates had the highest N/SRP and C/SRP ratios over the whole incubation, whereas the highest DOC, nutrient per gram of litter and the lowest C/N and C/SRP ratios were characteristic for the EA leachate ($p < 0.001$). The EA litter was the major producer of ammonium N and SRP during the decomposition (20-200 days) with the amounts of released nutrients increasing over time (see Figs. 4 and 5, Table S2). Ammonium N and SRP per gram of litter in the leachates were closely correlated with DN and Norg during the whole incubation ($r > 0.870$). The ammonium N and SRP per gram of litter in the leachates were closely correlated with litter respiration rates in the first two sampling times (21 and 70 days), with correlation coefficients ranging from 0.732 to 0.853. However, these correlations had weakened ($r < 0.510$) by the end of the incubation (200 days).

3.3. Identified LMW compounds in litter leachates

Particular groups of identified LMW compounds in the leachates (per gram of litter) were affected by litter type. The RDA (with time as a covariate) showed that the differences among litters explained 26.5% of the adjusted variability (pseudo-F = 8.7, $p < 0.01$) (Fig. 3B). Identically for all litters, the amount of identified LMW compounds in leachates decreased after 70 days of decomposition, although the DOC production remained similar (for more details see Tables S3, S4 and Fig. S2). The contribution of the identified LMW compounds to DOC (identified LMW compounds normalized on DOC) was also dependent on the litter type, but showed a different pattern (Fig. S3). The proportion of sugars in DOC was higher in the *Sphagnum* than in the vascular plant leachates while the proportions of amino acids and oligosaccharides were higher in *Sphagnum* and *Vaccinium* leachates as compared to *Eriophorum*.

Due to the used identification method, we have more specific information about the size and composition of the organic acids (OA) pool than about other groups of identified LMW compounds. In general, vascular plant litters released a higher amount of OA than *Sphagnum*, significantly decreasing till the end of the incubation (200 days) in all cases. *Sphagnum* released more OA from fresh litter than after it started

Table 1
Initial chemical characteristics of plant litter types: total contents of carbon, nitrogen, and phosphorus (all in %), their molar ratios, decay rate constant (in % of present C day^{-1}) and total C loss through respiration after 200 days (in % of initial C content): SA – *Sphagnum* aboveground, SB – *Sphagnum* belowground, EA – *Eriophorum* aboveground, EB – *Eriophorum* belowground, VA – *Vaccinum* aboveground, VB – *Vaccinium* belowground, (mean ± SD, n = 4).

Litter type	C_{TOT}	N_{TOT}	P_{TOT}	C/N	C/P	N/P	Decay rate constant	Respiration C loss
SA	**42.6** ± 0.1a	**0.96** ± 0.01abc	**0.076** ± 0.002a	**51.7** ± 0.6a	**1456** ± 27ac	**28.2** ± 0.84ac	**0.523** ± 0.17a	**8.8** ± 1.8a
SB	**42.3** ± 0.3a	**0.68** ± 0.01a	**0.046** ± 0.004b	**72.3** ± 0.4d	**2213** ± 9.5d	**30.6** ± 0.03acd	**0.246** ± 0.06b	**14,6** ± 0,8b
EA	**44.8** ± 0.1b	**1.50** ± 0.08d	**0.174** ± 0.019d	**34.9** ± 1.8b	**701** ± 62b	**20.0** ± 0.77b	**1.064** ± 0.17c	**21.3** ± 0.4c
EB	**45.5** ± 0.0b	**0.86** ± 0.01ab	**0.077** ± 0.006a	**61.8** ± 0.4c	**1503** ± 157ac	**24.3** ± 2.69abc	**0.498** ± 0.23ab	**9.5** ± 1.9a
VA	**47.1** ± 0.1c	**1.82** ± 0.02e	**0.112** ± 0.002c	**30.2** ± 0.3b	**1106** ± 5.2c	**36.6** ± 0.15d	**0.584** ± 0.07a	**18.6** ± 1.6c
VB	**49.0** ± 0.0d	**1.11** ± 0.02c	**0.079** ± 0.008a	**51.4** ± 1.1a	**1656** ± 24a	**33.4** ± 1.16ad	**0.449** ± 0.17a	**10.0** ± 1.2a

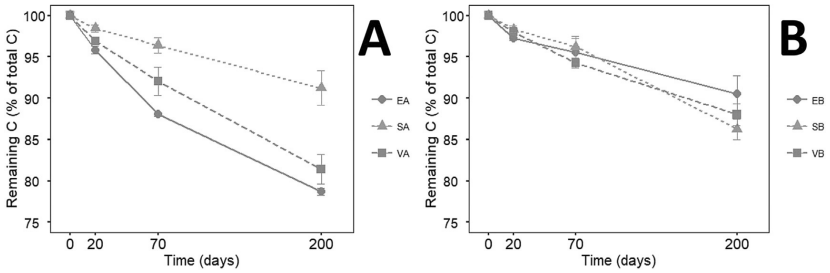


Fig. 1. Remaining C (expressed as percentage of total C content) in the studied litter types during the 200-day decomposition for A) aboveground and B) belowground litters. SA – *Sphagnum* aboveground, SB – *Sphagnum* belowground, EA – *Eriophorum* aboveground, EB – *Eriophorum* belowground, VA – *Vaccinium* aboveground, VB - *Vaccinium* belowground, (mean, ± SD, n = 4).

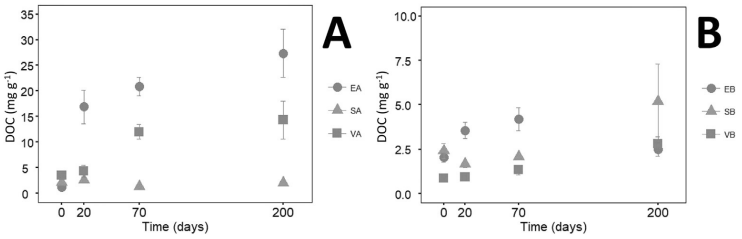


Fig. 2. DOC per gram of litter for the A) aboveground and B) belowground (with different scale on y axis) litter leachates during the 200-day decomposition. SA – *Sphagnum* aboveground, SB – *Sphagnum* belowground, EA – *Eriophorum* aboveground, EB – *Eriophorum* belowground, VA – *Vaccinium* aboveground, VB - *Vaccinium* belowground, (mean, ± SD, n = 4).

to decompose, while *Eriophorum* and *Vaccinium* litters released the highest amount of OA (especially acetic, propionic and shikimic acids) when the DOC in the leachates (per gram of litter) rapidly increased (20 and 70 days, respectively) (Table S4 and Fig. S4). The portion of total OA in the released DOC did not exceed 5% except for *Eriophorum* and *Vaccinium* after 20 (8.9%) and 70 (11%) days of incubation, respectively.

3.4. Molecular weight fractions: C and N distribution and aromaticity of DOC

The DOC distribution among the MW fractions did not significantly differ among the litter leachates at any sampling time, although it was less uniform at the beginning than at the end of the incubation (Fig. 6A). Generally, the majority (> 60%) of DOC was equally divided between the smallest and the highest MW fraction, while the remaining DOC was again similarly distributed between the two middle MW fractions. The N distribution among the MW fractions (Table S5) closely followed that of C till day 70, with correlation coefficients ranging from 0.549 to 0.916 ($p < 0.05$), but later, this relationship between C and N weakened.

The aromaticity index was below 4 for all *Sphagnum* DOC (Fig. 7). *Sphagnum* DOC (SA, SB) aromaticity was lower than that of vascular plant DOC (EA, EB, VA, VB) for all MW fractions during the whole incubation. We did not find any relation between the aromaticity and any other parameter measured in the leachate MW fractions.

3.5. Molecular weight fractions: P, Al and Fe distribution

Phosphorus distribution among the MW fractions differed between the litter types and was temporarily highly dynamic (Fig. 6B). The leachates from the fresh litters formed three specific groups. Both *Sphagnum* leachates contained nearly 100% of the total dissolved P in the highest MW fraction (> 100kDa), while, oppositely, the leachates from leaf litters (VA, EA) had the most P in the two small MW fractions (< 1 kDa and 1-10 kDa), with the P in the highest fraction (> 100 kDa) forming only up to 11% of the total dissolved P. Both root leachates (EB and VB) had 70% of the dissolved P in the two largest fractions (Fig. 6B). The P distribution was positively correlated to Al, Fe and C contents in all MW fractions (except the least abundant 10-100 kDa fraction) with correlation coefficients ranging from 0.529 to 0.826.

After 20 days, at least 50% of the dissolved P in all the leachates occurred in the 10-100 kDa fraction and its distribution was not related to any other measured element, with the exception of a correlation with

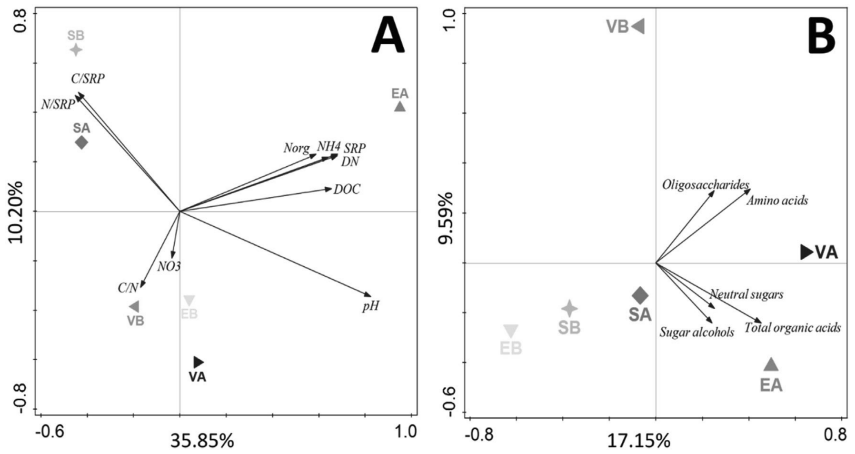


Fig. 3. A) Leachate characteristics (per gram) as affected by plant litter type (RDA with litter type as the explanatory variable and time as a supplementary variable); all four sampling events are included in the analyses. B) Sugars, amino acids and total organic acids (per gram) in leachate (RDA with litter type as the explanatory variable and time as a supplementary variable); all four sampling events are included in the analyses. SA – *Sphagnum* aboveground, SB – *Sphagnum* belowground, EA – *Eriophorum* aboveground, EB – *Eriophorum* belowground, VA – *Vaccinium* aboveground, VB - *Vaccinium* belowground. All data were log transformed and centered.

DOC in the > 100 kDa fraction (r = 0.584). The P from the 10-100 kDa fraction was later redistributed to the smaller MW fractions (< 1 kDa and 1-10 kDa) in leachates from the vascular plant litters and exclusively to the smallest MW fraction in the *Sphagnum* leachates. After 200 days, all the dissolved P in the *Sphagnum* leachates occurred only in the smallest and largest MW fractions, while it was distributed among all the MW fractions in all vascular plant leachates with the smallest contribution in the largest MW fraction (Fig. 6B). The occurrence of total dissolved P in the smaller MW fractions of < 1kDa and 1-10kDa was significantly correlated with the amounts of Al and Fe in those fractions (r ranging between 0.595 and 0.676). Moreover, there were strong correlations between the litter respiration rate and the occurrence of total dissolved P in either the 10-100 kDa (r = 0.732) or < 1 kDa (r = -0.730) fractions after 200 days.

3.6. DOC biodegradability

The DOC leached from the fresh litters decomposed by comparable decay rates of 7.2–9.6% of present C per day, with the exception of the leachate from fresh EA, which decomposed much faster (20% present C a day). The easily biodegradable compounds described by biodegradability formed 22-29% of *Sphagnum*, 38-40% of the root (EB and VB) and 44-48% of fresh leaf (EA and VA) DOC leachates with the only significant difference being between the *Sphagnum* and EA leachates (Table 2). In the first 70 days of litter decomposition, the contribution of easily biodegradable compounds to the leached DOC either remained similar (SA, EB, VA, VB) or slightly decreased (SB, EA). Accordingly, the leachate DOC decay rates remained the same or only slightly decreased, with the exception of the significant decrease in the EA leachate decay rate, which then became comparable with the others. After 200 days of litter decomposition, leachate DOC decay rates decreased to 4.8–9.5% of present C a day and SB leachate DOC decomposed even more slowly (decay rate of < 1% of present C a day), indicating the degradation of compounds of lower quality. This decay rate was so slow and temporarily stable that the DOC losses could not be fitted with any model to get the plateau. Therefore, the measured cumulative C-CO_2 loss was used instead. Generally, the DOC in the leachates from the decomposed litters contained only 12-23% of easily biodegradable compounds, which was significantly less compared to the leachates from fresh litters as well as from litters decomposing for only 70 days.

4. Discussion

4.1. Comparison of litter decomposition rates

In accord with our hypothesis, the vascular plant leaf litter decomposed faster than both *Sphagnum* and roots litter. The litter decomposition rates, calculated here using the respiratory C loss during 200 days of laboratory incubation, correlated well (correlation coefficient 0.893) with the decomposition rates measured *in situ* in our

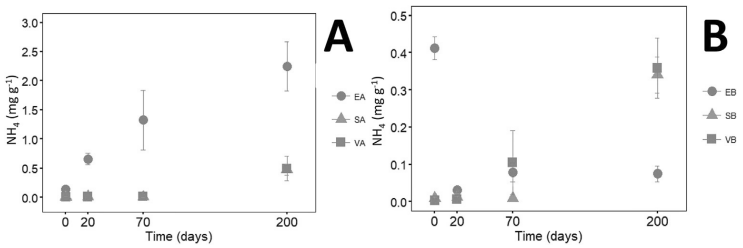


Fig. 4. Ammonium N in the leachate per gram of litter for the A) aboveground and B) belowground (with different scale on y axis) litters during the 200-day decomposition. . SA – *Sphagnum* aboveground, SB – *Sphagnum* belowground, EA – *Eriophorum* aboveground, EB – *Eriophorum* belowground, VA – *Vaccinium* aboveground, VB - *Vaccinium* belowground (mean, ± SD, n = 4).

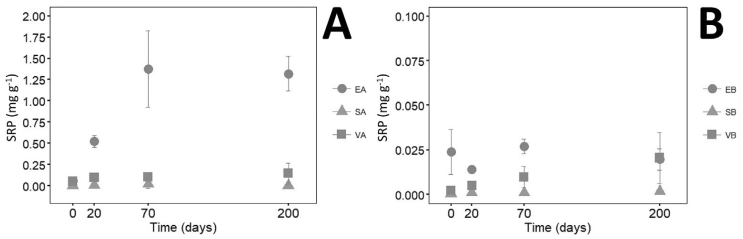


Fig. 5. Soluble reactive P in the leachate per gram of litter for the A) aboveground and B) belowground (with different scale on y axis) litters during the 200-day decomposition. . SA – *Sphagnum* aboveground, SB – *Sphagnum* belowground, EA – *Eriophorum* aboveground, EB – *Eriophorum* belowground, VA – *Vaccinium* aboveground, VB - *Vaccinium* belowground (mean, ± SD, n = 4).

previous study using litter bags (Kaštovská et al., 2017). During the first stage of decomposition (till the 70th day), litter decomposition was at least partly controlled by nutrient availability, because the litter respiration rate tightly correlated with the amounts of ammonium N and SRP per gram of litter in the litter leachates. The lowest decay rate in the SB litter corresponded well with its higher C/P and C/N ratios, while the fastest decomposing EA litter had the lowest C/P ratio. The weakened correlations between litter C mineralization and nutrient leaching in the later stage of decomposition (day 200) indicate that other factors than sufficient nutrient availability controlled the litter decay.

4.2. DOC leaching and its quality

Significantly more DOC per gram of its biomass was leached from decomposing litter of vascular plants than from *Sphagnum*. While very

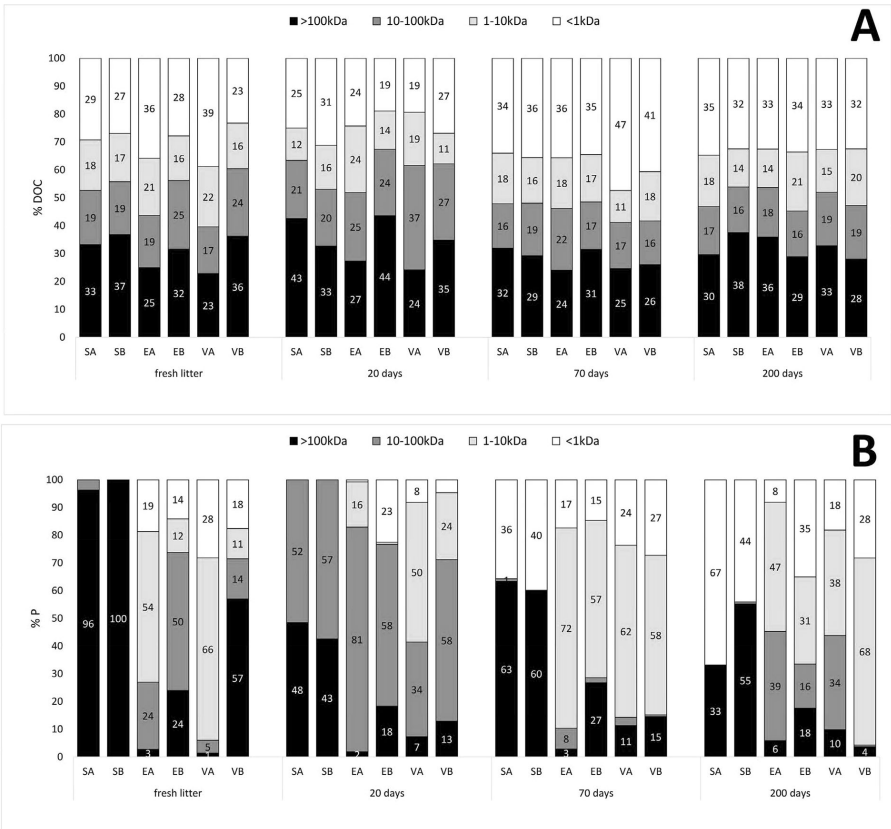


Fig. 6. A) Carbon (DOC) and B) phosphorus (total dissolved phosphorus) contents (%) in four DOC fractions separated according to their molecular weight (> 100 kDa, 10-100 kDa, 1-10 kDa, < 1 kDa), EA – *Eriophorum* aboveground, SA – *Sphagnum* aboveground, VA – *Vaccinium* aboveground, EB – *Eriophorum* belowground, SB – *Sphagnum* belowground, VB - *Vaccinium* belowground (mean; ± SD; n = 4).

