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Source and transformations of lignin in *Carex*-dominated peat

Judith Schellekens [a,*], Peter Buurman [b], Thomas W. Kuyper [c]

[a] Departamento de Edafoloxía e Química Agrícola, Universidade de Santiago de Compostela, Fac. de Bioloxía, Campus Universitario Sur, Santiago de Compostela, 15782 A Coruña, Spain
[b] Earth System Science Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands
[c] Department of Soil Quality, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands

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ABSTRACT

We identified the effects of vegetation changes, and aerobic and anaerobic decay on the lignin composition in the Penido Vello peat record (Galicia, Spain). The ombrotrophic part of this peat record was dominated by graminoids and has significant contributions of ericoids at some depths. The organic matter (OM) of different peat fractions (bulk, NaOH-extractable fraction, and non-extractable residues) of 15 samples from the upper meter was analysed with pyrolysis-gas chromatography/mass spectrometry (pyrolysis-GC/MS). In addition, the dominant plant species were analysed, including *Carex durieui*, *Agrostis curtisii*, *Molinia caerulea*, *Deschampsia flexuosa*, *Festuca rubra*, *Eriophorum angustifolium*, *Erica mackaiana* and *Calluna vulgaris*, and their lignin composition compared to that of the peat OM. The high abundance of guaiacol and 4-formylguaiacol in fresh plant tissue compared to peat OM suggests that in addition to *p*-coumaric and ferulic acid (which are abundant in graminoids), other non-lignin phenolic monomers are contributed by graminoid species. For the non-lignin phenolics, graminoids differed from ericoids in the high abundance of ferulic acid (4-vinylguaiacol), while *p*-coumaric acid (4-vinylphenol) showed high and similar abundances in ericoids and graminoids. This result suggests that ratios between *p*-hydroxyphenyl (or *p*-coumaric acid) and other lignin moieties in (pyrolysates of) peat cannot be used as source indicator. Comparison of plant and peat fractions using factor analysis allowed a distinction between the effects of source (plant identity) and decay on the lignin composition of the Penido Vello peat, and different stages of decomposition were identified. Preferential decay of guaiacyl over syringyl moieties was found for the first stage of decay. This preferential decay is probably related to the large abundance of guaiacyl moieties in easily degradable non-lignin phenolics. Preferential decay of syringyl moieties occurred during subsequent aerobic decay.

1. Introduction

In ombrotrophic peatlands, fluctuations in the water table are determined by variation in precipitation only. Bog hydrology strongly influences the botanical composition and decomposition process. The molecular composition of OM in peatlands, soils and sediments supplies information on the botanical sources (plant identity) and on the extent of decomposition during litter decay. Therefore, the molecular composition of OM is often used as a proxy for past environmental conditions (e.g. McClymont et al., 2011), or to obtain information on the rate of carbon sequestration that is a crucial element in the global carbon cycle (Clymo et al., 1998).

Lignin is a significant component of OM in anaerobic ecosystems such as peatlands, as decomposition of lignin primarily depends on the supply of oxygen (Williams and Yavitt, 2003). The knowledge of lignin transformations in soils and sediments, recently reviewed by Thevenot et al. (2010), is mainly based on aerobic systems and uses the abundance of syringyl, guaiacyl and *p*-hydroxyphenyl moieties, oxygen functionality and chain length reduction of alkyl side-chains, and demethoxylation. In anaerobic systems, however, the interpretation of these characteristics may be different. In addition, the lignin composition varies strongly between plant species, plant parts and elements of plant cells, and its resistance to decay may show similar differences (Machinet et al., 2011). Because changes in hydrology drive changes in both plant species composition and degree of decomposition, the interpretation of chemical changes in peat in terms of decomposition is complex (Yeloff and Mauquoy, 2006).

Many methods have been developed to study the lignin composition in plant and soil OM (Lu and Ralph, 2010). Characterisation of lignin is difficult because it is not possible to isolate

* Corresponding author. Tel.: +31 653832087.
E-mail address: schellekens.j@hetnet.nl (J. Schellekens).

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lignin in its intact state. Distinguishing non-lignin phenolics also found in the cell wall from macromolecular lignin can be difficult. In soil OM studies, the lignin composition is usually analysed with the CuO oxidation method (Thevenot et al., 2010), but also pyrolysis-gas chromatography/mass spectrometry (pyrolysis-GC/MS) and tetramethylammonium hydroxide thermochemolysis (Filley et al., 2006; Nierop, 2001) are frequently applied. Although pyrolysis-GC/MS is a destructive method and rearrangements may occur during the pyrolysis process, most lignin fragments produced during pyrolysis retain the substitution patterns of the lignin macromolecule (Martín et al., 1979; Ralph and Hatfield, 1991; Stout et al., 1988). In addition to the lignin composition, pyrolysis-GC/MS gives detailed information on the overall molecular composition, which benefits the interpretation. The method has been successfully applied to characterise peat deposits and provides valid information on both vegetation and decomposition characteristics (e.g. Kuder and Kruge, 1998; Schellekens et al., 2009).

