The biogeochemistry of *Sphagnum* mosses - the effects of substrate source on their phenolic composition

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Abstract
Several species of peat moss and vascular plants were collected from field incubation litterbag experiments set up in a peatland located in the boreonemoral zone of central Sweden. These were then analysed using THM in the presence of TMAH which revealed the distributions of both lignin- and *Sphagnum*-derived phenols within specific species. THM of seven species of *Sphagnum* indicated the presence of four *Sphagnum*-specific biomarkers; methylated 4-isopropenylphenol, methylated *cis* and *trans* 3-(4'-hydroxyphen-1-yl)-but-2-enoic acid, and methylated 3-(4'-hydroxyphen-1-yl)-but-3-enoic acid.

Key Words
Thermochemolysis, tetramethylammonium hydroxide.

Introduction

Northern peatlands cover an area of around 350 x 10^6 ha, and store around one-third of global soil carbon (C) (Gorham 1991), however the effect that increasing temperatures and elevated CO_2 levels will have on these systems is of great uncertainty (Davidson and Janssens 2006). *Sphagnum* moss litter is the dominant input of C_{org} into ombrotrophic bogs. It contains phenolics that act both as structural support components and as inhibitors of microbial decomposition of the organic matter (Verhoeven and Liefveld 1997; Freeman *et al.* 2001). There are also vascular plants associated with peatlands which will contribute lignin and other polyphenols. *Sphagnum* does not contain lignin (Mauseth, 1998) and instead biosynthesizes other phenylpropanoids including *trans*-Sphagnum acid (Rasmussen *et al.* 1995). Thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH), otherwise known as TMAH thermochemolysis, can be used to identify lignin phenols (e.g. Mason *et al.* 2009). Thermochemolysis was first introduced as an analytical pyrolysis technique with *in situ* derivatisation using TMAH (Challinor 1989; Kaal and Janssen 2008). The aim of this presentation is to characterize and compare the phenolics of both *Sphagnum* and *Polytrichum* moss species as well as some vascular plant species which contribute to the peat litter of a bog located in central Sweden.

Materials and methods

Site and samples

Samples were collected from Ryggmossen in the boreonemoral zone of central Sweden. In this study, 45 samples were analysed from four distinct sets of samples. A field incubation litterbag experiment of fresh litter had been set up on the site. These litterbags were sampled twice: sampled first in September 2007 giving set 1(0001-0009), and again in April 2008 resulting in set 2 (0020-0029). These samples consisted of seven species of *Sphagnum* (*S. capillifolium, S. fuscum, S. majus, S. centrale, S. balticum, S. magellenicum, S. angustifolium*), and one species of *Polytrichum (P. commune)*. The third set of samples (10F-19D), were collected from the field incubation reciprocal transplant experiment in September 2007. Fresh and degraded samples were taken from each habitat; fresh samples from the near surface oxic horizon, and the degraded samples from just below the water table. The final sample sets (0030-0045) are non-*Sphagnum* species sampled from a field monitoring site in April 2008.

TMAH thermochemolysis

On-line thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH) was performed using a pulsed mode open pyrolysis system, specifically a CDS 1000 pyroprobe unit (Chemical Data Systems, USA) fitted with a platinum coil and a CDS 1500 valved interface. Approximately 1.2 mg of peat sample was weighed into a quartz pyrolysis tube plugged with pre-extracted silica wool. An internal standard, 5α-androstane was added to the samples to enable accurate quantitative

Table 1. The peatland comprised of four stages.

	Stage	Description
SF	Swamp Forest	seasonally flooded woodland with an understory of shrubs and forest mosses.
FL	Fen Lagg	poorly minerotrophic peatland with a diverse assemblage of sedges, peat mosses, and other plant groups.
BM	Bog Margin	well drained ombrotrophic peatland with low, open tree cover and an understory of dwarf shrubs and peat mosses.
BP	Bog Plateau	ombrotrophic peatland with a distinct microtopography of dry hummocks and wet hollows.

analysis to be carried out on peaks within the chromatogram, and 10 µl of an aqueous solution of TMAH (25%; w/w) was added to the sample immediately prior to THM. The pyroprobe interface was maintained at 340 °C and THM was carried out at 450 °C for 10 s (20 °C/ms temperature ramp) with the products passing into an HP5890 gas chromatograph with an open split (40 mL/min) and a 60 m HP5-MS column (0.25 mm internal diameter, 0.25 µm film thickness, J&W Scientific, USA). Helium was used as carrier gas at a flow rate of 1 ml/min. The GC oven was programmed to start at 100 °C, held for 2 minutes, then raised to 320 °C at a rate of 3 °C /min, where it was held for 16 min. Product detection was carried out using an HP5000 series mass selective detector in full scan mode (*m/z* 50 to 700) with compound identification based on the NIST98 mass spectral library, on ion fragmentation patterns and following the conventions described in other studies (Clifford *et al.* 1995; Hatcher *et al.* 1995), together with the comparison of mass spectra and relative retention times with the literature (Mason *et al.* 2009; Vane 2003).