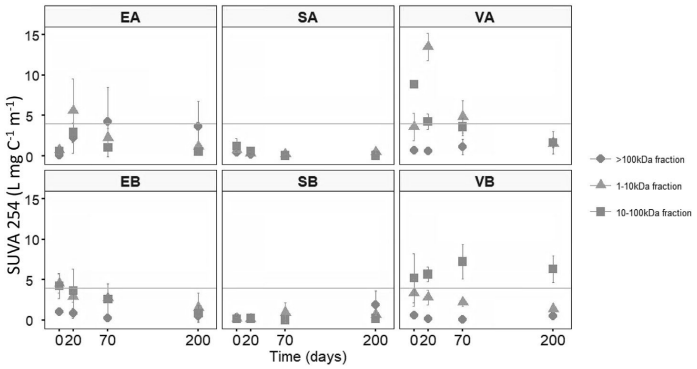


Fig. 7. Specific UV absorbance measured at 254 nm (SUVA 254; index of aromaticity; L mg $C^{-1} m^{-1}$) for the three DOC fractions separated according to their molecular weight (> 100 kDa, 10-100 kDa, 1-10 kDa), EA – *Eriophorum* aboveground, SA – *Sphagnum* aboveground, VA – *Vaccinium* aboveground, EB – *Eriophorum* belowground, SB – *Sphagnum* belowground, VB - *Vaccinium* belowground (mean; ± SD; n = 4). The grey line indicates the aromaticity index, above which aromatic compounds prevail (mean, ± SD, n = 4).

fresh vascular plant litter did not produce much DOC, significant DOC leaching occurred relatively soon after the litters started to decompose. Both leaf litters were major DOC sources, with more leached from *Eriophorum* than *Vaccinium* leaves. Similar to the faster decay rates, both *Eriophorum* litters leached significant DOC amounts already after 20 days of decomposition, while this occurred later for *Vaccinium* leaves. DOC production was most likely connected with different nutrient content and chemical composition of the litters (Kaštovská et al., 2017).

Despite the species differences in litter composition (C, N, P content), decay rates and DOC production rates, the DOC leached from the litters did not significantly differ in the C and N distributions among the MW fractions. This finding contrasts with our hypothesis that leaf litter leachates will be richer in lower molecular weight compounds. Additionally, contrary to other studies (e.g. Kiikkila et al., 2006; Kiikkila et al., 2013), which reported a temporal decrease in the contributions of the < 1 kDa and > 100 kDa fractions during decomposition, we did not find a significant C redistribution among the MW fractions during decomposition for any litter type. This may suggest that microbes equally utilized organic compounds from all MW fractions and/or that the uptake of compounds from particular MW fractions is continuously compensated by the input of depolymerisation products from higher MW fractions. The high MW fractions are likely formed mainly by nucleic and humic acids, and supramolecular associations of small molecular mixtures of plant and microbial derived components (Sutton and Sposito, 2005; Hardie et al., 2007), which can be supplied by exoenzymatic cleavage of the insoluble litter itself. In summary, the simple characterization of DOC by the proportional C (N) distribution among the MW fractions cannot be used as a predictor of DOC or litter decomposability.

We further characterized compounds in the separated MW fractions by their aromaticity index. The DOC aromaticity index reflected well the litter content of lignin-like compounds, which increased in the order *Sphagnum* < *Eriophorum* < *Vaccinium* (Kaštovská et al., 2017), This index also differentiated between *Sphagnum* DOC, which had an aliphatic character (in all the fractions), and the more aromatic DOC originating from the vascular plant litters. The aliphatic character of *Sphagnum* DOC was also reported by Castells et al. (2005), Giudice and Lindo (2017) and Weishaar et al. (2003), who estimated that *Sphagnum* DOC contains less than 15% of aromatic compounds likely due to high concentrations of the pectin-like polysaccharide sphagnan (Hájek et al., 2011). In contrast, and in agreement with our results, *Vaccinium* was found to leach highly hydrophobic and aromatic DOC (Peuravuori and Pihlaja, 2007; Volk et al., 2002). We further detected temporal changes in the aromaticity of particular DOC fractions leached from litters in different decomposition stages, showing alterations in their compound composition. However, these changes were not systematic either in the fractions or the litters, which is in contrast to some other studies (e.g. Don and Kalbitz, 2005). In summary, similarly to the DOC distribution to MW fractions, the aromaticity index (of the fractions > 1 kDa MW) did not explain either the differences in biodegradability among the fresh litter DOC leachates or their decreasing biodegradability with ongoing decomposition.

Particular groups of identified LMW compounds in the leachates (per gram) were affected by litter type, with their content changing during incubation and some even dissappeared by the end of incubation. The increase of total organic acids (namely lactic, acetic (glycolic) and propionic acids) in the leachates (per gram of litter) indicate

Table 2
Decay rate (in % of present C day^{-1}) and biodegradability of DOC (in % of initial C, plateau of the model) leached from the decomposing litter after 0, 20, 70 and 200 days, estimated from leachate DOC loss during 42 days of incubation using a one-phase decay model. EA – *Eriophorum* aboveground, SA – *Sphagnum* aboveground, VA – *Vaccinium* aboveground, EB – *Eriophorum* belowground, SB – *Sphagnum* belowground, VB - *Vaccinium* belowground (mean; ± SD; n = 4). Different letters indicate significant differences (Tukey HSD) between litter types (in row).

Time		SA	SB	EA	EB	VA	VB
0 day	Decay rate	**7.68** ± 2.05a	**7.23** ± 0.02.3a	**20.00** ± 2,88b	**8.72** ± 1.43a	**8.09** ± 1.55a	**9.62** ± 01.53a
	Biodegradability	**21.7** ± 2.2a	**29.2** ± 3.3ab	**48.0** ± 1.1c	**38.3** ± 2.2bc	**44.1** ± 3.1bc	**40.2** ± 2.2bc
20 days	Decay rate	**18.30** ± 4.85a	**7.65** ± 3.86bc	**9.34** ± 2.36bc	**8.07** ± 3.02bc	**6.43** ± 0.63b	**12.57** ± 3.21ac
	Biodegradability	**10.9** ± 0.7a	**16.5** ± 2.3ad	**37.0** ± 2.7bcd	**23.7** ± 2.5ad	**47.2** ± 1.4c	**30.2** ± 2.3d
70 days	Decay rate	**6.65** ± 2.21a	**7.44** ± 2.53a	**10.86** ± 5.16a	**6.28** ± 2.4a	**5.26** ± 2.57a	**5.68** ± 2.40a
	Biodegradability	**19.4** ± 1.0a	**19.0** ± 2.2a	**41.1** ± 5.8a	**19.9** ± 2.7a	**42.3** ± 8.4a	**37.7** ± 5.9a
200 days	Decay rate	**4.84** ± 1.53a	**0.73** ± 0.71a	**9.57** ± 3.11a	**6.09** ± 1.05a	**6.59** ± 2.94a	**4.92** ± 1.00a
	Biodegradability	**11.8** ± 1.5a	23.3 ± 3.2%[a]	**16.8** ± 3.1a	**15.7** ± 1.0a	**20.5** ± 3.4a	**12.6** ± 1.1a

[a] DOC biodegradability for SB leached after 200 days of decomposition was not estimated using the model because the DOC loss rate remained linear during the 42 days of incubation. Therefore, the measured DOC loss was used instead.

occurring of fermentation processes). The presence of shikimic acid indicates synthesis of aromatic amino acids (Tzin et al. 2012) and monomeric and polymeric phenols (Lattanzio, 2013). Shikimic acids were produced more in *Vaccinium* and *Eriophorum* DOC due to aromatic precursors (compounds), which are more abundant in their litter than in *Sphagnum* litter.

The decreasing portion of identified LMW compounds in DOC till the end of incubation (from day 70 till 200) indicates that these compounds were decomposed and replaced by other compounds with low molecular weight. These could be simple aromatic compounds (like phenolic compounds) (Li et al., 2004; Richard et al., 2007; Kiikkila et al., 2012), oligopeptides and oligonucleotides, but we cannot show it by our data. The decrease in LMW compounds before the end of our experiment corresponded with decreased DOC biodegradability.

Our results (this experiment; Kaštovská et al., 2017) suggest nutrient (especially P) limitation of litter decomposition and soil microbial activities in peatlands, similarly as in many other works (Hill et al., 2014, Sottocornola et al., 2007 and others), In the present experiment, the highest C/P ratio of the *Sphagnum* litter corresponded well to the much higher C/SRP ratios of the *Sphagnum* leachates as compared to the vascular plants. This indicates that P availability could limit decomposition of *Sphagnum* litter and can contribute to the lower biodegradability of its leached DOC. In the case of the vascular plant litters, leaf litter leachates were always richer in SRP relative to C than those from the root litter of the respective species during the first stage of decomposition (first 70 days), which was mirrored in their higher DOC biodegradabilities (although the differences were not significant). Together with significant DOC leaching, both leaf litters, and especially the nutrient-richest *Eriophorum* leaf litter, released also significant amounts of mineral P and N, which could subsequently be available to plants and soil microbes in the surrounding litter. During the ongoing decomposition, from 70 to 200 days of incubation, the C/SRP and C/N ratios of litter leachates did not change significantly, or even decreased in some cases, while DOC biodegradability decreased for all four vascular plants litters. The low DOC biodegradability thus cannot be related to nutrient limitation and points again to the significant change in DOC chemistry, for example the marked decrease in the contribution of easily decomposable LMW compounds.

We suggest that the various P release from the decomposing litters is related to differences in the character of the P containing compounds in the litters. This was indicated by the very litter-type specific P distribution among the MW fractions in the litter leachates, differing between the *Sphagnum*, root and leaf litters of the vascular plants. Moreover, differently from the distribution of C and N among the MW fractions, P distribution was highly dynamic and reflected the ongoing litter decomposition. *Sphagnum* leachates differed from all other litters during the whole incubation. The P in the leachates from fresh *Sphagnum* was almost all associated with the highest MW fraction (> 100 kDa), which could be predominantly composed of DNA and its degradation products (Pietramellara et al., 2008). This could be related with the negligible SRP leaching from *Sphagnum* as shown by the extremely high C/SRP ratios of the leachates (> 5000). During the ongoing decomposition, a part of the P was firstly redistributed to the intermediate 10-100 kDa fraction (day 20) and later to the lowest one < 1 kDa (day 70 and 200) as a result of depolymerisation, hydrolysis and other chemical processes like precipitation with metals (Stutter et al., 2012). However, this P redistribution was not followed by an increase in SRP leaching. It is likely that co-precipitation (complexation) of P with Al and Fe could take place within the lowest MW fraction, indicated by the strong positive correlations between P with Fe and Al in < 1kDa fraction at the end of incubation. This co-precipitation could decrease the availability of P for microorganisms and DOC biodegradability, especially when DOC is co-precipitated with Al. Scheel et al. (2007) found that DOC-Al complexes are 6–10 times less degradable compared to non-precipitated DOC, while DOC co-precipitation with Fe did not form microbial inaccessible organic matter (Eusterhues et al., 2014). The co-precipitation of P with Al (and Fe) could also partially explain the very low biodegradability of DOC leached after 200 days of decomposition from EB, which had the highest proportion of Al in the < 1kDa fraction from all the litters at that time (see Table S5). Moreover, low P availability in this fraction is indicated by the negative correlation of the proportion of P in this fraction with the litter mineralization rate. In contrast, the leaves of vascular plants, both of which leached significant amounts of P during their decomposition, contain a substantial proportion of P-binding compounds in the 1-100 kDa fractions. In those fractions, P was positively related to DOC but not with metallic ions. Moreover, the proportion of P in the 10-100 kDa fraction was positively correlated with respiration rate, which indicates that P in this fraction was available for microbes. Moreover, the P rich EA litter was especially an important source of available P.

4.3. DOC biodegradability

DOC biodegradability was more strongly affected by the time of incubation (decomposition stage) than by litter origin. Among the DOC leached from fresh litters, only EA DOC decomposed much faster than the other litter leachates and was also significantly more degradable, although *Sphagnum* DOC tended to be less degradable than leachates from vascular plant litters and root litters were less degradable than the respective leaf leachates. At the end of the incubation, DOC biodegradability significantly decreased and became uniform for all litters. Similar results were also shown by both Don and Kalbitz (2005) for forest tree litter and Pinsonneault et al. (2016) with peatland plants. This could be ascribed to changes in DOC quality (in our case a decrease in the portion of easily degradable sugars, amino acids and organic acids in the DOC) and/or in the case of *Sphagnum* to the accumulation of compounds inhibiting heterotrophic microbial activities and P limitation. In our incubation, the biodegradability of DOC leached from litters after 200 days incubation ranged within 12–20%. These values are close to the values for biodegradabilities of DOC extracted from undisturbed peat cores from our study sites, which varied between 8 and 15% (unpublished results). Therefore, based on our results, the DOC leached from fresh litter may act as a source of easily available organic compounds and nutrients for the first two or three months of the decomposition process (depending on the rate of decomposition), while the litter leachates in the later stages of decomposition are much less concentrated and degradable. Still, the leaf litter of vascular plants in the later stage of decomposition (mainly that of *Eriophorum*) may still leach significant amounts of nutrients which then become available for microbes and plants.

4.4. Upscale to ecosystem level

Using our experimental data, we estimated potential DOC fluxes from the litters of the three studied plants to the soil and their effect on whole ecosystem functioning. For this estimation, we used the average primary production of *Vaccinium*, *Eriophorum*, and *Sphagnum* biomass measured at our study sites (Table 3; Edwards, unpublished data). Our measured biomass of *Sphagnum* was close to the average primary production for *Sphagnum fallax* published in Gunnarsson (2005). We further assumed that all *Vaccinium* leaves, but only 40% of *Eriophorum* leaves, senesce in the autumn (Wein, 1973), 50-90% of annual root production dies-back, and the whole annual *Sphagnum* primary production starts to decompose. To calculate the flush of DOC per area to a 10 cm depth (mg C m^{-2}) and its contribution to the DOC in the site (%), we used the portion of litter C transformed to DOC measured at day 20 (Table 3), a water volume of 100 litres in this soil layer and the DOC concentrations measured under each plant dominant *in situ* in our previous study (Table 3; Kaštovská et al., 2017).

Based on these calculations, only leaf litter of the vascular plants can leach significant amounts of DOC, which occurs mainly during the early stage of decomposition. The vascular plant and *Sphagnum* litters

Table 3
DOC fluxes and their contribution to the *in situ* soil DOC calculated for the litter of three studied plant dominants: *Sphagnum fallax,* EA – *Eriophorum* aboveground, VA – *Vaccinium* aboveground, EB – *Eriophorum* belowground, VB - *Vaccinium* belowground, (mean ± SD, n = 4).

	unit	Sphagnum fallax	Eriophorum vaginatum		Vaccinium myrtillus	
			EA	EB	VA	VB
Primary production	g DW m^{-2} $year^{-1}$	377	95.8	7.69	145	147
Primary production	g C m^{-2} $year^{-1}$	159	42.9	3.50	68.3	72.0
Portion of DOC leached from litter	% of total C	0.4	3.78	0.78	0.93	0.2
Potential litter DOC leached from litter	mg C L^{-1}	6.32	6.49	0.14–0.25	6.38	0.71–1.57
Potential litter DOC leached from litter	mg C L^{-1}	6.32	6.63–6.74		7.09–7.95	
Total soil porewater DOC in situ	mg C L^{-1}	73	67		92	
Contribution of litter DOC to soil porewater DOC	%	8.0–9.0	9.0–11.0		7.0–9.0	

could contribute 7–11% to soil DOC. We assume that the contributions could be even higher when litter (especially that of *Sphagnum*) will decompose under anaerobic conditions, because the percentage of litter transformed to DOC could be then higher (e.g. Kim et al., 2014). During the first decomposition stage, the vascular plant litters will produce highly degradable DOC and nutrient rich leachate, which will be important mainly in the autumn and during winter. *Sphagnum* DOC will be less degradable with very low nutrient contents (especially low in P), which can enter the soil during the whole vegetation season.

5. Conclusions

(i) The leaves of vascular plants (*Eriophorum vaginatum* and *Vaccinium myrtillus*) decomposed the fastest, releasing higher amounts of nutrients and easily degradable organic acids, sugars and amino acids to the leachate, compared to their roots and *Sphagnum*. *Eriophorum* leaf litter is a significantly larger DOC and nutrient source than *Vaccinium*.

