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Quality of DOC produced during litter decomposition of peatland plant dominants

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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

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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.