The effects of decomposition on monocotyledon tissues have been studied by few authors (Kuder et al., 1998). The ombrotrophic part of the Penido Vello peat record was dominated by *Carex durieui* and grasses (*Agrostis curtisii*, *Molinia caerulea*, *Deschampsia flexuosa* and *Festuca rubra*) with significant contributions of *Erica mackaiana* and *Calluna vulgaris*, *Eriophorum angustifolium* and mosses at some depths. *M. caerulea*, *E. angustifolium* and mosses indicate wetter conditions, the other plant species drier conditions (Fraga et al., 2005). The molecular composition of the high-resolution sampled ombrotrophic Penido Vello bog (Galicia, Spain) has been previously studied with pyrolysis-GC/MS (Schellekens et al., 2011). Combined interpretation of vegetation markers, groups of pyrolysis products and present-day vegetation composition enabled a reconstruction of bog hydrology. Wet and dry periods identified by molecular chemistry agreed well with other European studies. Thus, the Penido Vello bog, with its known changes in vegetation composition and hydrology, is particularly suited to study the effects of decomposition on the lignin composition in peat.

The purpose of this study is to identify and separate the effects of source and decomposition (aerobic and anaerobic) on the lignin composition of the Penido Vello peat record. Because polysaccharides and lignin-like phenolic monomers are associated with lignin (see Section 4.1), the effects of source and decomposition on polysaccharides and phenolic monomers were also studied.

2. Material and methods

2.1. Location and sampling

The Penido Vello peat is an ombrotrophic bog located in the Xistral mountains (Galicia, NW Spain). Location and bog are described in detail by Martínez-Cortizas et al. (1997, 2002). The 3 m thick peat record dates back to 6000 years BC. For the purpose of this study only the high-resolution sampled (slices of 2 cm thick) upper meter of the peat record was used, as it showed better correlations between vegetation markers than the deeper part, which was sectioned into 5 cm thick slices (Schellekens et al., 2011). The upper meter (51 samples) represents around 2000 years of peat accumulation.

2.2. Samples

Bulk samples of plant species and peat were previously analysed with pyrolysis-GC/MS (Schellekens et al., 2011). In addition, pyrolysates of NaOH-extractable and non-extractable OM of a selection of 15 samples, previously analysed by Buurman et al. (2006) were used. The selection of these samples was based on differences in quantified ^{13}C NMR data. The extractable fraction more reflects decomposed OM and the non-extractable residue more closely resembles intact plant material (Buurman et al., 2006; Schellekens et al., 2009; Schellekens and Buurman, 2011; Zaccone et al., 2008). Comparison of pyrolysates of fresh plant tissues and the extractable and non-extractable peat fraction allows the distinction between effects of source and those of decomposition on the lignin fraction.

2.3. Extraction

An aliquot (0.5 g) of each peat sample was extracted with NaOH (0.1 M, 20 mL), shaken for 24 h under N_2 and centrifuged (1 h) at 4000 g. The extract was decanted and the extraction repeated a second time. The extracts were combined, acidified to pH 1 with HCl, shaken for 24 h, dialysed against distilled water (cut off 10,000 D) and freeze-dried. The residues were acidified, dialysed against distilled water and freeze-dried.

2.4. Pyrolysis-GC/MS

The samples were pyrolysed using a Horizon Curie-Point pyrolyser (Curie temperature 600 °C) connected to a Carlo Erba GC8000 gas chromatograph. The pyrolysis products were separated on a non-polar fused silica column (Chrompack 25 m, 0.25 mm i.d.) coated with CP-Sil 51 b (film thickness 0.40 μm), with He as carrier gas. The initial oven temperature was 40 °C and the heating rate 7 °C min^{-1}. The final temperature, 320 °C, was maintained for 15 min. The GC column was connected to a Fisons MD800 mass spectrometer (m/z 45–650, cycle time 1 s).

2.5. Quantification

In addition to the 106 pyrolysis products quantified for the bulk samples (Schellekens et al., 2011), a number of less common lignin pyrolysis products were quantified. This resulted in a total of 120 quantified pyrolysis products. These 120 pyrolysis products were also quantified for the peat extracts and residues and for the plant samples. Quantifications were based on the peak area of two major fragment ions of each pyrolysis product (Appendix). All quantifications were checked manually. For each sample, the sum of the peak areas was set at 100% and relative proportions were calculated with respect to this sum. The resulting quantification gives the abundance of each pyrolysis product, expressed as percentage of the total ion current (TIC).

The pyrolysis products were grouped, according to probable origin and chemical similarity, into a number of source groups: polysaccharides, aliphatic biopolymers, methylketones, lignins, phenols, catechols, (other) aromatics, polyaromatics, nitrogen compounds, fatty acids, steroids and triterpenoids. Factor analysis was applied using Statistica® Version 6 (Statsoft Inc, Tulsa).

3. Results

The abundance of groups of pyrolysis products for plant and peat OM are given in Table 1. The abundance of lignin-derived pyrolysis products as percentage of the total quantified pyrolysis products are given in Table 2. Based on analysis of 18 plant species markers were found for graminoids, ericoids, and woody species (including ericoids but not exclusively ericoids; Schellekens et al., 2011).

3.1. The composition of lignin-derived pyrolysis products in plant species

To interpret the chemical composition of the lignin–carbohydrate complex (LCC), the ratio of lignin to polysaccharides is used in addition to groups of pyrolysis products (Table 1). In graminoids the