(ii) DOC distribution to the four molecular weight fractions (< 1, 1-10, 10-100 and > 100 kDa) did not differ among the litters. The C/N/P stoichiometry of the leachates differed among the different litter types, reflecting their original C/N/P stoichiometry, but this was not directly related to DOC biodegradability. Nevertheless, the extremely high C/P ratio of the *Sphagnum* leachate indicates possible P limitation of litter as well as DOC decay. Neither the DOC distribution to the fractions nor leachate C/N/P stoichiometry were identified as important factors driving DOC biodegradability. On the contrary, total dissolved P distribution to the four molecular weight fractions significantly differed among the litters and likely affected SRP leaching from the litters. Especially *Sphagnum* was very specific: The P in fresh litter was associated with high molecular weight DOC which later was redistributed to lower molecular weight fractions and complexed with Al and Fe. The P complexation could retard SRP leaching, which was negligible especially in the case of *Sphagnum* litter.

(iii) DOC biodegradability was higher for vascular plant leaves than for *Sphagnum* litter in the early stages of decomposition (0-20 days), but later it decreased and became more uniform for all litters. The temporal differences related with litter decomposition stage were more pronounced than those caused by litter origin.

The leaf and to a much lesser extent root litters of the vascular plants can release significant amounts of DOC, which occurs mainly during the early stage of their decomposition. Compared to *Sphagnum*, the leachates from the vascular plants litter contain more aromatic DOC with higher biodegradability and more nutrients, especially P. Therefore, DOC leaching from decomposing vascular plants litter can temporarily but markedly contribute to soil DOC, change its quality, increase nutrient availability and stimulate heterotrophic microbial activities. Thanks to the patchy distribution of vascular plants in peatlands, litter DOC leaching may contribute to the spatial heterogeneity of peatland DOC concentrations, chemistry and microbial activity.

Acknowledgements:

Financial support was provided by Czech Science Foundation (13-17398S). The article was supported by project of MEYS (LM2015075, EF16_013/0001782) - SoWa Ecosystems Research. We thank Tomáš Hubáček, Terezia Říhová, Daniel Vaněk, Kateřina Kučerová, Iveta Chomová and Martina Petrlíková for technical support and chemical analyses.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.03.018.

References

Castells, E., Peñuelas, J., Valentine, D.W., 2005. Effects of plant leachates from four boreal understory species on soil N mineralization, and white spruce (*Picea glauca*) germination and seedling growth. Annals of Botany 95, 1247–1252.
Clark, J.M., Lane, S.N., Chapman, P.J., Adamson, J.K., 2008. Link between DOC in near surface peat and stream water in an upland catchment. The Science of the Total Environment 404, 308–315.
Don, A., Kalbitz, K., 2005. Amounts and degradability of dissolved organic carbon from foliar litter at different decomposition stages. Soil Biology and Biochemistry 37, 2171–2179.
Eusterhues, K., Neidhardt, J., Hadrich, A., Küsel, K., Totsche, K.U., 2014. Biodegradation of ferrihydrite-associated organic matter. Biogeochemistry 119, 45–50.
Giudice, R.D., Lindo, Z., 2017. Short-term leaching dynamics of three peatland plant species reveals how shifts in plant communities may affect decomposition processes. Geoderma 285, 110–116.
Gorham, E., 1991. Northern peatlands: role in the carbon cycle and probable responses to climatic warming. Ecological Applications 1, 182–195.
Gunnarsson, U., 2005. Global patterns of Sphagnum productivity. Journal of Bryology 27, 269–279.
Hájek, T., Balance, S., Limpens, J., Zijlstra, M., Verhoeven, J.T.A., 2011. Cell-wall polysaccharides play an important role in decay resistance of *Sphagnum* and actively depressed decomposition in vitro. Biogeochemistry 103, 45–57.
Hardie, A.G., Dynes, J.J., Kozak, L.M., Huang, P.M., 2007. Influence of polyphenols on the integrated polyphenol-maillard reaction humification pathway as catalysed by birnessite. Annals of Environmental Science 1, 91–110.
Hill, B.H., Elonen, C.M., Jicha, T.M., Kolka, R.K., Lehto, L.L.P., Sebestyen, S.D., Seifert-Monson, L.R., 2014. Ecoenzymatic stoichiometry and microbial processing of organic matter in northern bogs and fens reveals a common P limitation among peatland types. Biogeochemistry 120, 203–224.
Kaštovská, E., Straková, P., Edwards, K., Urbanová, Z., Bárta, J., Mastný, J., Šantrůčková, H., Picek, T., 2017. Cotton-grass and blueberry have opposite effect on peat characteristics and nutrient transformation in peatland. Ecosystems online ISSN: 1432-9840. https://doi.org/10.1007/s10021-017-0159-3.
Kiikkilä, O., Kitunen, V., Smolander, A., 2006. Dissolved organic matter from surface organic horizons under birch and conifers: degradation in relation to chemical characteristics. Soil Biology and Biochemistry 38, 737–746.
Kiikkilä, O., Kitunen, V., Smolander, A., 2012. Chemical and biological characterization of dissolved organic matter derived from Norway spruce litter divided into fractions according to molecular size. European Journal of Soil Biology 50, 109–111.
Kiikkilä, O., Smolander, V., Kitunen, V., 2013. Degradability, molecular weight and adsorption properties of dissolved organic carbon and nitrogen leached from different types of decomposing litter. Plant and Soil 373, 787–798.
Kim, Y., Ullah, S., Moore, T.R., Roulet, N.T., 2014. Dissolved organic carbon and total

dissolved nitrogen production by boreal soils and litter: the role of flooding, oxygen concentration, and temperature. Biogeochemistry 118 (1–3), 35–48.
Kopáček, J., Hejzlar, J., 1995. Semi-micro determination of total phosphorus in soils, sediments, and organic materials: a simplified perchloric acid digestion procedure. Communication in Soil Science and Plant Analysis 26 (11–12), 1935–1946.
Lattanzio, V., 2013. Phenolic compounds: introduction. In: Ramawat, K., Mérillon, J.M. (Eds.), Natural Products. Springer, Berlin, Heidelberg.
Leenheer, J.A., Croue, J.P., 2003. Characterizing aquatic dissolved organic matter. Environmental Science & Technology 37 (1), 18–26.
Li, L., Zhao, Z., Huang, W., Peng, P., Sheng, G., Fu, J., 2004. Characterization of humic acids fractionated by ultrafiltration. Ogranic Geochemistry 35, 1025–1037.
McDowell, W.H., Zsolnay, A., Aitkenhead-Peterson, J.A., Gregorich, E.G., Jones, D.L., Jodemann, D., Kalbitz, K., Marschner, B., Schwesig, D., 2006. A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. Soil Biology and Biochemistry 38, 1933–1942.
Moore, T.R., Dalva, M., 2001. Some controls on the release of dissolved organic carbon by plant tissues and soils. Soil Science 166, 38–47.
Painter, T.J., 1991. Lindow man, Tollund man and other peat-bog bodies—the preservative and antimicrobial action of sphagnan, a reactive glycuronoglycan with tanning and sequestering properties. Carbohydrate Polymers 15, 123–142.
Peuravuori, J., Pihlaja, K., 2007. Characterization of freshwater humic matter. In: Nollet, L.M.L. (Ed.), Handbook of Water Analysis. CRC Press, Boca Raton, London, New York, pp. 435–448.
Pietramellara, G., Ascher, J., Borgogni, F., Ceccherini, M.T., Guerri, G., Nannipieri, P., 2008. Extracellular DNA in soil and sediment: fate and ecological relevance. Biology and Fertility of Soils 45, 219–235.
Pinsonneault, A.J., Moore, T.R., Roulet, N.T., Lapierre, J.-L., Pinsonneault, A.J., Moore, T.R., Roulet, N.T., Lapierre, J.F., 2016. Biodegradability of vegetation-derived dissolved organic carbon in a cool temperate ombrotrophic bog. Ecosystems 19 (6), 1023–1036.
Richard, C., Guyot, G., Rivaton, A., Trubetskaya, O., Trubetskoj, O., Cavani, L., Ciavatta, C., 2007. Spectroscopic approach for elucidation of structural peculiarities of Andisol soil humic acid fractionated by SEC-PAGE setup. Geoderma 142, 210–216.
Robroek, B.J.M., Albrecht, R.J.H., Hamard, S., Pulgarin, A., Bragazza, L., Buttler, A., Jassey, V.E.J., 2016. Peatland vascular plant functional types affect dissolved organic matter chemistry. Plant and Soil 407 (1–2), 135–143.
Rousk, J., Jones, D.L., 2010. Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in H_2O and 0.5 M K_2SO_4 soil extracts. Soil Biology and Biochemistry 42, 2331–2335.
Scheel, T., Dorfler, C., Kalbitz, K., 2007. Precipitation of dissolved organic matter by aluminium stabilizes carbon in acidic forest soils. Soil Science Society of America Journal 71, 64–74.
Soong, J.L., Calderon, F.J., Betzen, J., Cotrufo, M.F., 2014. Quantification and FTIR characterization of dissolved organic carbon and total dissolved nitrogen leached from litter: a comparison of methods across litter types. Plant and Soil 385, 125–137.
Sottocornola, M., Boudreau, S., Rochefort, L., 2007. Peat bog restoration: effect of phosphorus on plant re-establishment. Ecological Engineering 31, 29–40.
Stalheim, T., Balance, S., Christensen, B.E., Granum, P.E., 2009. Sphagnan - a pectin-like polymer isolated from Sphagnum moss can inhibit the growth of some typical food spoilage and food poisoning bacteria by lowering the pH. Journal of Applied Microbiology 106, 967–976.
Sutton, R., Sposito, G., 2005. Molecular structure in soil humic substances: the new view. Environmental Science & Technology 39 (23), 9009–9015.
Stutter, M.I., Shand, C.A., George, T.S., Blackwell, M.S.A., Bol, R., MacKay, R.L., Richardson, E.A., Condron, L.M., Turner, B.L., Haygarth, P.M., 2012. Recovering phosphorus from soil: a root solution? Environmental & Science Technology 46, 1977–1978.
Tfaily, M.M., Hamdan, R., Corbett, J.E., Chanton, J.P., Glaser, P.H., Cooper, W., 2013. Investigating dissolved organic matter decomposition in northern peatlands using complimentary analytical techiques. Geochimica et Cosmochimica Acta 112, 116–129.
Thacker, S.A., Tipping, E., Gondar, D., Baker, A., 2008. Functional properties of DOM in a stream draining peat. The Science of the Total Environment 407, 566–573.
Tzin, V., Galili, G., Aharoni, A., 2012. Shikimate Pathway and Aromatic Amino Acid Biosynthesis. eLS. John Wiley & Sons Ltd, Chichester.
Verhoeven, J.T.A., Liefveld, W.M., 1997. The ecological significance of organochemical compounds in *Sphagnum*. Acta Botanica Neerlandica 46, 117–130.
Verhoeven, J.T.A., Toth, E., 1995. Decomposition of *Carex* and *Sphagnum* litter in fens: effect of litter quality and inhibition by living tissue homogenates. Soil Biology and Biochemistry 27, 271–275.
Volk, C., Wood, L., Johnson, B., Robinson, J., Zhu, H.W., Kaplan, L., 2002. Monitoring dissolved organic carbon in surface and drinking waters. Journal of Environmental Monitoring 4, 43–47.
Wein, R.W., 1973. Biological flora of the british Isles:*Eriophorum vaginatum* L. Journal of Ecology 61, 601–615.
Weishaar, J.L., Aiken, G.R., Bergamaschii, B.A., Fram, M.S., Fugii, R., Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science & Technology 37, 4702–4708.
Wickland, K.P., Neff, J., Aiken, G.R., 2007. Dissolved organic carbon in Alaskan boreal forest: sources, chemical characteristics, and biodegradability. Ecosystems 10, 1323–1340.
Worrall, F., Reed, M., Warburton, J., Burt, T., 2003. Carbon budget for a British upland peat catchment. The Science of the Total Environment 312, 133–146.

Paper IV

Microorganisms responsible for priming effect on peatland DOC decomposition induced by root exudates: an incubation study

Mastný J., Kaštovská E., Bárta J., Picek T.

2019

Manuscript

Microorganisms responsible for priming effect on peatland DOC decomposition induced by root exudates: an incubation study

Jiří Mastný[1], Jiří Bárta[1], Eva Kaštovská[1], Tomáš Picek[1]

1 Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, Branišovská 1760, České Budějovice 37005, Czech Republic

Keywords: priming effect, peatland, DOC, r-strategic bacteria, microbial community, microbial functions

One sentence summary: This study demonstrates the effect of root exudates addition on decomposition of recalcitrant dissolved organic carbon and on microbial community composition, dynamics and functions.

Abstract

A positive priming effect (PE) on microbial decomposition of organic matter in peatland ecosystems may become an important phenomenon in the near future after the expected plant community composition shift from *Sphagnum* to vascular plants with climate change with increased root exudates input to the soil. The aim of our study was to evaluate the effect of root exudates on recalcitrant dissolved organic carbon (DOC) decomposition and to identify the microorganisms responsible for this process. We incubated peatland recalcitrant DOC under laboratory conditions together with a mixture of organic compounds simulating root exudate addition, in two additions (2% or 5% of present DOC) and differing in C/N stoichiometry (C/N 7, 25, 50). Stable isotope technique (addition of ^{13}C labelled compounds) was used to evaluate PE, the microbial community composition and quantity was accessed by targeted gene sequencing and quantitative PCR (qPCR), respectively. The low level of root exudates addition caused negative PE (from -7.9 to -1.2 %) whereas positive PE (from 3.2 to 19.9 %) occurred with high level of root exudates and with increasing C/N ratio. The microbial community was limited by nutrients resulting in increased "microbial nutrient mining". R-strategic bacteria were probably the more important group causing the positive PE than K-strategists. High level of added root exudates stimulated microbial functional groups with potential to decompose complex compounds. Detailed analysis of the microbial community revealed several genera with distinct effects on positive PE. After root exudates addition, *Burkholderia*, *Pseudomonas* and *Mucilaginibacter* were the most important groups of r-strategic bacteria later followed by the K-strategists *Bryocella* and *Candidatus Solibacter.*

Introduction

In the peatlands, decomposition of soil organic carbon (SOC) is restricted as the result of anoxic conditions, low pH, nutrient limitation and low decomposability of the plant material. Consequently, peatland dissolved organic carbon (DOC) is also hardly decomposable and is thus referred as recalcitrant (Tfaily et al., 2013) and peatlands ecosystems thus represent a substantial source of DOC to surface water (Clark et al., 2008; Thacker et al., 2008). Vascular plants and mosses release from their roots and steles variety of organic compounds including low molecular weight and easily decomposable exudates, such as organic acids, carbohydrates and amino acids (Jones et al., 2009). Input of such compounds stimulates microbial growth and activity, including a production of extracellular enzymes, which may accelerate decomposition of peat and peatland DOC – a phenomenon called positive priming effect (PE) (van Huisstede et al., 2006; Lu and Conrad, 2005). Positive PE has been reported in peatland ecosystems (Hamer and Marschner 2002; Basiliko et al., 2012), although its significance is considered as minor (Basiliko et al., 2012) or unclear (Hamer and Marschner, 2002). However, plant community composition on peatlands shifts from *Sphagnum* to vascular plants (graminoids and ericoids) due to ongoing climate change (Weltzin et al., 2000; Buttler et al., 2015; Dieleman et al., 2015). Therefore, the addition of decomposable root exudates will increase and a role of positive PE in dynamics of peatland C cycle and DOC export may become more important (Frolking et al., 2002).

Because of the microaerophilic or anaerobic nature of peatlands, several studies showed that bacteria dominate in the microbial community (Myers et al., 2012; Winsborough and Basiliko, 2010), while fungi are highly suppressed, and their abundance is usually increases only in oxic microsites. *Sphagnum* mosses are the most common vegetation usually accompanied by ericoid shrubs and graminoids. Edwards et al. (2018) described the species-specific and seasonal changes in quantity and quality of soluble rhizodeposites of *Vaccinium myrtillus* (ericoid), *Eriophorum vaginatum* (graminoid) and *Sphagnum fallax*. The highest production of exudates was found for *Eriophorum*, lower for *Vaccinium* and the lowest for *Sphagnum.* Generally, the low-molecular exudates were dominated by organic acid with much lower contribution of sugars and amino acids and their C/N ratio ranged from 8 to 80 depending on plant and vegetation season. Generally, *Sphagnum* exudates had higher C/N ratios compare to exudates from *Eriophorum* and *Vaccinium,* which contained more organic and inorganic N, and exudate C/N ratio of all species increased from spring to autumn. Root exudation of vascular plants contributed from 1 to 5 % to the peatland DOC (Edwards et al., 2018).

The addition of exudates to the soil is mostly shown to stimulate decomposition of pre-existing organic matter, causing a positive PE. However, negative or no PE can also occur. A meta-analysis of data from terrestrial ecosystems (Zhu et al., 2014) showed that addition of organic compounds can do both suppress SOC decomposition rate (up to 50 %) and stimulate it (up to 400 %). Similarly, a modelled PE of simple organic compounds on recalcitrant DOC in aquatic ecosystems can range from -130% to +370% (Hotchkiss et al., 2014). The response to addition of easily decomposable compounds significantly varies among systems. However, it can be generalized that the extent and direction of induced PE is influenced by the amount and type of entering organic compounds (Blagodatskaya and Kuzyakov, 2008; Guenet et al., 2010). Generally, a low level of easily decomposable compounds addition rather leads to negative or no PE, while the high labile C addition may induce strong positive priming (Liu et al., 2017). Further, it is known that organic acids addition stimulate SOC decomposition more than addition of carbohydrates (Shi et al., 2011). Besides organic acids and carbohydrates, root exudates also contain N in amino acids (Grayston et al., 1997; Bolton et al., 1992) and inorganic forms (Wardle and Greenfield, 1991). After root exudates addition, growing microbial biomass has enhanced N and P demand (Chen et al., 2014; Miao et al., 2017), which may lead to "nutrient mining" from native SOC and DOC (Chen et al., 2014; Fang et al., 2018). Accordingly, larger PE was found with high C/N ratio of exudates (Qiao et al., 2016) and under N limitation in the soil (Fontaine et al., 2011) compare to replete N conditions. Therefore, high C/N ratio of root exudates may lead to increased PE due to "nutrient mining" from native DOC and such phenomena could play an important role in nutrient limited peatland ecosystems.