Results and discussion

THM in the presence of TMAH of the peat mosses and herbaceous plants yields methylated phenols including those with guaiacyl, syringyl and *p*-hydroxyphenyl lignin-derived structures as well as the methyl esters of the cinnamyl phenols, ferulic acid and *p*-coumaric acid. However it is important to note that not all of these particular phenolics are necessarily specific only to lignin. For example, 3,4,5-trimethoxy benzoic acid methyl ester can be formed from other biopolymers such as tannins (e.g. Mason *et al.* 2009). There was one compound identified in the TMAH thermochemolysis products of *P. commune* that was absent during the THM of all of the other moss and vascular plant samples, namely 3,4-dimethoxybenzenepropanoic acid methyl ester. This compound is therefore tentatively suggested as being a marker for *P. commune*. Four distinct phenols were found exclusively in all of the different species of *Sphagnum* mosses collected in this study. The mass spectra and relative retention times for these four potential *Sphagnum* biomarkers (Figure 1) were consistent with the following assignments: methylated 4-isopropenylphenol (*m/z* 133,148), methylated *cis* and *trans* 3-(4'-hydroxyphen-1-yl)-but-2-enoic acid (*m/z* 175, 206), and methylated 3-(4'-hydroxyphen-1-yl)-but-3-enoic acid (*m/z* 133, 148, 206). These compounds were not present in the thermochemolysis products from *Polytrichum commune*. TMAH thermochemolysis of the other mosses and herbaceous plants confirmed that these components are specific to *Sphagnum*. The herbaceous plants and the *P. commune* yielded a full range of lignin-derived phenols as well as 1,3,5-trimethoxybenzene, a significant component of the vascular plant distributions which was absent from the *Sphagnum* thermochemolysis products.

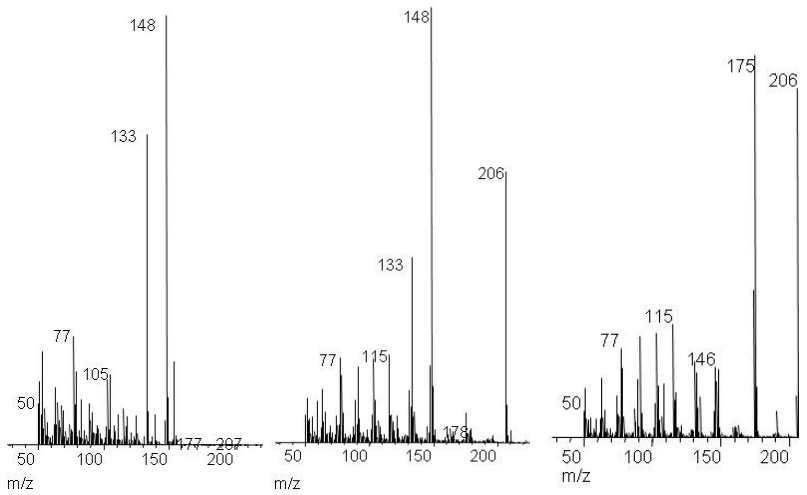

Figure 1. Mass spectra of *Sphagnum* marker 1; 4-isopropenylphenol (*m/z* 148) and *Sphagnum* marker 3; methylated 3-(4'-hydroxyphen-1-yl)-but-3-enoic acid (*m/z* 133, 148, 206), and *Sphagnum* markers 2 and 4; *cis* and *trans* 3-(4'-hydroxyphen-1-yl)-but-2-enoic acid (*m/z* 175, 206).

Conclusions

Lignin is a component of the peat litter and arises from the herbaceous plants present in the litterbag experiments. Biomarkers for a non-lignin source, which is suggested to be *Sphagnum,* have been identified which are the *cis* and *trans* isomers of the methyl esters of 3-(4'-hydroxyphen-1-yl)-but-2-enoic acid as well as 3-(4'-hydroxyphen-1-yl)-but-3-enoic acid methyl ester.

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