The root exudates addition influences the composition and functioning of the rhizosphere microbial community. First it stimulates the growth of r-strategists (Kuzyakov, 2010), commonly Gram-negative bacteria such as *Gammaproteobacteria* and *Bacteriodetes* but also fungi (Wild et al., 2014). Due to a fast growth, this population requires sufficient amounts of N and P, which will be immobilized in its biomass (Elser et al., 1996; Makino et al., 2003). After depletion of easily degradable compounds, r-strategists die-back and their necromass serves as a substrate for a development of K-strategic population (contributed e.g. by *Acidobacteria* and *Planctomycetes*) (Schimel and Schaeffer, 2012; Allison et al., 2010). The stimulated enzymatic production of these microbes may lead to a targeted or co-metabolic decomposition of pre-existing, more stable SOC and finally result in positive PE (Fontaine et al., 2003; Blagodatskaya et al., 2014). The composition of microbial community actively utilizing the substrates, its succession and a potential contribution of particular bacterial and fungal representatives to PE

will depend not only on the quantity and quality of entering exudates but also on initial composition of microbial community (Garcia-Pausas and Paterson, 2011).

In this study, we incubated peatland water with original recalcitrant DOC under laboratory conditions together with a mixture of organic compounds simulating root exudate addition. The exudate addition differed in quantity (2% or 5% of present DOC) and in the C/N stoichiometry (C/N ratios 7, 25, 50) simulating different scenarios of root exudation of peatland plant species during vegetation season. We aimed to (i) evaluate the effect of root exudates on recalcitrant DOC decomposition with focus on its dynamics and resulting PE and (ii) identify the microorganisms responsible for this process.

Based on the literature survey we hypothesized:

(i) Addition of exudates will stimulate decomposition of recalcitrant peatland DOC resulting in a positive PE. The positive PE will increase with increasing level of added exudates and their increasing C/N ratio.

(ii) Addition of exudates to DOC will lead to successional changes in the present microbial community: Initially, the presence of fresh exudates will increase the abundance and proportion of r-strategic species, while later the proportion of K-strategist will increase albeit the community will be smaller. Namely, K-strategic species with a larger potential to produce extracellular enzymes will be responsible for the positive PE.

Methods

Peatland water collection and preparation

Peatland water originated from a spruce swamp forest located in the Šumava Mountains southwest Czech Republic (49°1’28.04”N, 13°32’32.14”E) and it was collected in October 2015. The water was filtered through the low-protein-binding Express PLUS Polyethersulfone membrane (GPWP) with a 0.22 µm pore size (Merck Millipore Ltd., Ireland). The filtrate was analysed for dissolved organic C (DOC) (60 mg L^{-1}) on a LiquiTOC II (Elementar, Germany) and for pH (pH = 4).

Experimental design of incubation

Peatland water was incubated in 120 ml glass NTS vials. The control treatment consisted of the 75 ml of the filtrated peatland water with the final DOC concentration of 60 mg C L^{-1}. In experimental treatments, the peatland water was enriched by a mixture of simple organic compounds with different molar C/N ratio

simulating root exudates. The basic mixture of artificial root exudates with the molar C/N ratio of 50:1 consisted of acetic acid, glucose and glutamic acid (^{13}C labelled compounds, 99 at. % of ^{13}C, Sigma Aldrich), which contributed to the total C in the mixture by 75 %, 15 % and 10 %, respectively (Drake et al., 2013; Eiland et al., 2001; Edwards et al., 2018). The molar C/N ratios of 25:1 and 7:1 were adjusted by an addition of ammonium nitrate to the basic mixture of organics. The artificial root exudates were added to the peatland water in two concentrations representing 2 % and 5 % of the recalcitrant DOC concentration in four replicates. Then the samples were inoculated by 0.5 ml of unfiltered supernatant prepared from the peat sampled at the same locality as the peatland water (10 g of peat shaken in 100 ml of distilled water for 1 h at 20°C, and centrifuged at 1000 g for 5 minutes). After inoculation the vials were air-tightly closed with rubber stoppers and incubated on the roll-and-roll shaker at 20°C for 25 days.

At time 0, 4, 11 and 25 days after start of the incubation the solution was taken from each sample, filtered through the low-protein-binding Express PLUS Polyethersulfone membrane (GPWP) with a 0.22 µm pore size (Merck Millipore Ltd., Ireland)) and immediately analysed for soluble reactive P (SRP), ammonium N and nitrate N collorimetrically on flow injection analyser (FIA Lachat QC8500, Lachat Instruments, USA).

Respiratory C losses from the samples and priming effect

The CO_2 and O_2 concentration in the headspace of each flask was measured at 1, 2, 3, 4, 7, 11, 18 and 25 days of incubation using a HP 6850 gas chromatograph (Agilent, USA). After each measurement, the flasks were opened, ventilated and closed again. The CO_2 data were used to calculate respiration rates and cumulative respiratory losses for each treatment, which were used as a proxy for decomposition losses.

At days 1, 4, 11 and 25, isotopic composition of the evolved CO_2 was analysed by Gasbench II (Finnigan, Germany) connected with IRMS Delta X Plus (Finnigen, Germany). To calculate the amount of CO_2 derived from exudates and pre-existing DOC, the following mass balance equations based on atom % were used:

$$C_T\delta_T = C_{EX}\delta_{EX} + C_{DOC}\delta_{DOC}$$

where C_T ($C_T = C_{EX} + C_{DOC}$) is the total amount of CO_2 during the considered time interval, δ_T is the corresponding isotopic composition, C_{EX} is the amount of CO_2 derived from the added exudates, δ_R is its isotopic composition in the exudates, C_{DOC} is the amount of CO_2 derived from DOC and δ_{DOC} is its isotopic composition.

The primed DOC-derived CO_2 is the difference between the C_{DOC} of exudate-amended samples and C-CO_2 efflux from control samples ($C_{control}$). The extent of PE was expressed as relative (%) to respiration rate of control at particular sampling time.

$$PE\ (\%) = 100 \times (C_{DOC} \text{ in exudate-amended samples} - C_{control}) / C_{control}$$

Nucleic acid extraction and quantification

10 ml of peatland water were filtered through the low-protein-binding Express PLUS Polyethersulfone membrane (MILLGPWP) with a 0.22 µm pore size (Merck Millipore Ltd., Ireland). Filters were kept frozen until nucleic acid (DNA) extraction. Filters were cut into the small pieces to fit bead-beating tube. DNA was extracted from the filters according to modified bead-beating protocol (Urich et al., 2008). Total DNA was quantified fluorometrically using Quantus fluorometer (Promega, USA) with Quantus DNA Start-Up Kit. Quality of DNA was also verified by agarose gel electrophoresis.

DNA sequencing and microbial community analyses

The aliquots of DNA extracts were sent to SEQme company (Czech republic) for the preparation of a library and sequencing using HiSeq2500 platform. The Earth Microbiome Project (EMP) protocol was used for library preparation with modified universal primers 515FB/806RB (Caporaso et al., 2011) and ITS1F/ITS2 (Gardes and Bruns, 1993) for prokaryotic 16S rDNA and fungal ITS1 amplicons, respectively. Bacterial 16SrDNA raw pair-end reads (150 bp) were joined and quality filtered using USEARCH v. 10.0.240 to obtain reads of approx. 250bp length (Edgar, 2013). The fungal ITS1 region was extracted from reads using ITSx algorithm (Bengtsson-Palme et al., 2013). Both 16S and ITS1 amplicons were trimmed to equal lengths. Bacterial and fungal unique reads were grouped to zero-radius OTUS (zOTUs) using an UNOISE 3.0 algorithm, which includes also the removal of potential chimeric sequences (Edgar, 2013). Taxonomic assignment of each bacterial and fungal zOTU was done using blast algorithm (e-value = 0.001) and curated ARB Silva 132 database (Quast et al., 2013) and UNITE v 7.2 (Koljalg et al., 2013). Raw sequencing data were deposited in European Nucleotide Archive (ENA) under the study ID PRJEB29666.

Quantification of prokaryotic and eukaryotic microbial community

Quantification of bacterial, archaeal and fungal SSU rRNA genes was performed using the FastStart SybrGREEN Roche® Supermix and Step One system (Life Technologies, USA). Each reaction mixture (20 µl) contained 2 µl DNA template (~1-2 ng DNA), 1 µl each primer (0.5 pmol µl-1 each, final concentration), 6 µl dH2O,

10 µl FastStart SybrGREEN Roche® Supermix (Roche, France) and 1 µl BSA (Fermentas, 20 mg μl^{-1}). Initial denaturation (3 min, 95°C) was followed by 30 cycles of 30s at 95°C, 30s at 62°C (bacteria), 15s at 72°C, and completed by fluorescence data acquisition at 80°C used for target quantification. Product specificity was confirmed by melting point analysis (52°C to 95°C with a plate read every 0.5°C) and amplicon size was verified with agarose gel electrophoresis. Bacterial and archaeal DNA standards consisted of a dilution series (ranging from 10^1 to 10^9 gene copies μl^{-1}) of a known amount of purified PCR product obtained from genomic *Escherichia coli* ATCC 9637 DNA by using the SSU gene-specific primers 341F/534R (Muyzer et al., 1993). R^2 values for the standard curves were > 0.99. Slope values were > 3.37 giving an estimated amplification efficiency of > 93%. The qPCR conditions for fungal quantification were as follows: initial denaturation (10 min, 95°C) followed by 40 cycles of 1 min at 95°C, 1 min at 56°C, 1 min at 72°C, and completed by fluorescence data acquisition at 72°C used for target quantification. Fungal DNA standards consisted of a dilution series (ranging from 10^1 to 10^7 gene copies μl^{-1}) of a known amount of purified PCR product obtained from genomic *Aspergillus niger* DNA by using the SSU gene-specific primers nu-SSU-0817-5' and nu-SSU1196-3' (Borneman and Hartin, 2000). R^2 values for the fungal standard curves were > 0.99. The slope was between -3.32 to -3.5 giving estimated amplification efficiency between 97 and 91%, respectively. Detection limits for the various assays (i.e. lowest standard concentration that is significantly different from the non-template controls) were less than 100 gene copies for each of the genes per assay. Samples, standards and non-template controls were run in duplicates.

Analyses of metabolic potential of the prokaryotic community

Two independent pipelines (PICRUSt, FAPROTAX) were used for in-silico prediction of the functional potential of the prokaryotic community. For PICRUSt (Langille et al., 2013) and FAPROTAX (Louca et al., 2016) analyses the rarified OTU tables to 2000 sequences generated by Qiime 1.9.1 were used with taxonomic classification based on GreenGenes database ver. 13.05. In general, the PICRUSt pipeline first normalized each OTU abundances by small ribosomal subunit gene (SSU) copy variation in bacterial genomes based on the most similar taxa. The resulting normalized table was then used for OTU functional annotation using known bacterial and archaeal genomes (Langille et al., 2013). From the normalized OTU table we were additionally able to calculate community average genome SSU copy number (ACN) in each sample (Thompson et al., 2017). The ACN was calculated from the raw and normalized OUT table (1^{st} step in PICRUSt pipeline). SSU gene copies range from 1 to 15 in microbial genomes. Copiotrophic microbes are assumed

to have more SSU gene copies in genome, therefore the higher average ACN shows the higher proportion of the copiotrophic taxa in the microbial community.

Statistical analyses

The effects of exudates additions, their C/N ratios and time on each parameter (respiration rate, priming effect, nutrient concentrations, bacterial and fungal abundance) were tested by full-factorial repeated–measures ANOVA (Statistica 12, Dell, USA) followed by post hoc comparison (unequal N HSD test). The significant differences (Welchs t-test, two-sided, Bonferroni correction) of phyla and genera between different treatments and control were analysed in STAMP v2.1.3 (Parks et al. 2014).

Results

Total respiration rates and priming effect

Samples with exudate addition had initially lower total respiration rates than control (original peatland water) (Fig. 1). However, from day 2, the enriched samples respired significantly more than control. The stimulation effect of exudate addition on total respiration rate was higher and lasted longer for 5% exudates level than for 2% level. Specifically, samples with 2% exudate level respired more than control only till day 4 (Fig. 1a), while samples with 5% exudates level till day 11 (Fig. 1b). The exudate C/N ratio did not affect total respiration rate in any treatment and on any time. After 25 days incubation, from 17.1 to 23.5% of total C (DOC + exudate C) were transformed to CO_2.

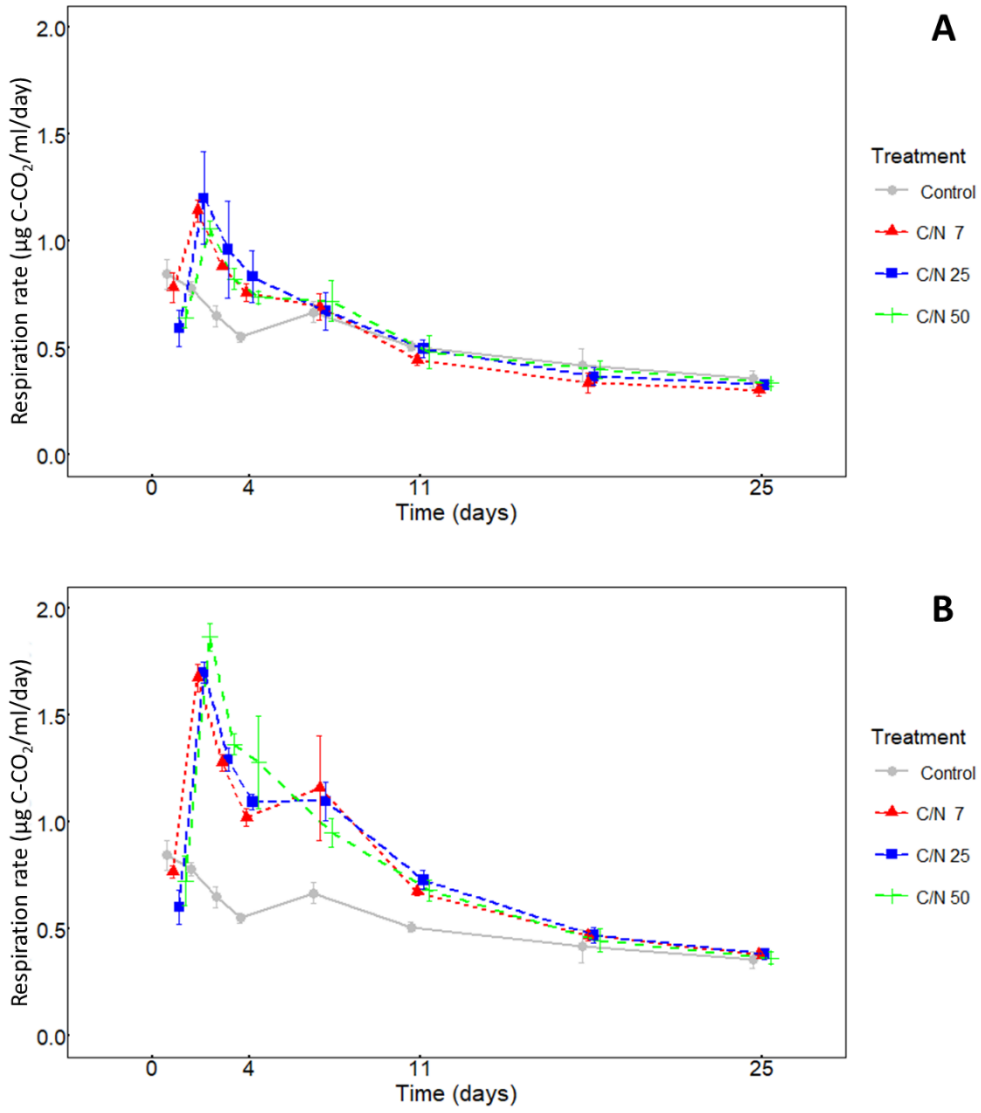


Figure 1: Respiration rate of control samples and samples amended by artificial exudates with different C/N ratios **A/** in concentration of 2 % of total DOC and **B/** in concentration of 5 % of total DOC during 25 days incubation (means, ± standard deviations, n=4).

The priming effect (PE) on DOC induced by exudate addition was dynamic in term of time. The addition of exudates at both concentrations initially induced negative PE (Fig. 2 a, b) till day 4, whereas later between day 4 and 11, the PE turned to significantly positive for 5% exudate level ($p<0.001$). The exudates with C/N ratio of

50 induced larger positive priming as compared to the exudates with lower C/N on the day 11 ($p<0.01$) and only that one remained positive also on the day 25 ($p<0.05$) (Fig. 2 b). Differently, no PE or slightly negative was measured for 2% exudate addition till the end of experiment, with no effect of exudate C/N ratio on PE (Fig. 2 a). Final PE ranged from -7.9 to -1.2% for low exudates level while the PE from 3.2 to 19.9% was measured for high exudates level. Significantly higher PE values were found for C/N ratio 50 than 7 for high level of exudates addition.

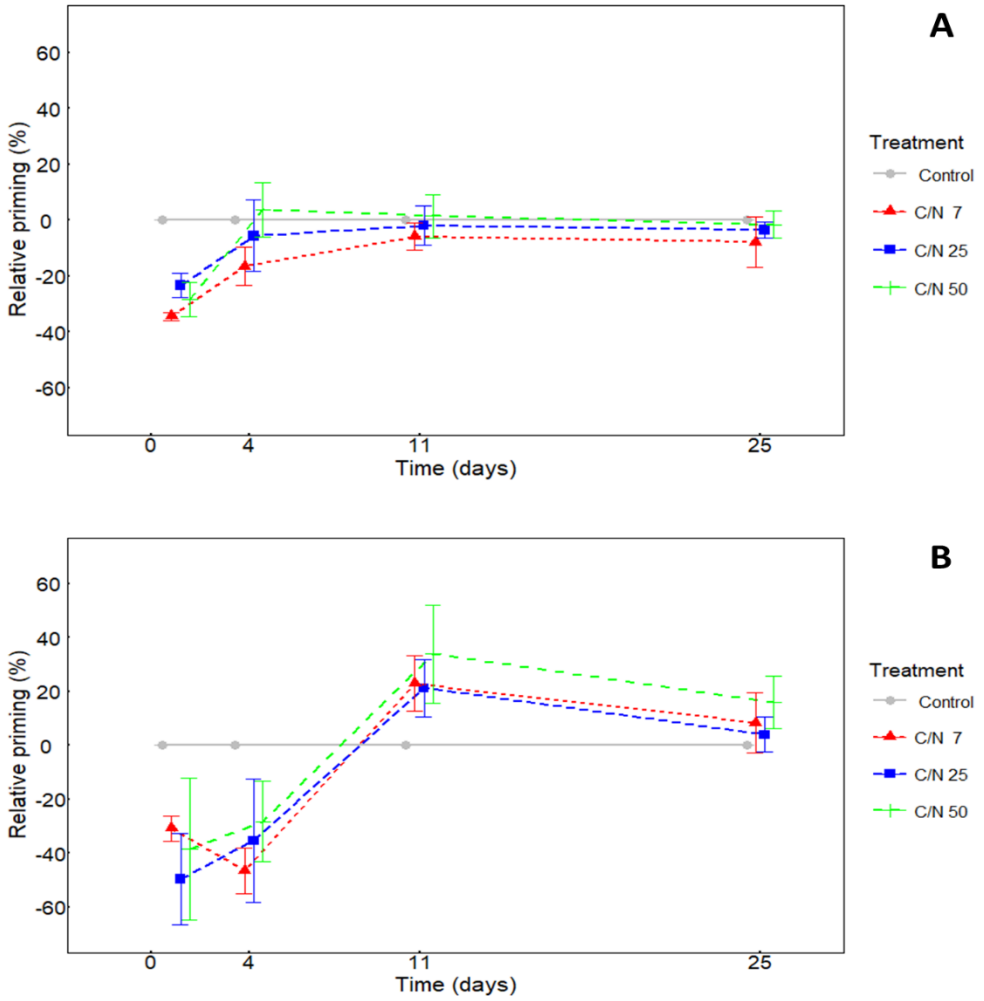


Figure 2: Relative priming effect on DOC induced by artificial exudates with different C/N ratios **A/** in concentration of 2 % of total DOC and **B/** in concentration of 5 % of total DOC during 25 days incubation (means, ± standard deviations, n=4).

Nutrient concentrations as affected by exudates addition

Concentration of ammonium N in soil solution of samples enriched with exudates significantly decreased during first four days (except 2% exudates with C/N ratio 7) ($p<0.001$) and then slowly increased back close to its original levels till day 25. Similar dynamics of ammonium N occurred also in the control but the changes were much less pronounced and the decrease lasted till day 11 (Fig. 3 a, b). Differently, no significant changes were found for nitrate (Fig. S2 a,b). Soluble reactive phosphorus (SRP) concentration decreased in time ($p<0.001$), with the fastest decline between day 0 and 4, followed by relatively stable phase between day 4-11 and another but slower decrease till the end of incubation (Fig. 4 a, b). The decline of SRP in the solution was more pronounced under 5% than 2% level of exudates addition ($p<0.001$). The SRP declined also in controls but the decline was stable, without an initial sharp decrease. After 25 days of the incubation, samples with 5% exudates had still significantly lower SRP concentration than control, while those with 2% exudates did not differ from the control ($p<0.01$).

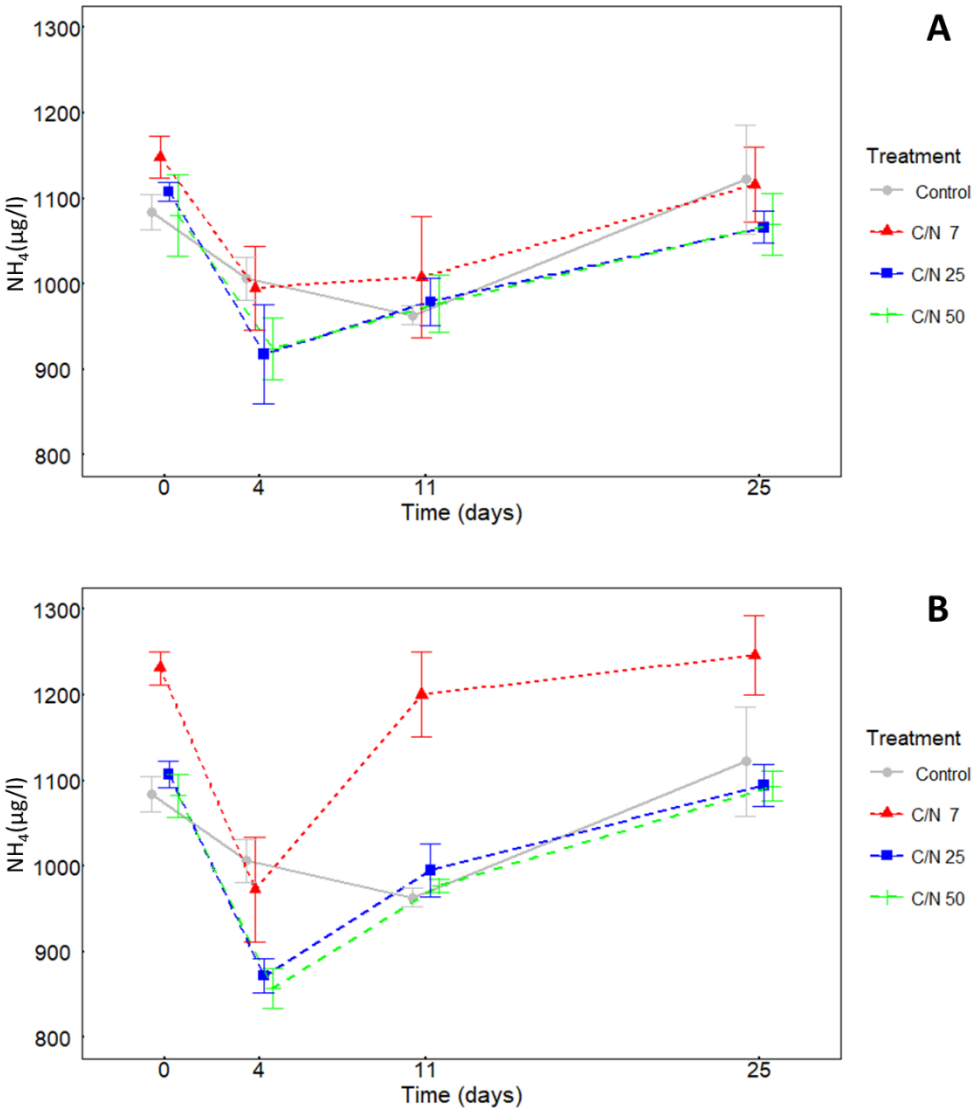


Figure 3: Ammonium N concentration in control samples and samples amended by artificial exudates with different C/N ratios **A/** in concentration of 2 % of total DOC and **B/** in concentration of 5 % of total DOC during 25 days incubation (means, ± standard deviations, n=4).

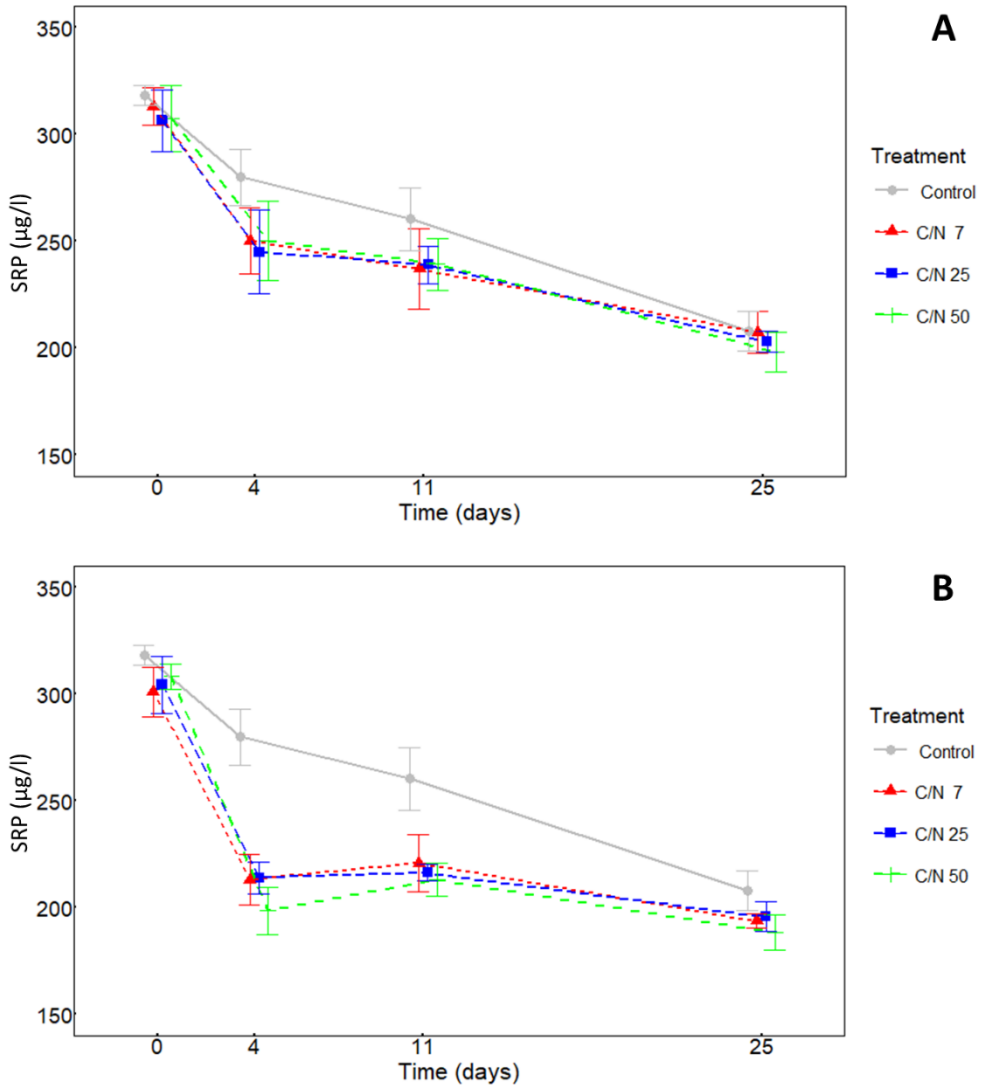


Figure 4: Soluble reactive phosphorus (SRP) concentration in control samples and samples amended by artificial exudates with different C/N ratios **A/** in concentration of 2 % of total DOC and **B/** in concentration of 5 % of total DOC during 25 days incubation (means, ± standard deviations, n=4).

Temporal changes in abundance and composition of microbial communities

Generally, microbial community in peatland water was dominated by bacteria. Fungi formed minor part of microbial community, which is shown by a low fungi to bacteria ratio (F/B ratio), which ranged from $1.15*10^{-5}$ to 0.026 (Fig. S3 a,

b). Further, bacterial abundance was more dynamic than fungal one after exudate addition. Abundance of bacterial gene increased during the first 4 days of incubation (Fig. 5). This growth was followed by a significant decrease till day 11 for 5% level addition (Fig. 5b). For 2% level addition, the bacterial abundance was similarly dynamic only for exudates with C/N 50, while they did not change significantly for other exudate C/N ratios (Fig. 5a). Fungal abundance increased from day 11 till day 25 and, therefore, the F/B ratio increased compare to original conditions ($p<0.05$) (Fig. 5 a, b). Finally, samples with both 2% and 5% exudate addition had higher F/B ratio than control and, additionally, those enriched with exudates with C/N ratio of 7 had higher F/B ratios than other treatments ($p<0.01$). The changes of bacterial and fungal community in time are given in Fig. 6 (control is shown in Fig. S4).

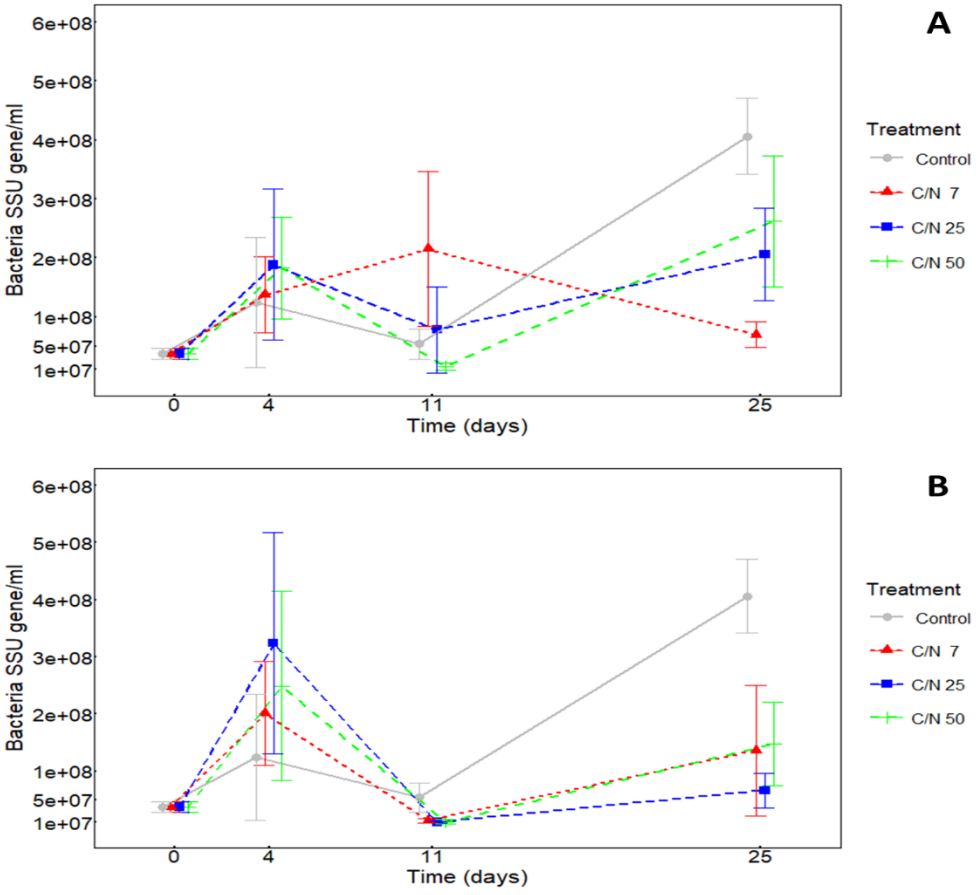


Figure 5: Bacteria SSU gene (per ml) in control samples and samples amended by artificial exudates with different C/N ratios **A/** in concentration of 2 % of total DOC and **B/** in concentration of 5 % of total DOC during 25 days incubation (means, ± standard deviations, n=4)´

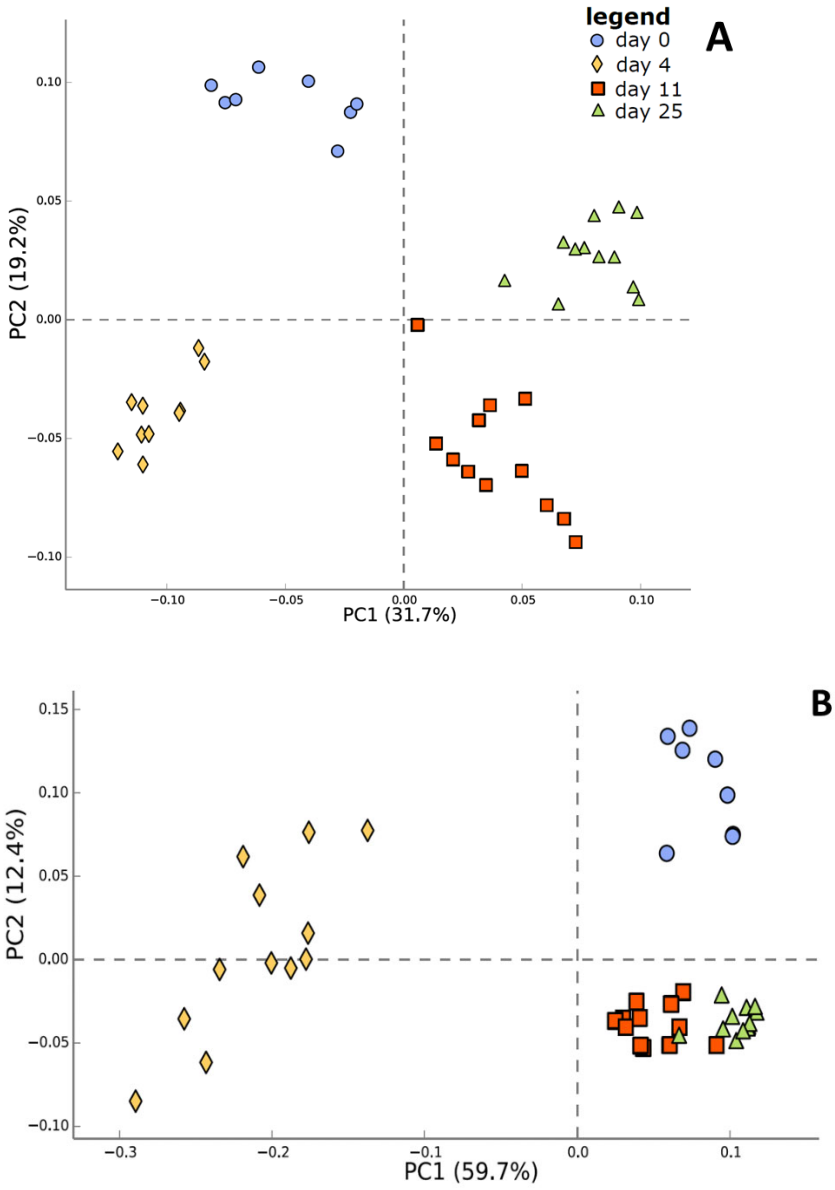
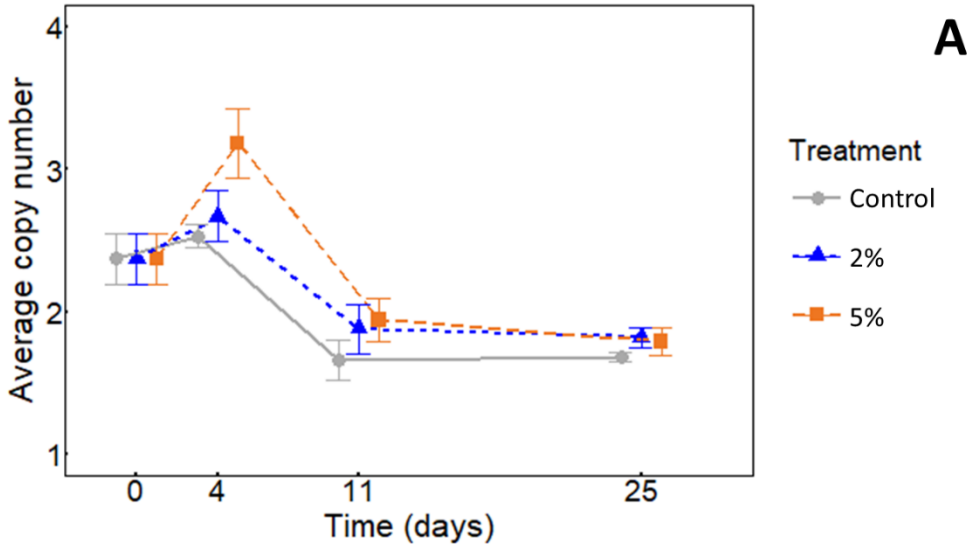


Figure 6: Distribution of bacterial and fungal community in time in the samples amended by artificial exudates **A/** in concentration of 2 % of total DOC and **B/** in concentration of 5 % of total DOC during 25 days incubation. (n=4).

Temporal changes of r- and K-strategist of prokaryotic community

Average 16SrRNA gene copy number per genome (ACN) can be used as the proxy for characterizing the r- or K-strategy nature of bacterial community, as higher average ACN shows the higher proportion of the copiotrophic taxa. High level of exudates addition significantly rise ACN in the bacterial community till the day 4 (Fig. 7 a b c), which indicates increased abundance of r-strategic species. Till 11 days, the ACN decreased, but was still significantly higher than control ($p<0.05$), while later on ACN did not differ among treatments. The ACN was negatively correlated with SRP concentration and positively correlated with respiration rate ($r=-0.71$ and $r=0.69$, respectively; $p<0.05$) at day 4 of the incubation. However, these correlations disappeared at day 11 and later.



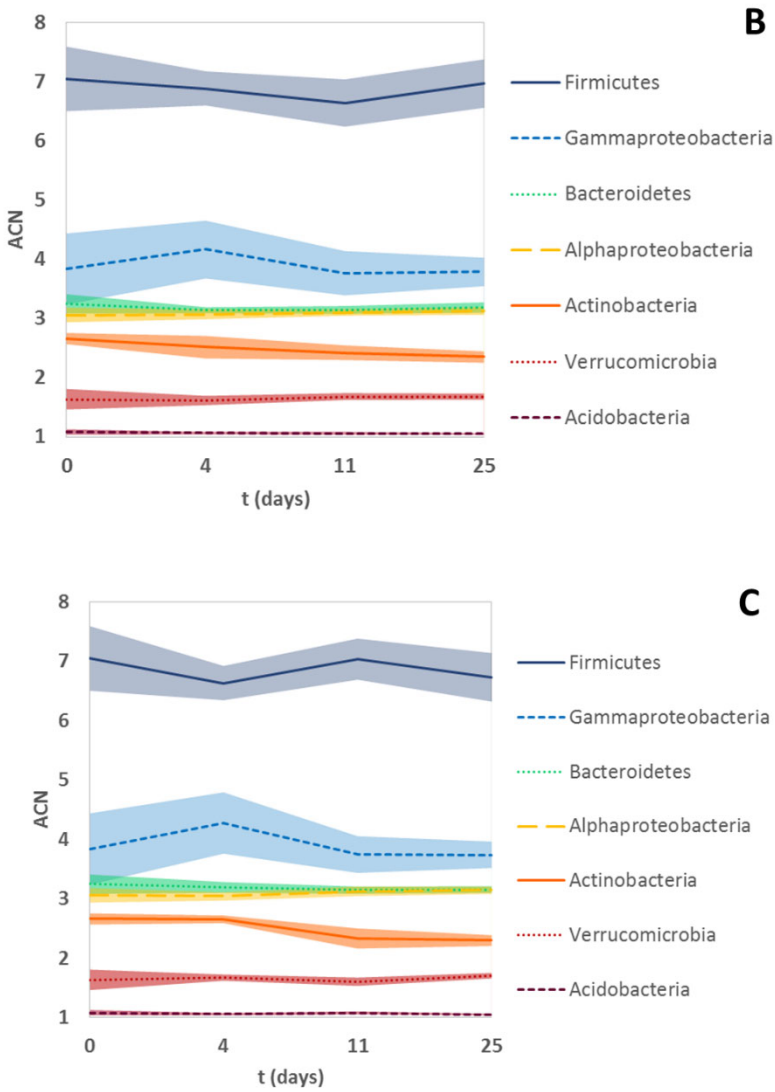


Figure 7: A/ r and K strategists in microbial community according to average 16rRNA copy number per genome (ACN) in control and samples amended by artificial exudates in concentration of 2 % and 5 % of total DOC during 25 days incubation. **B**/ Changes in each particular bacterial phylum in samples amended by artificial exudates in concentration of 2 % of total DOC during 25 days incubation and **C**/ in concentration of 5 % of total DOC during 25 days incubation. Enhanced ACN in the prokaryotic community indicates increased abundance of r-strategic species (means, ± standard deviations, n=12).

The overall prokaryotic community composition and especially the relative abundance of the main phyla and classes changed substantially during the 25 days of incubation after the exudate addition (Fig. 8 a,b).

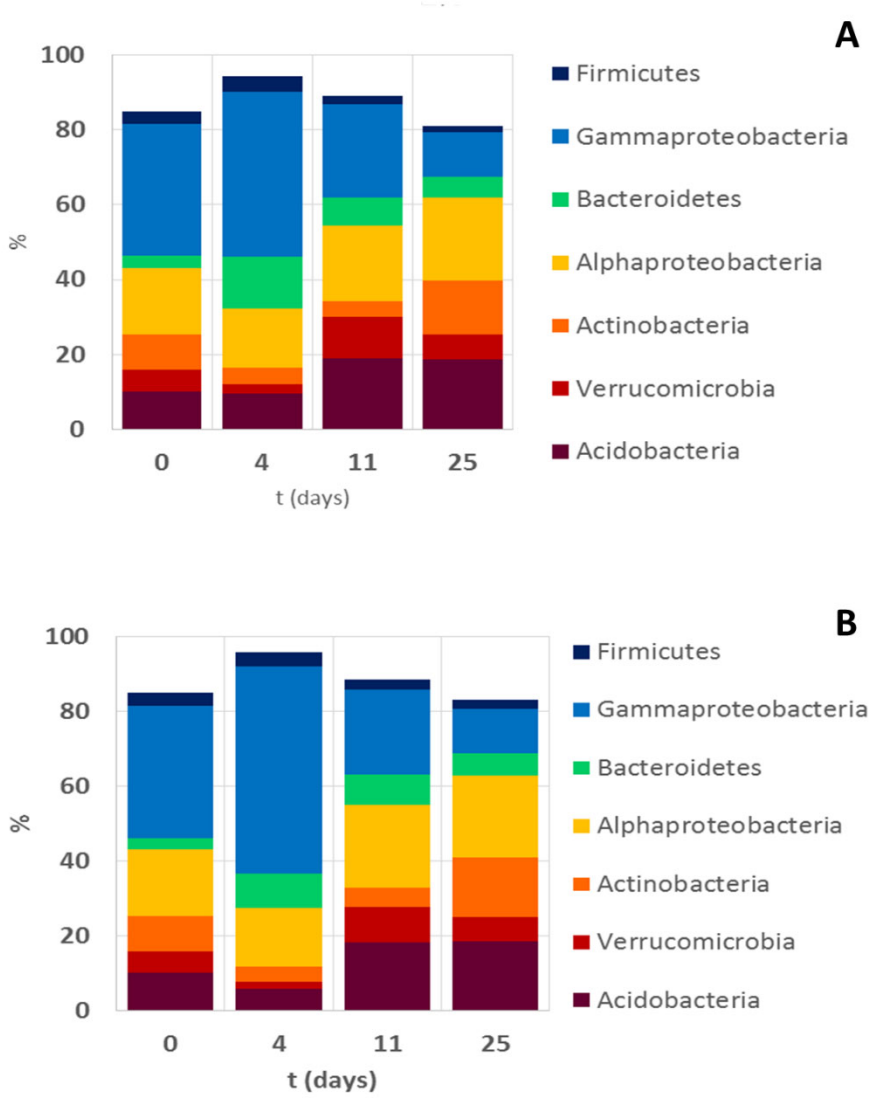


Figure 8: The relative abundance of the main phyla and classes in samples amended by artificial exudates in concentration of **A**/ 2 % of total DOC and **B**/ 5 % of total DOC during 25 days incubation.

In the early stage of the decomposition *Proteobacteria* were the most enriched phylum, specifically at higher dose of exudates (Table 1). This was caused by a significant increase in relative abundance of *Pseudomonas* (at both levels of exudate addition but more pronounced at 5% level) and *Burkholderia* (only at 5% level of exudate addition) (Table 2). Other genus from *Gammaproteobacteria, Masillia* responded oppositely and its proportion in the community decreased after exudate addition. At high level of exudates addition, *Mucilaginibacter* (*Bacteroidetes*) was also substantially enriched, although the proportion of *Bacteriodetes* phylum within the community decreased (Table 2).

Table 1: Significant differences in relative abundance (%) of bacterial phyla between the control and treatments with added root exudates (2% or 5%) at particular sampling times.

Time (day)	4		11		25	
Treatment	2%	5%	2%	5%	2%	5%
Acidobacteria	0.06	-0.73	0.28	2.13	-3.69	-3.92
Actinobacteria	0.17	0.07	0.15	0.46	0.17	0.26
Bacteroidetes	0.93	-1.21	1.42	2.62	0.19	1.50
Firmicutes	1.19	0.09	0.64	0.22	0.86	0.29
Proteobacteria	3.35	15.1	2.82	8.67	0.18	-0.60
Verrucomicrobia	0.05	-1.06	1.18	-4.31	1.03	1.3

Table 2: Significant differences in relative abundance (%) of bacterial genera between the control and treatments with added root exudates (2% or 5%) at particular sampling times.

Time (day)	4		11		25	
Treatment	2%	5%	2%	5%	2%	5%
Acidobacteria						
Bryocella	---	---	---	1.43	0.28	0.66
Candidatus_Solibacter	---	---	0.03	---	0.08	0.96
Granulicella	---	---	0.02	---	-2.71	-4.4
Telmatobacter	---	-0.3	---	---	-1.2	-1.13
Gammaproteobacteria						
Burkholderia	---	20.55	---	---	---	0.08
Legionella	---	---	1.16	4.42	0.11	0.29
Massilia	-5.63	-7.41	---	-0.38	---	---
Pseudomonas	5.56	10.95	0.33	0.49	0.02	0.02
Bacteroidetes						
Mucilaginibacter	0.79	2.67	0.21	0.51	0.1	0.08

At day 11, community composition largely differed from initial stage. *Proteobacteria* were still enriched, but *Bacteriodetes* and *Acidobacteria* phyla also increased their relative abundance in amended samples compare to control (Table 1). This was mainly attributed to *Legionella* and *Pseudomonas* (*Proteobacteria*) and *Bryocella* (*Acidobacteriaceae* (*Subgroup* 1)), which were the most enriched genera in this stage of decomposition (Table 2).

After 25 days of incubation, microbial communities of amended samples had significantly lower proportions of *Acidobacteria* as compared to control. However, looking deeper into the taxonomy of *Acidobacteria*, there were large differences in the response of specific genera. *Granulicella* and *Telmatobacter* (both belonging to *Acidobacteriaceae* (*Subgroup* 1)) decreased their proportion in the community, while *Candidatus Solibacter* (*Acidobacteria*, *Subgroup 3*) and *Bryocella* (Acidobacteriaceae (Subgroup 1)) were enriched (Table 2; for more details about bacterial and fungal community see Tab. S1 and S2, respectively).

We investigated in the detail a response of microbial community after 5% exudate addition with the C/N ratio 50 (Table S3), because this addition induced the most pronounced changes in bacterial community and was connected with the largest positive PE (Fig. 2). In the early stage of decomposition, *Methylocella* was

enriched in the treatment with high exudate C/N ratio up to 0.18% as compared to both C/N ratio 7 and 25 ($p<0.05$; Tab. S3). At day 11, the C/N ratio 50 had the highest increase of fungal genus *Rhodotorula* by 25.1% as compared to C/N ratio 7 ($p<0.05$) and was enriched by 0.4 % with *Methylobacterium* as compared to both C/N ratio 7 and 25. A significant positive response on exudates with C/N ratio 50 was found also for *Candidatus Solibacter* as compared to C/N ratio 7 and for *Legionella* and *Byssovorax* as compared to C/N ratio 25 by 0.5%, 1.5% and 0.3%, respectively (Tab. S3).

Temporal changes of functional potential of prokaryotic community

Bacterial community contained 4096 zOTU, from which 37% was assigned to metabolic or other ecologically relevant functions using FAPROTAX bioinformatic pipeline. The main metabolic guilds showed distinct temporal behavior after 2% and 5% exudate addition (Fig. 9). At day 4, the 5% level of exudates addition significantly increased the proportion of chemoheterotrophic prokaryotes while the proportion of anaerobes including fermenting bacteria decreased. This trend was almost identical for all exudates C/N ratios. Contrary, the day 11 was characteristic by diminished chemoheterotrophy and relative increase of chemolitotrophs (mainly ammonia oxidizers) across exudates C/N ratios. Similar changes occurred also after 2% level of exudate addition but these changes were mostly not significant. The 5% level of exudates addition also increased the proportion of anaerobes at the end of incubation (25^{th} day) at all C/N ratios but specifically at highest C/N ratio.

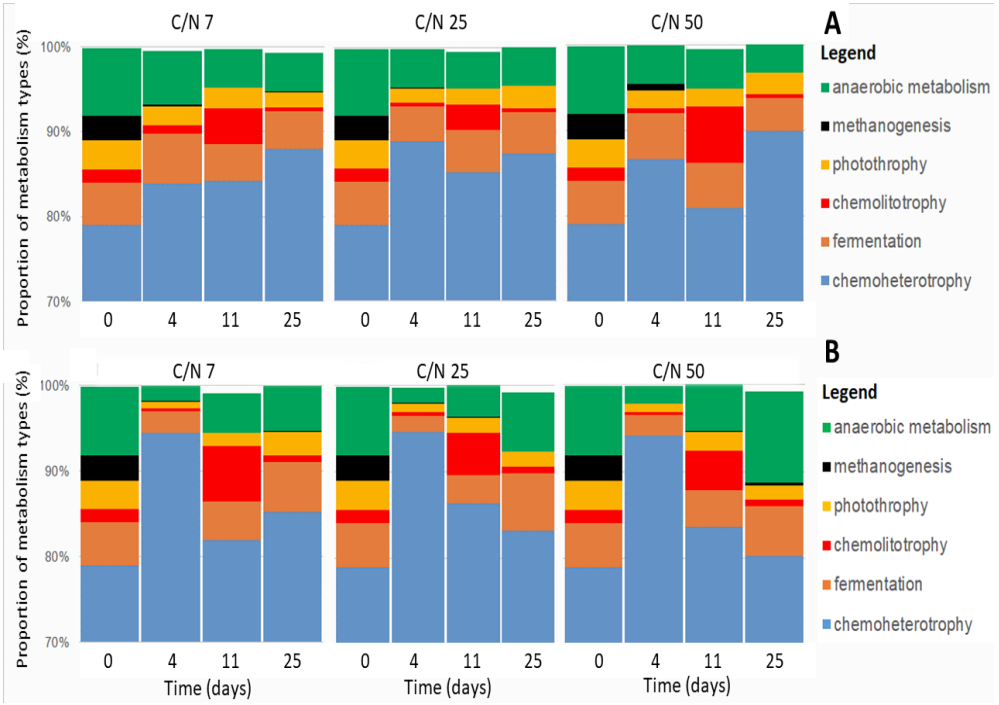


Figure 9: Temporal development of metabolism types of prokaryotic community in samples amended by artificial exudates with different C/N ratios **A/** in concentration of 2 % of total DOC and **B/** in concentration of 5 % of total DOC during 25 days incubation. Type of metabolism was assigned according to known bacterial and archaeal genomes using FAPROTAX algorithm (Louca et al. 2016).

PICRUSt, another functional analysis, revealed that bacteria decomposed more complex organic matter as it is shown by bacteria capable of aromatic compounds degradation. These bacteria were significantly enriched during first four days in all treatments including control, but specifically after the 5% level of exudates addition, which significantly differed from both control and low level of exudate addition ($p<0.001$). Afterwards, both amended treatments were enriched compare to control till the end of incubation (Fig. 10).

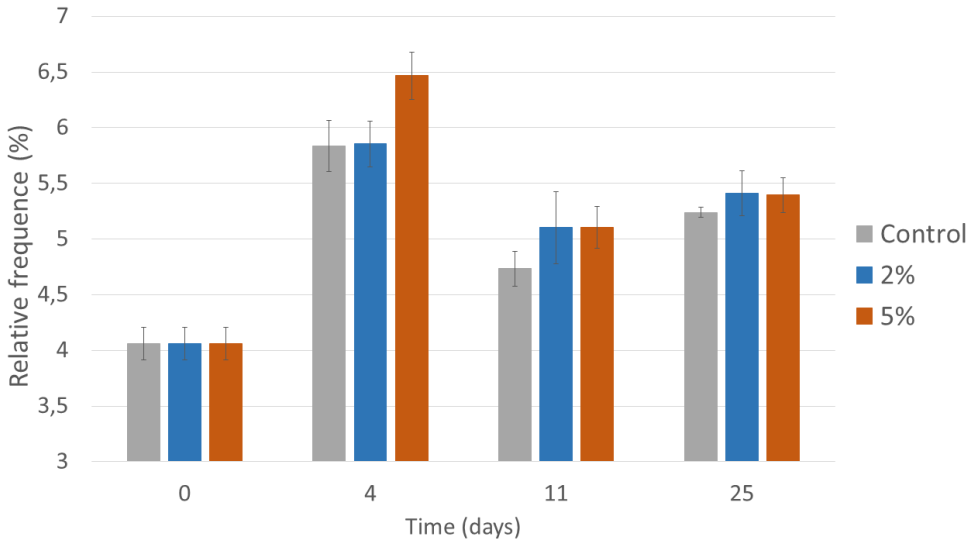


Figure 10: Relative frequency of bacteria capable of aromatic compounds biodegradation and metabolism in control and in samples amended by artificial exudates with concentration of 2 % and 5 % of total DOC during 25 days incubation (means, ± standard deviations).

Discussion

Priming effect was affected by quantity and C/N ratio of added exudates

Microbial community was dominated through the whole incubation by bacteria. Addition of root exudates caused a significant successional development of bacterial community size (Fig. 4) and composition (Fig. 6) which was also observed by Shi et al. (2011). In our incubation the highest shift in the bacterial community composition was between 4th and 11th day, Fig. 6). The compositional change was more pronounced after the addition of higher dose. Both levels of exudate addition influenced decomposition of recalcitrant peatland DOC which was accompanied by the shift in the composition, life strategy and functioning of microbial community. The PE was primarily dependent on the level of added C and on the magnitude of induced changes in the microbial growth and functioning (Fig. 2).

The low level of exudate addition enabled only limited microbial growth during the incubation. A nutrient demand of communities developing after low exudates addition was mostly covered from the available pools of nutrients, which was shown by a decrease in concentrations of SRP and extractable ammonium N. DOC mineralization during the incubation did not enhance compare to control.

Therefore, the 2% exudate addition finally resulted in a negative (for C/N ratio 7) or no PE (for C/N ratio 25 and 50) on pre-existing peatland DOC.

Contrary, higher dose of exudates (5% of DOC) evoked significant microbial growth at the initial phase of exudate decomposition (Fig. 4a) and significant shift in the composition of bacteria (Fig. 7c, 8b). This was related with a pronounced growth of especially r-strategic chemoheterotrophic *Gammaproteobacteria* (e.g. *Burkholderia*) after the 4th day. Rapid growth of bacteria probably also enhanced bacterial grazing and N mineralization and accelerated the N turnover (Meier et al. 2017). The enhanced bacterial grazing can be ascribed to the following steep decrease of bacteria (mainly Gammaproteobacteria, Fig. 8b) between the 4th and 11th day (Fig. 4a). Moreover, accelerated N mineralization was confirmed by the increase of ammonia on 11th day (Fig. S1b). The increase of ammonia could support chemolithotrophic ammonia oxidizers on the same day (Fig. 9).

The 5% level of exudate addition also caused significant positive PE at day 11th day at all tested C/N ratios (Fig. 2b). The PE was the largest for C/N 50 and lasted till the day 25. This prolonged PE could be connected with larger co-metabolism of more recalcitrant DOC, biosynthesis of extracellular enzymes which stimulated “N mining” from native DOM in order to cover nutrient demands of fast-growing microbial biomass at the C/N ratio of 50 compare (Chen et al., 2014; Fang et al., 2018).

Our results are in the line with findings of Liu et al. (2017) who found that low level of C addition lead to a negative or no PE during 7 week incubation, while the high level of C addition induced positive priming and of Wang et al. (2015), who observed an increasing PE with increasing C/N ratio of crop residues added to the soil. Our results also indicate that the exudate dose close or above the 5% of peat DOC, can largely influence the functioning and composition of bacterial community and evoke the positive priming effect. Moreover, our temporal sampling allowed us to clearly distinguish two phases of microbial growth and community change, related to a different utilization of added exudates and original DOC, and available nutrients from the solution.

The early stage – preferential use of exudates and negative PE

The early stage of incubation immediately following the exudate addition (till day 4) was characteristics by a preferential use of the added compounds by the community, resulting in a strong negative PE (Fig. 1). The addition induced mainly bacterial growth connected with rapid decline of SRP and DIN which were immobilized into the newly formed biomass. The R-strategic bacteria increased as

indicated by the increased ACN of the community (Fig. 7a) and by the proportional increase of *Gammaproteobacteria* and *Bacteriodete*s, known as opportunistic bacteria, fast growers with a rapid turnover (Fierer et al., 2016), which are often stimulated by the presence of labile substrates like root exudates (Fierer et al., 2007; Jenkins et al., 2010). The highest increase was observed for *Burkholderia* and *Pseudomonas,* the typical rhizosphere copiotrophs (Buée et al., 2009).

Pseudomonas is able to degrade complex aromatic compounds including lignin and other phenolics (Bandounas et al., 2011) and produce chitinolytic enzymes (Brabcová et al., 2016). It belongs among the most efficient phosphate solubilizers due to production of organic acids (Rodriguez and Fraga, 1999) and can facilitate iron uptake of plants by producing pyoverdines (Vansuyt et al., 2007). Therefore, *Pseudomonas* could facilitate nutrients (mainly P) uptake by plants, especially in nutrient limited environment like peatlands.

Among *Bacteroidetes*, the *Mucilaginibacter* which was the most enriched at day 4 after exudates addition is also able to degrade complex biopolymers like cellulose (Štrusková et al., 2012). In our experiment, *Burkholderia, Pseudomonas* and *Mucilaginibacter* were the most enriched copiotrophic bacteria which are also typical for the large metabolic versatility (Lladó et al., 2015; Sun et al., 2016). Our results showed that the bacterial community very quickly (4^{th} day after the 5% exudate addition) had a large potential to degrade complex organic compounds, including cellulose and aromatics. We therefore argue that this community dominated by the r-strategic taxa and their extracellular enzymes, can largely contribute to the following positive PE, which was observed at 11^{th} day (Fig. 2). This partly contradicts a classical understanding of the mechanisms of PE. It suggests that the main role in production of exoenzymes responsible for a degradation of pre-existing SOC is played by K-strategists, which follow in the succession, when the r-strategic community dies after a depletion of simple substrates (Kuzyakov 2010).

Because the 5% of peat DOC addition and C/N ratio of 50 induced the largest PE, we focused on specific bacterial taxa responding significantly at this high C/N ratio. We found that *Methylocella* was enriched in C/N 50 (Table S3). *Methylocella* species are a facultative methanotrophs, and can grow on various simple C compounds including acetate, succinate, malate etc. (Dedysh et al., 2005) and are able to fix atmospheric N_2 (Dunfield et al., 2003, Dos Santos et al., 2012). The ability to fix N_2 may probably favour *Methylocella* species at high C/N ratios Similarly, Raja et al., (2006) found another methylotrophic and putative N_2 fixing bacteria, *Methylobacterium*, enriched in treatments with high C/N ratio. Because methanotrophs produce unique enzyme methane monooxygenase (mmo) and because monooxygenases are produced by the bacteria which degrade aromatics it was suggested that also methanotrophs might contribute to their degradation

(Hanson and Hanson, 1996). However, up to date there has been no evidence that either *Methylocella or Methylobacterium* are capable of complex aromatic compounds degradation (Semrau et al., 2011). More likely, *Methylocella* species used the open niche and utilized the added compounds and their ability to compensate the N deficiency by fixing the atmospheric N_2.

The later stage of the incubation – dieback of r-strategic community

Exudate depletion from the solution occurred before day 4 as indicated by a sharp decrease in microbial respiration rates (Fig. 1). Consequently, bacterial abundance decreased between days 4 and 11 mainly due to dieback of r-strategists and a new, smaller community composed of different groups of bacteria was established. The ACN per cell decreased (Fig. 7), which indicated a shift towards K-strategists like *Alphaproteobacteria* and *Acidobacteria*. *Bryocella* and *Candidatus Solibacter* from Acidobacteria were the main enriched genera between 11-25 days of incubation (Table 2). *Acidobacteria* in general are reported as the dominant microbial group in peatlands because they prefer low pH and oligotrophic conditions (Lin et al., 2012, Amha et al., 2015). Members of *Acidobacteria* are also efficient cellulose decomposers (Haichar et al., 2007; Pankratov et al., 2011; Štursová et al., 2012; Lladó et al., 2015) and their high abundance in microbial community may drive the cellulose degradation in acidic *Sphagnum* peat (Pankratov et al., 2011). They could contribute to the degradation of leaf/root litter in the peatlands especially in the rhizosphere with high level exudates addition. The *Bryocella* species were shown to have high enzymatic activity (Lladó et al., 2015) and thus may contribute to the significant positive PE observed at day 11 (Fig. 2). On the other hand, *Candidatus Solibacter* was enriched mainly at the end of the incubation (day 25) of the incubation and was one of a few genera enriched under high C/N ratio. This genus is capable of cellulose, hemicellulose and chitin degradation and may contribute to cellulose degradation in the later stage of decomposition. Therefore, *Bryocella* and *Candidatus Solibacter* species could play important role in the second stage of PE in peatland DOC. We thus suggest that the pool of enzymes causing the observed positive PE at days 11 and 25 is a mixture of enzymes produced by r-strategists directly activated by the exudate addition in the early stage, which persisted after their dieback and continued their function, and enzymes produced by later incoming K-strategists.

Differently from the sharp decrease of nutrient concentration in the solution observed during the fast growth of previous r-strategic community, we observed an increase of ammonia concentration during the later decomposition stage (Fig. 3). It can originate from the dieback of r-strategic bacteria or by increased

predation which leads to the release of ammonia back to the environment. Coincidently, the metabolic potential of prokaryotic community showed an increase of ammonia oxidizers at day 11 (Fig. 8). Ammonia oxidizers belong to slow growing chemolitotrophs, which can use the available NH_4^+ in their energetic metabolism. Additionally, high level of added root exudates with high C/N ratio lead to the higher proportion of anaerobes in the late stage of the incubation (25 day), which may relate to larger release of simple organic compounds from necromass of r-strategists (Blagodatsky et al., 2000).

Upscale to ecosystem level (a potential role of plant exudates in stimulation of peatland DOC decomposition *in situ*)

Edwards et al. (2018) estimated that root exudates of peatland vascular plants, which were easily degradable, could contribute from 1% up to 5% to the pre-existing peatland DOC *in situ*. Our results evoke that a lower input of root exudates, achieving around 2% of DOC has a significant effect on composition of rhizosphere microbial community, but is insufficient to induce significant positive PE on recalcitrant peatland DOC. However, when the exudate input reaches the level of 5% of the present DOC, it may induce a transient positive priming effect lasting several days. With exudates poor for N (C/N ratio of 50), the induced positive priming may persist for more than two weeks and result in a enhanced decomposition of the old DOC. According to Edwards et al. (2018), the situation, when exudation input is high enough, occurs at the top of the season especially in the presence of graminoid species such as *Eriophorum vaginatum*. However, the exudates at that time were relatively rich in N, therefore likely not causing significant peatland DOC losses. We suggest that the plants may rather benefit from the changes in the composition of microbial community, which the exudates induce in their rhizosphere. According to our results, root exudates input supports growth of r-strategic species, which are able to immobilize high amounts of nutrients in their biomass, keep them in the vicinity of plant roots, protect them from losses with the leaching DOC and potentially release them during their turnover. The rhizosphere community in enriched in species like *Pseudomonas,* which can mobilize P and others (e.g. *Burkholderia* and *Mucilaginibacter)* with high metabolic potential. The presence of microbial communities able to keep a lot of nutrients in the rhizosphere of peatland vascular plants is further supported by our previous results from the field. In Kaštovská et al. (2018) we showed that soil microbial biomass associated with *Eriophorum vaginatum* and *Vaccinium myrtillus* immobilize large amounts of N and P present in the system. Additionally, the association of

ericaceous shrub with mycorrhizal fungi increase P mobilization and increases its availability to the plant (Read et al. 2004).

Differently from root exudates of vascular plants, *Sphagnum* "exudates" is not expected to cause a significant positive priming effect on DOC decomposition. Although the *Sphagnum*-released compounds can contribute up to 20 % to the peatland DOC, they are of low degradability being only around 15 % (Edwards et al., 2018). Additionally, the compounds leached from *Sphagnum* were shown to immobilize phosphorus by its incorporation to the high molecular weight complexes and by co-precipitation with metals (Mastný et al., 2018) and they are known by their antimicrobial effects (Painter 1991).

In accordance with the study of Basiliko et al., (2012), we suggest that an input of root exudates from vascular plants may induce a positive priming effect on organic matter decomposition in the peatlands, but its importance for C transformation and ecosystem C balance is likely minor under current conditions. However, if ongoing climate changes will result in a significant shift in plant community composition from *Sphagnum* to vascular plants dominance, a priming effect caused by the enhanced root exudation could lead to higher dynamics of C cycle in peatlands.

Conclusions:

1/ Priming effect was affected by level of exudates addition: Low level of root exudates addition ~ (2% of total DOC) to peatland water cause negative priming effect (PE) whereas positive PE can occur with increasing level of root exudates ~ (5% of total DOC). Increasing C/N ratio from 7 to 50 enhances PE. Growing microbial community is limited by nutrients and it leads to increased "microbial nutrient mining" and positive PE.

2/ Bacteria played more important role than fungi in our experiment as indicated by the ratio lower than 0,005. After root exudates addition, r-strategic bacteria increased with level of root exudates addition and their growth was connected with increased uptake of SRP and ammonium N from peatland water. High level of added root exudates ~ (5% of total DOC) stimulated microbial functional groups with potential to decompose complex compounds. Therefore, our experiment indicates that r-strategic bacteria (mainly *Gammaproteobacteria*) may be more important group causing positive PE on peatland DOC decomposition than K-strategists. The positive PE found at 11^{th} day in the treatments with high level of exudates addition, could be caused by both the enzymes produced by K-strategic bacteria and by the enzymes produced from r-strategic bacteria before their die-back after 4^{th} day.

Detailed analysis of microbial community revealed several genera with distinct effect on positive priming (indicated by their high enzymatic activity); after root exudates addition *Burkholderia*, *Pseudomonas* and *Mucilaginibacter* were the most important groups of r-strategic bacteria; later followed by K-strategists *Bryocella* and *Candidatus Solibacter.*

Funding

This work was supported by the Grant Agency of the Czech Republic [13-17398S]. The article was further supported from MEYS; projects [LM2015075] and [EF16_013/0001782] – SoWa Ecosystems Research.

Acknowledgements

We thank Ondřej Žampach, Daniel Vaněk, Kateřina Kučerová and Hana Bošková for technical support and chemical analyses and Keith Raymond Edwards for language corrections.

References

Allison, S.D., Wallenstein, M.D., Bradford, M.A. 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3, 336–340.

Amha, Y., Bohne, H., Alsanius, B. 2015. Patterns of Fungal and Bacterial Carbon Mineralization Across Northern European Peatlands. Geomicrobiology Journal 32(10), 914-923.

Bandounas, L., Wierckx, N.J.P., de Winde, J.H., Ruijssenaars, H.J. 2011. Isolation and characterization of novel bacterial strains exhibiting ligninolytic potential. BMC Biotechnology 11, 94–105.

Basiliko, N., Stewart, H., Roulet, N., Moore, T. 2012. Do root exudates enhance peat decomposition? Geomicrobiology Journal 29, 374–378.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, M., de Sousa, F., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, M.K., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods in Ecology and Evolution 4, 914–919.

Blagodatskaya, E.V., Kuzyakov, Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils 45, 115-131.

Blagodatskaya, E., Khomyakov, N., Myachina, O., Bogomolova, I., Blagodatsky, S. & Kuzyakov, Y. 2014. Microbial interactions affect sources of priming induced by cellulose. Soil Biology and Biochemistry 74, 39–49.

Blagodatsky, S.A., Heinemeyer, O., Richter, J. 2000. Estimating the active and total soil microbial biomass by kinetic respiration analysis. Biology and Fertility of Soils 32, 73–81.

Bolton, H., Fredrickson, J.K., Eliott, L.F. 1992. Microbial ecology of the rhizosphere. In Soil Microbial Ecology, Metting FB (ed.) Marcel Dekker: New York, 27-63.

Borneman, J., Hartin, R.J. 2000. PCR primers that amplify fungal rRNA genes from environmental samples. Appl Environ Microbiol 66, 4356-4360.

Brabcová V, Nováková M, Davidová A, Baldrian P. 2016. Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community. New Phytologist 210, 1369–81.

Buée M, De Boer W, Martin F, van Overbeek L, Jurkevitch E. 2009. The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant and Soil 321, 189–212.

Buttler, A., Robroek, B.J.M., Laggoun-Défarge, F., Jassey, V. E. J., Pochelon, C., Bernard, G., … Bragazza, L. 2015. Experimental warming interacts with soil moisture to discriminate plant responses in an ombrotrophic peatland. Journal of Vegetation Science 26, 964–974.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R. 2010. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences, USA 108 (Suppl 1), 4516–4522.

Clark J.M., Lane S.N., Chapman P.J., Adamson J.K. 2008. Link between DOC in near surface peat and stream water in an upland catchment. Science of the Total Environment, 404, 308–315.

Dedysh, S.N., Knief, C., and Dunfield, P.F. 2005. Methylocella species are facultatively methanotrophic. Journal of Bacteriology 187, 4665–4670.

Dieleman, C.M., Branfireun, B.A., McLaughlin, J.W., Lindo, Z. 2015. Climate change drives a shift in peatland ecosystem plant community: implications for ecosystem function and stability. Global Change Biology 21, 388–395.

Dos Santos, P.C., Fang, Z., Mason, S.W., Setubal, J.C., Dixon, R. 2012. Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. BMC Genomics 13, 162.

Drake, J.E., Darby, B.A., Giasson, M.A., Kramer, M.A., Phillips, R.P., Finzi, A.C. 2013. Stoichiometry constrains microbial response to root exudation – insights from a model and a field experiment in a temperate forest. Biogeosciences 10, 821–838.

Dunfield, P.F., Khmelenina, V.N., Suzina, N.E., Trotsenko, Y.A., and Dedysh, S.N. 2003. Methylocella silvestris sp. nov., a novel methanotrophic bacterium isolated from an acidic forest cambisol. Journal of Systematic and Evolutionary Microbiology 53, 1231–1239.

Edgar, R.C., Robert, C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10, 996–998.

Edwards, K.R., Kaštovská, E., Borovec, J., Šantrůčková, H., Picek, T. 2018. Species effects and seasonal trends on plant efflux quantity and quality in a spruce swamp forest. Plant Soil 426, 179–96.

Eiland, F., Klamer, M., Lind, A.M., Baath, E. 2001. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. Microbial Ecology 41, 272–280.

Elser, J.J., Dobberfuhl, D.R., MacKay, N.A., Schampel, J.H. 1996. Organism size, life history and N:P stoichiometry. BioScience 46, 674–684.

Fang, Y., Nazaries, L., Singh, B.K., Singh, P.B. 2018. Microbial mechanisms of C priming effects revealed during the interaction of crop residue and nutrient inputs in contrasting soils. Global Change Biology 24(7), 2775-2790.

Fierer, N., Bradford, M.A., Jackson, R.B. 2007. Toward an ecological classification of soil bacteria. Ecology 88,1354–1364.

Fontaine, S., Mariotti, A., Abbadie, L. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology and Biochemistry 35, 837-843.

Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J. M. G., Maire, V., et al. 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biology and Biochemistry 43, 86–96.

Frolking, S., Roulet, N.T., Moore, T.R., Lafleur, P.M., Bubier, J.L., Crill, P.M. 2002. Modelling seasonal to annual carbon balance of Mer Bleue Bog, Ontario, Canada. Global Biogeochemical Cycles 16, 1029–1040.

Garcia-Pausas, J., Paterson, E. 2011. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. Soil Biology and Biochemistry 43, 1705-1715.

Gardes, M., Bruns, T.D. 1993. Its Primers with Enhanced Specificity for Basidiomycetes - Application to the Identification of Mycorrhizae and Rusts. Molecular Ecology 2, 113-118.

Grayston, S.J., Vaughan, D., Jones, D. 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5, 29–56.

Guenet, B., Danger, M., Abbadie, L., Lacroix, G. 2010. Priming effect: bridging the gap between terrestrial and aquatic ecology. Ecology 91, 2850–2861.

Haichar, F.Z., Achouak, W., Christen, R., Heulin, T., Marol, C., Marais, M.F., Mougel, C., Ranjard, L., Balesdent, J., Berge O. 2007. Identification of cellulolytic bacteria in soil by stable isotope probing. Environmental Microbiology 9, 625–634.

Hamer, U., Marschner, B. 2002. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 165, 261-268.

Hanson, R.S., Hanson, T.E. 1996. Methanotrophic bacteria. Microbiology Reviews, 60(2), 439-471.

Hotchkiss, E.R., Hall, R.O., Baker, M.A., Tank J.L. 2014. Modeling priming effects on microbial consumption of dissolved organic carbon in rivers. Journal of Geophysical Research—Biogeosciences 119, 982–995.

Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X. et al. 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Global Change Biology 20, 2356–2367.

Jenkins, S.N., Rushton, S.P., Lanyon, C.V., Whiteley, A.S., Waite, I.S., Brookes, P.C., Kemmitt, S., Evershed, R.P., O'Donnell, A.G., 2010. Taxon-specific responses of soil bacteria to the addition of low level C inputs. Soil Biology and Biochemistry 42, 1624–1631.

Jones, D.L., Nguyen, C., Finlay, R.D. 2009. Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant and Soil 321, 5-33.

Kaštovská, E., Straková, P., Edwards, K., Urbanová, Z., Bárta, J., Mastný, J., Šantrůčková, H., Picek, T., 2018. Cotton-Grass and Blueberry have Opposite Effect on Peat Characteristics and Nutrient Transformation in Peatland. Ecosystems 21, 443–458.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., et al. 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22, 5271–5277.

Kuzyakov, Y. 2010. Priming effects: Interactions between living and dead organic matter. Soil Biology and Biochemistry 42, 1363-1371.

Langille, M., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J., Clemente, J., Burkepile, D., Vega Thurber, R., Knight, R., Beiko, R., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology 31, 814–821.

Lin, X., Green, S., Tfaily, M.M., Prakash, O., Konstantinidis, K.T., Corbett, J.E., Chanton, J.P., Cooper, W.T., Kostka, J.E. 2012. Microbial community structure and activity linked to contrasting biogeochemical gradients in bog and fen environments of the glacial Lake Agassiz peatland. Applied Environmental Microbiology 78: 7023–7031.

Liu, X.-J.A., Sun, J., Mau, R.L., Finley, B.K., Compson, Z.G., van Gestel, N., Brown, J.R., Schwartz, E., Dijkstra, P., Hungate, B.A., 2017. Labile carbon input determines the direction and magnitude of the priming effect. Applied Soil Ecology 109, 7-13.

Lladó, S., Žifčáková, L., Větrovský, T., Eichlerová, I., Baldrian, P. 2016. Functional screening of abundant bacteria from acidic forest soil indicates the metabolic potential of Acidobacteria subdivision 1 for polysaccharide decomposition Biology and Fertility of Soils 52, 251–260.

Louca, S., Parfrey, L.W., Doebeli, M. 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353, 1272-1277.

Lu, Y., Conrad, R. 2005. Stable isotope probing of methanogenic archaea in the rice rhizosphere. Science 309, 1088–1090.

Makino, W., Cotner, J.B., Sterner, R.W., Elser, J.J. 2003. Are bacteria more like plants or animals? Growth rate and substrate dependence of bacterial C : N : P stoichiometry. Functional Ecology 17, 121–130.

Mastný, J., Kaštovská, E., Bárta, J., Chronáková, A., Borovec, J., Šantrůčková, H., Urbanová, Z., Edwards, K. R., Picek, T. 2018. Quality of DOC produced during litter decomposition of peatland plant dominants. Soil Biology and Biochemistry 121, 221–230.

Meier I.C., Finzi A.C., Philiips R.P. 2017. Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. Soil Biology and Biochemistry 106, 119–128.

Miao, S., Ye, R., Qiao, Y., Zhu-Barker, X., Doane, T.A., Horwath, W.R., 2017. The solubility of carbon inputs affects the priming of soil organic matter. Plant and Soil 410, 129–138.

Muyzer, G., Dewaal, E.C., Uitterlinden, A.G. 1993. Profiling of Complex Microbial-Populations by Denaturing Gradient Gel-Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes-Coding for 16s Ribosomal-Rna. Applied and Environmental Microbiology 59, 695-700.

Myers, B., Webster, K.L., McLaughlin, J.W., Basiliko, N. 2012. Microbial activity across a boreal peatland nutrient gradient: the role of fungi and bacteria. Wetlands Ecology and Management 20, 77– 88.

Painter, T.J., 1991. Lindow man, Tollund man and other peat-bog bodies—the preservative and antimicrobial action of sphagnan, a reactive glycuronoglycan with tanning and sequestering properties. Carbohydrate Polymers 15, 123–142.

Pankratov, T.A., Ivanova, A.O., Dedysh, S.N., Liesack, W., 2011. Bacterial populations and environmental factors controlling cellulose degradation in an acidic Sphagnum peat. Environmental Microbiology 13, 1800-1814.

Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G. 2014. STAMP: Statistical analysis of taxonomic and functional profiles. Bioinformatics 30, 3123–3124.

Raja, P., Uma, S., Sundaram, S. 2006. Non-nodulating pink-pigmented facultative Methylobacterium sp. with a functional nifH gene. World Journal of Microbiology & Biotechnology 22, 1381-1384.

Rodriguez, H., Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances 17, 319–339.

Qiao, N., Xu, X., Hu, Y., Blagodatskaya, E., Liu, Y., Schaefer, D., Kuzyakov, Y., 2016.Carbon and nitrogen additions induce distinct priming effects along an organic-matter decay continuum. Scientific Reports 6, 1-8.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J.,Glockner, F.O. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41, 590-596.

Semrau, J.D. 2011. Bioremediation via methanotrophy: overview of recent findings and suggestions for future research. Frontiers in Microbiology 2, 209.

Shi, S., Richardson, A.E., O'Callaghan, M., DeAngelis, K.M., Jones, E.E., Stewart, A., Firestone, M.K., Condron, L.M., 2011. Effects of selected root exudate components on soil bacterial communities. FEMS Microbiology Ecology 77, 600-610.

Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3 (348), 1-11.

Sun, D., Meng, J., Xu, G.E., Chen, W. 2016. Microbial community structure and predicted bacterial metabolic functions in biochar pellets aged in soil after 34 months. Applied Soil Ecology 100, 135–143.

Štursová, M., Zifcafova, I., Leigh, M.B., Burgess, R., Baldrián, P., 2012. Cellulose utilization in forest litters and soils: identification of bacterial and fungal decomposers. FEMS Microbiology Ecology 80, 735-746.

Tfaily, M.M., Hamdan, R., Corbett, J.E., Chanton, J.P., Glaser, P.H., Cooper, W.T. 2013. Investigating dissolved organic matter decomposition in northern peatlands using complimentary analytical techniques. Geochimica et Cosmochimica Acta 112, 116–129.

Thacker, S.A., Tipping, E., Gondar, D., Baker, A., 2008. Functional properties of DOM in a stream draining peat. The Science of the Total Environment 407, 566–573.

Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., Prill, R.J., Tripathi, A., Gibbons, S.M., Ackermann, G., et al., 2017. A communal catalogue reveals Earth's multiscale microbial diversity. Nature 551, 457-463.

Urich, T., Lanzén, A., Qi, J., Huson, D.H., Schleper, C., Schuster, S.C., 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PloS One 3, e2527.

van Huissteden, J., van den Bos, R., Alvarez, I.M., 2006. Modelling the effect of watertable management on CO2 and CH4 fluxes from peat soils. Netherlands Journal of Geosciences-Geologie en Mijnbouw 85, 3-18.

Vansuyt, G., Robin, A., Briat, J.F., Curie, C., Lemanceau, P., 2007. Iron acquisition from Fe-pyoverdine by Arabidopsis thaliana. Molecular Plant-Microbe Interactions 20, 441-447.

Wang, H., Boutton, Thomas, W., Xu, W.H., Hu, G.Q., Jiang, P., Bai, E., 2015. Quality offresh organic matter affects priming of soil organic matter and substrate utili-zation patterns of microbes. Scientific Reports 5, 10102.

Wardle, D.A., Greenfield, L.G. 1991. Release of mineral nitrogen from plant root nodules. Soil Biology and Biochemistry 9, 827–832.

Weltzin, J.F., Pastor, J., Harth, C., Bridgham, S.D., Updegraff, K.& Chapin, C.T. 2000. Response of bog and fen plant commu-nities to warming and water-table manipulations. Ecology 81, 3464–3478.

Wild, B., Schnecker, J., Alves, R.J.E., Barsukov, P., Bárta, J., Čapek, P., Gentsch, N., Gittel, A., Guggenberger, G., Lashchinskiy, N., Mikutta, R., Rusalimova, O., Santrůčková, H., Shibistova, O., Urich, T., Watzka, M., Zrazhevskaya, G., Richter, A., 2014. Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. Soil Biology and Biochemistry 75, 143-151.

Winsborough, C., Basiliko, N., 2010. Fungal and bacterial activity in Northern peatlands. Geomicrobiology Journal 27, 315-320.

Zhu, B., Gutknecht, J.L.M., Herman, D.J., Keck, D.C., Firestone, M.K., Cheng, W., 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biology and Biochemistry 76, 183-192.

7 Curriculum vitae

Personal information

First name(s) / Surname(s)	Jiří Mastný
Address(es)	Doudleby 7, 370 07 České Budějovice, Czech Republic
Telephone	+420 38 7772347 (office)
E-mail:	mastny.jiri@seznam.cz
	http://kbe.prf.jcu.cz/en/jiri-mastny
Nationality	Czech
Date of birth	05 June 1988
Gender	Male
University address	Department of Ecosystem Biology, Faculty of Science, University of South Bohemia Branišovská 1760 CZ – 370 05 České Budějovice Czech Republic

Education and training

2014 – present: Ph.D. program, University of South Bohemia, České Budějovice

(Faculty of Science, Department of Ecosystem Biology)

student of ecosystem biology

(supervisor: Ing. Tomáš Picek, Ph.D.)

2011 – 2014: Master of Biology (Mgr.), University of South Bohemia, České Budějovice

(Faculty of Science, Department of Ecosystem Biology)

student of soil biology

(supervisor: Ing. Tomáš Picek, Ph.D.)

2007 – 2011: Bachelor of Biology (Bc.), University of South Bohemia, České Budějovice (Faculty of Science, Department of Ecosystem Biology)

student of soil biology (supervisor: Ing. Tomáš Picek, Ph.D.)

Scientific focus

The effect of peatland plant dominants on quality of dissolved organic matter

Work and science experience

Collaborator on the project: Species diversity and biogeochemical interactions in pristine, drained and restored boreal and temperate spruce swamp forests

Member of a field works in the Sumava, Czech republic, 2014-17 (collection and treatment of soil samples, field measurement of microbial activity)

International Courses and Certifications

Course of Plant and soil, Tartu, Estonia (2014), TOEFL ITP (2012), TOEIC L+R (2016)

Personal Skills and Competences

Native language: Czech

Other languages: English, C1

German, A2

Driving license holder B

Participation in International Conferences and Workshops

„Quality and biodegradability of dissolved organic matter originating from litter of spruce swamp forest vegetation dominants.“ 5th International Symposium on Soil Organic Matter (SOM) 2015 - Gottingen, Germany (20-24th September 2015).

„DOM quality and microbial community as affected by litter of spruce swamp forest plant dominants.“ Biogeomon 2017 – Litomyšl, Czech Republic (20-24th August 2017).

„Quality of DOM produced during litter decomposition of peatland plant dominants.“ workshop Carbon Cycling in Boreal Peatlands and Climate Change II“ - Hyytiälä, Findland (25-29th September 2017).

mastny.jiri@seznam.cz

Effect of peatland plant dominants on quality and quantity of organic matter in spruce swamp forest soils

Ph.D. Thesis Series, 2020, No. 1

Printed in the Czech Republic by Typodesign s.r.o.

Edition of 20 copies

University of South Bohemia in České Budějovice

Faculty of Science

Branišovská 1760

CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 776 201

www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz