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Comparison of extraction efficiencies for water-transportable phenols from different land uses

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ABSTRACT

The composition and quantification of vascular plant-derived phenols in dissolved organic matter (DOM) is of importance in understanding and estimating carbon flux from soils under different land uses. Solid phase extraction (SPE) was used to extract waterborne organic matter (WBM), and thermally assisted hydrolysis (THM) using tetramethylammonium hydroxide (TMAH) was compared with gas chromatography-flame ionization detection (GC-FID) for the quantification of oxygenated aromatics in WBM, from freshwater samples from grazed grassland, woodland and moorland land uses in southwest England, UK.

WBM recovered with SPE correlated with water total organic carbon (TOC) content. SPE followed by THM was shown to be the approach for isolating and quantifying water-transportable phenols. All the different land uses exported similar amounts of lignin per unit weight of OC to the drainage water. We also conclude that a significant proportion of lignin phenols is lost from soils as a component of WBM in a particulate form, so the magnitude of total phenol loss is likely greater than previously thought.

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1. Introduction

Dissolved organic matter (DOM) represents an important fraction of organic carbon (OC) since it is the most mobile fraction, affecting many biogeochemical cycles in terrestrial and aquatic environments (Bolan et al., 2011). The recent intensification of the hydrological cycle with changing climate (Durack et al., 2012) emphasizes the need to characterise the molecular composition of DOM in different land uses. Riverine dissolved OC (DOC) concentration varies from ca. 1 mg/l in alpine environments (Meybeck, 1982) to 25 mg/l for rivers draining swampy areas, e.g. the Satilla River, Georgia (Berner and Berner, 2012), influenced by climate variables, such as a wide annual mean temperature with sufficient precipitation (Tian et al., 2013). The worldwide average of 5.75 mg/l (Meybeck, 1982) equates to a flux of 0.25×10^{15} g riverine DOC/yr transported to the ocean (Hedges et al., 1997). The most important sources of DOM in soils are decomposed plant litter, root exudates and microbial biomass (Kalbitz et al., 2000), comprising, amongst others, lignin-derived phenols, carbohydrate-derived compounds, n-alkanoic acids,

n-alkanes and smaller amounts of N-containing compounds such as amino acids (Frazier et al., 2003, 2005; Bowen et al., 2009).

In soils, there is evidence that lignin phenols protect soil OM (SOM) from oxidation, contributing to the antioxidant capacity of soils, by scavenging reactive free radicals, thereby terminating the oxidative chain reaction (Rimmer, 2006; Rimmer and Abbott, 2011). Aromatics in DOM, such as lignin-derived compounds, can be preferentially stabilized by sorption to soil minerals, thereby contributing to stable forms of SOM, compared with more labile components such as carbohydrates (Kalbitz et al., 2005). This is supported by the observation of decreasing dissolved lignin phenols concentration with increasing depth in mineral soils in forest ecosystems, attributed to their sorption to the soil matrix (Guggenberger and Zech, 1994). However, fractionation of phenols can arise where more oxidised (carboxylated) phenols tend to remain in soil solution (Guggenberger and Zech, 1994), also observed in their preferential dissolution in leachates directly from plant litter (Hernes et al., 2007). Hedges and Parker (1976) proposed a parameter for the total amount of lignin phenols in a sediment (Λ) by summing the weights of phenols equivalent to S (S4, S5 and S6) and G (G4, G5 and G6) moieties normalised to OC. This was later corrected to take into account the input of cinnamyl phenols (G18 and P18) to the sedimentary OM (Hedges and Mann, 1979b). Reported total dissolved lignin phenol concentration from

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the USA ranges from 0.07 mg/100 mg OC in island drains from the Sacramento-San Joaquin River Delta (Eckard et al., 2007) to 8.13 mg/100 mg OC (for the 1000 Da–0.2 μm DOC fraction) for the Big Pine Creek watershed (Dalzell et al., 2005), determined from CuO oxidation. Assuming an average dissolved lignin phenol concentration of 2.65 mg/100 mg OC (the mean of published riverine values; Ertel et al., 1986; Benner and Opsahl, 2001; Frazier et al., 2003; Dalzell et al., 2005; Eckard et al., 2007), this amounts to a riverine flux of 6.61×10^{12} g dissolved lignin phenols/yr, which clearly represents a substantial loss of terrestrial C in the form of lignin. In addition to the large losses of dissolved lignin phenols, the molecular structure of lignin monomers is unique to vascular plants (Hedges and Mann, 1979a), allowing them to be used as terrestrial biomarkers in aquatic ecosystems (Gardner and Menzel, 1974; Goni et al., 1997).

Solid phase extraction (SPE) has been widely used to isolate DOC from aqueous solutions (Aiken et al., 1979; Meyersschulte and Hedges, 1986; Moran et al., 1991; Lara and Thomas, 1994; Simpson, 2000; Wang et al., 2012). Comparison of hydrophobic sorbents (C_2, C_8, C_{18}, cyclohexyl and phenyl XAD-2) and ultrafiltration to extract marine humic substances, found that C_{18} achieved the greatest extraction efficiency (Amador et al., 1990). Comparison of different solid phase "sorbents" silica-based octadecyl bonded phases (C_{18}, C_{18}EWP and C_{18}OH), silica-based octyl bonded phase (C_8) and modified styrene divinyl benzene polymers (PPL and ENV) – on surface seawater samples from the north Brazilian shelf off the Maracaçumé Estuary showed that PPL achieved the highest extraction efficiency for DOC, although C_{18} was more selective for terrigenous compounds (Dittmar et al., 2008). The extract from C_{18} SPE in disc form and original DOC from riverine samples had a similar distribution of functional groups, indicated from nuclear magnetic resonance (NMR) analysis (Kim et al., 2003). Therefore, many studies investigating dissolved lignin degradation products from freshwater (Louchouarn et al., 2000), estuarine water (Dittmar et al., 2007; Bianchi et al., 2009), oceans (Hernes and Benner, 2006) and stalagmites (Blyth and Watson, 2009) have favoured C_{18} SPE. Comparison of solvents such as MeOH or MeCN to activate and elute sorbed fresh, estuarine and marine DOC from C_{18} SPE cartridges found little difference in cinnamyl:vanillyl (C:V), syringyl:vanillyl (S:V) and acid:aldehyde ratios, although greater recovery of freshwater Λ_8 lignin phenols was achieved using MeOH (76.2–91.1%, mean 86.0%) than MeCN (48.3–77.4%, mean 67.4%) when compared with a sample prepared using rotary evaporation (Spencer et al., 2010).

Thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH), sometimes known as TMAH thermochemolysis, has been used off-line to analyse DOC, soils and vegetation (Martín et al., 1995; del Rio et al., 1998; Huang et al., 1998; Frazier et al., 2003). It has also been employed on-line with gas chromatography – mass spectrometry (GC–MS) to characterise the molecular components of plant litter, soils, aquatic humic substances (Saiz-Jimenez et al., 1993; Challinor, 1995; Clifford et al., 1995; Mason et al., 2009, 2012)

and terrestrial OM preserved in stalagmites (Blyth and Watson, 2009). One of its most interesting applications is in the characterisation of phenolic compounds with side chains extending up to three carbons formed from the TMAH-induced cleavage of ether and ester bonds in plant-derived polyphenols present in soils (e.g. Mason et al., 2012) and peat (e.g. Schellekens et al., 2015).

The aims of this study were to: (i) assess the yield from C_{18} SPE during the extraction of water-transportable lignin phenols from a range of natural freshwaters, and compare cold on-column GC and on-line THM using TMAH to detect extracted waterborne phenols, (ii) compare water TOC, total phenol concentration and phenolic diversity from different land uses – grazed grassland, woodland and moorland – chosen because they represent the three dominant land use types in southwest England.

2. Material and methods

2.1. Sampling sites and collection

River, soil drainage and pond water samples were collected in triplicate from six sites (Table 1) in the vicinity of Rothamsted Research North Wyke, Devon, southwest England, UK [50°45′N, 4°53′W] across different land uses (grazed grassland, woodland and moorland). The first replicate from each site was collected on May 11, 2010. The second and third replicates from the sites Grassland 1, Grassland 2, Woodland 1, Grassland 3 and River were collected on July 21, 2010. The second and third replicates from Woodland 2 were collected on January 24, 2011 since this site was dry until then. Water samples were collected in a bucket (15 l) to ca. half-full, before transferring a subsample (5 l) to two amber glass bottles (2.5 l) on-site. Sample pH was measured and all samples were adjusted to pH 2 by adding sufficient drops of concentrated HCl (Trace analysis grade, 37%; Fisher Scientific) and stored in a fridge until extraction.

2.2. Soil and livestock dung sampling

The soils were sampled from each of the grassland, woodland, and moorland land use types (Table 1) which had adjacent water outlets within 100 m for sampling. Fifteen soil cores (25 mm di., < 30 cm depth) were taken in 3 replicates of 5 in a 'W' spatial sampling pattern using a soil auger for each land use. The O and A horizons were separated for analysis, and the impermeable clay B horizon was discarded. The 5 samples constituting each of the 3 replicates were homogenised.

Representative samples of fresh cattle and sheep dung solids ($n = 3$) were collected from each of the grassland plots (Table 1).

2.3. Sample extraction

Total OC (TOC) content was determined for water samples (CA14 Formacs, Skalar (UK) Ltd.). The carrier gas was purified air, supplied by a TOC gas generator (scrubbed of CO_2 and moisture),

Table 1
Geographical positions of sampling sites with Ordnance Survey national grid reference (G.R.) and land use descriptions. River is a mixed land use sample (moorland, grazed grassland and woodland).

Sample	Position and description	G.R.
Grassland 1	Taw Barton Farm. Grazed grassland artificial drainage water	SX654971
Grassland 2	Sticklepath. Mix of spring water & grazed grassland drainage	SX641939
Grassland 3	Josephs Carr Pond in a wet mire	SX654988
Woodland 1	Tributary of River Taw. *Quercus robur* dominated woodland	SX665979
Woodland 2	Orchard Dean Copse. Seasonally flowing ditch in woodland	SX653982
River	River Taw, Moorland source, through grazed grassland and woodland	SX653984

and the inorganic catalyst was 2% orthophosphoric acid. Water samples were then extracted using FD or SPE (described below) and analysed on the basis of a split-split-plot experimental design.

SPE of water samples was carried out using a published method (Louchouarn et al., 2000) except that samples were not initially filtered (0.2 μm). Reversed phase C_{18} end capped SPE cartridges (60 ml, 10 g, Mega-Bond Elut; Agilent Technologies) were mounted on a vacuum manifold (VAC ELUT-20, 13 × 75 mm, Varian) connected to a vacuum pump (Gast Diaphragm pump, model: DOA-P504-BN; Gast Manufacturing, Inc., USA) via a liquid trap (Carboy Bottle 20 l, part 2226-0050 with filling venting closure, part 2161-0830; Varian Ltd.), enabling up to 10 SPE cartridges to be used simultaneously. Each cartridge was preconditioned with 100 ml MeOH (HPLC grade; Fisher Scientific) followed by 50 ml pure water (MilliQ Gradient A10) acidified to pH 2 (Trace analysis grade HCl acid, 37%; Fisher Scientific). Water (2.5 l) was drawn through the SPE cartridges at an average rate of ca. 20 ml/min via a Teflon transfer pipe (1/8 in. × 0.1 in.; Part AL20096, Varian Ltd.) and adapters (part 12131004, Varian Ltd.) to seal the SPE cartridge. After samples were extracted, cartridges were rinsed with 50 ml acidified pure water (pH 2) to remove any residual salts. Louchouarn et al. (2000) rinsed with 1 l acidified water as they analysed saline in addition to freshwater samples. Then, collection bottles (60 ml; Part BTF-543-030X, Fisher Scientific) were placed inside the vacuum manifold under each SPE cartridge prior to eluting the retained WBM in one fraction with 50 ml MeOH. The MeOH was evaporated from the collection bottles at 40 °C under a stream of N_2 and transferred quantitatively to a 5 ml glass vial before final evaporation to dryness and being capped and stored under N_2 in a freezer until analysis. Procedural blanks (ultrapure Milli-Q water) were analysed separately in order to assess potential contamination during sample handling.

2.4. Sample derivatisation and analysis

The dry WBM residues (ca. 3 mg) extracted using FD and SPE were analysed for total C and total N using a Carlo Erba NA2000 analyser (CE Instruments, Wigan, UK) and a SerCon 20–22 isotope ratio mass spectrometer (SerCon Ltd., Crewe, UK) at Rothamsted Research North Wyke. Wheat flour (1.91% N, 41.81% C, 4.80 δ^{15}N and −26.41 δ^{13}C) calibrated against IAEA-N-1 by Iso-Analytical, Crewe, UK was used as a reference standard.

An aliquot of SPE (ca. 2 mg) extract was derivatised with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in a GC vial (part: STV12-02L, Kinesis) with limited volume insert (part: INWC-01, Kinesis), by dissolving in 50 μl pyridine, and whirlimixed for 15 s with BSTFA (5 drops). Trimethylchlorosilane (TMCS, 1%) was added before capping the vials and heating the samples at 60 °C for 1 h. The samples were then blown down to dryness under N_2. 5α-Androstane (3 μl; 0.1 mg/ml) was added to each sample as an internal standard. Samples were redissolved in dichloromethane (DCM) and analysed using cold on-column GC with flame ionisation detection (GC-FID) for quantification and GC–MS for identification of the trimethyl (TMS) derivatives.

Cold on-column GC-FID (HRGC 5160 Mega Series; Carlo Erba Strumentazione) was used with a 60 m HP-5, 0.25 mm i.d., 0.25 μm film thickness column (Agilent J&W). The GC oven programme was 40 °C (2 min) to 300 °C (held 1 min) at 2 °C/min, then to 320 °C (held 40 min) at 20 °C/min (run time 174 min). The carrier gas was H_2 at 2 ml/min. Injection volume was 1 μl.

GC–MS analysis of the BSTFA-derivatised total solvent extract was performed with an Agilent 7890A GC split/splitless injector (280 °C) linked to an Agilent 5975C mass selective detector (electron voltage 70 eV, source 230 °C, quad 150 °C electron multiplier (EM) 1800 V, interface 310 °C). Samples were manually injected (1 μl) in splitless mode for 1 min before switching to an open split

(30 ml/min) using a 60 m HP5 ((5% phenyl)-methylpolysiloxane) column (0.25 mm i.d., 0.25 μm film thickness; Agilent J&W Scientific, USA) using the same GC oven temperature programme as for GC-FID. H_2 was the carrier gas at 1 ml/min. Product detection was carried out in full scan mode (m/z 50–700), EM voltage was 2176 V. Acquisition was controlled with a HP Compaq computer using Chemstation software.

An aliquot of SPE extracted WBM was analysed using THM with TMAH. Extracted sample (ca. 1 mg) was weighed into a quartz pyrolysis tube plugged with solvent-extracted glass wool. The glass wool had been extracted with DCM:MeOH (93:7, v/v) in a Soxhlet apparatus for 24 h. 5α-Androstane in DCM (3 μl; 0.1 mg/ml) was added as internal standard to the pyrolysis tube. Immediately prior to analysis, TMAH (5 μl; 25%; w/w in aqueous solution) was added. The tube was inserted into the Pt pyrolysis coil and flash pyrolysed at 610 °C for 10 s (20 °C/ms ramp). The temperature and ramp rate were chosen as they were successful in previous studies investigating lignin phenols (Clifford et al., 1995; Huang et al., 1998; Mason et al., 2009, 2012) and *Sphagnum*–derived phenols (Abbott et al., 2013). The pyroprobe interface was maintained at 340 °C with the products passing into an HP6890 GC instrument with an open split (30 ml/min) and a 60 m HP5-MS column (0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, USA). He was the carrier gas at 1 ml/min. The GC oven was programmed from 50 °C to 220 °C (held 1 min) at 1.5 °C/min, and then to 320 °C (held 16 min) at 15 °C/min. Detection was carried out using an HP5973 series mass selective detector (MSD) in full scan mode (m/z 50–700). Identification was based on the NIST98 mass spectral library as well as comparison with relative retention times and mass spectra reported in other studies (Clifford et al., 1995; del Rio et al., 1998; Chefetz et al., 2000; Nierop, 2001; Vane et al., 2001; Vane, 2003; Robertson et al., 2008; Mason et al., 2009, 2012).

2.5. Data presentation and statistical data analysis

Total lignin phenol concentration was normalised to 100 mg OC for each site. The statistical data analysis was carried out using analysis of variance (ANOVA) with GenStat 64-bit Release 14.1 and correlation using Microsoft Excel. Statistical significance was tested at the 95% level, and Tukey's 95% confidence intervals test was used to identify statistical differences.

3. Result and discussion

3.1. WBM extraction yield

SPE recovered 11.92 ± 2.69, 0.30 ± 0.30, 20.97 ± 6.67, 8.97 ± 3.17, 6.93 ± 0.97 and 3.24 ± 0.95 mg/l of WBM from Grassland 1, Grassland 2, Grassland 3, Woodland 1, Woodland 2 and River sites, respectively (Fig. 1b). The total weights of WBM recovered with SPE are reported in Table 2.

Expressed as a concentration, the amount of WBM recovered from the six water samples by SPE (Fig. 1b) correlated with water TOC (Fig. 1a, r^2 0.9542, $P < 0.001$). This confirms the organic nature of the isolated WBM.

3.2. Phenol extract yield from THM and GC-FID

Phenols were components of the TMAH thermochemolysis products from the SPE-extracted WBM at all 6 sites (Fig. 2) with total phenol concentration ranging between 0.09 ± 0.05 (Grassland 2) and 0.82 ± 0.37 mg/100 mg OC (Grassland 1). Previously, C_{18} SPE has demonstrated excellent recovery of lignin phenols (101 ± 4%) and repeatability from freshwater samples compared with direct dry-down (Louchouarn et al., 2000). The total concentration values
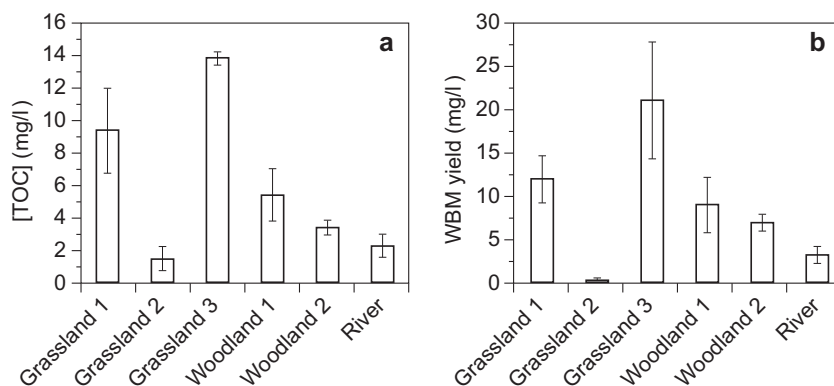
Fig. 1. Mean (± standard error of mean, $n = 3$) of (a) TOC content of the water samples from different land use sites (Table 1); (b) total extract yield from SPE waterborne matter (WBM).

Table 2
Mean (± standard error of mean in parentheses, $n = 3$) water sample pH, waterborne matter (WBM) yield and TOC in solid phase extracted (SPE) WBM solid residues for 6 water samples from sites described in Table 1 (n.d., not detected).

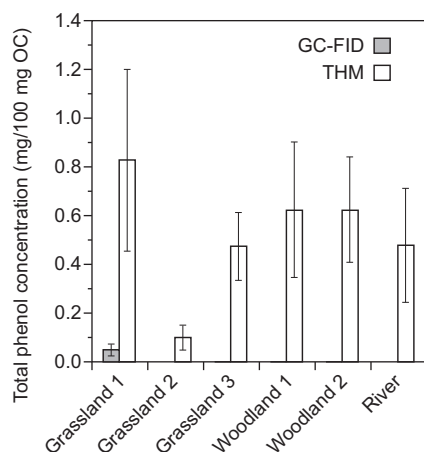| Site	pH	WBM yield (mg)	TOC of WBM residue (%)
Grassland 1	7.33 (0.20)	30.6 (7.9)	47.2 (2.2)
Grassland 2	6.39 (0.06)	2.5 (1.0)	59.1 (4.8)
Grassland 3	6.50 (0.21)	38.3 (14.0)	46.5 (3.4)
Woodland 1	7.14 (0.09)	23.9 (8.5)	44.2 (3.7)
Woodland 2	6.54 (0.01)	17.8 (0.9)	41.9 (2.9)
River	7.35 (0.18)	9.9 (2.7)	50.6 (2.0)

Fig. 2. Mean (± standard error of mean, $n = 3$) total phenolic concentration from SPE water samples detected using cold on-column GC-FID and THM. Sites are described in Table 1.

3.3. Phenol diversity in SPE extracts detected with THM or GC-FID

Following SPE and detection with GC-FID, some benzoic acids were found as their respective TMS ethers and esters (abbreviated to P6, G6, PA and S6) in 4 of the water samples (Grassland 1, Grassland 3, Woodland 1 and Woodland 2; Table 3). Grassland 1 contained the highest OC-normalised amounts with P6, PA and G6 having 38.8, 3.4, and 3.6 µg/100 mg OC, respectively. No phenols were detected in Grassland 2 or River sites. For Grassland 2, this may be due to the very low level of total phenols (Fig. 2), whereas for the River sample, it may be due to a large proportion of phenols existing in oligomeric or particulate form not identifiable via GC-FID. PA is the only acid in both Woodland 1 and Woodland 2 water samples, which have a significant input from *Quercus robur* (oak). In its underivatised form PA is 3,4-dihydroxybenzoic acid (protocatechuic acid) and has also been detected in oak dominated soils in the Netherlands (Nierop and Filley, 2007).

TMAH thermochemolysis yielded significantly more phenols at all 6 sites than from the GC-FID analysis of the WBM isolated from each of the water samples with SPE (Fig. 2). These included vascular plant-derived phenols released by guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) lignin units (Ralph et al., 2004) as well as phenols (P) from other sources.

Thermochemolysis releases phenols produced from the TMAH-induced cleavage of ether and ester bonds in the lignin macromolecule (Hatcher et al., 1995; Wysocki et al., 2008). A structurally diverse range of phenolics, in higher concentration as well as in a less oxidised form, were detected with THM than with GC-FID. This suggests that a significant proportion of dissolved lignin phenols is leached from the soil in an oligomeric form, rather than as monomers.

3.4. Water TOC and phenols from different land uses

The TOC and lignin phenol parameters for the grazed grassland and woodland water samples reflected ecosystem level inputs, incorporating any interaction between the contributing inputs within each ecosystem. The River sample was also subject to catchment scale processes, including interactions between grazed grassland, woodland and moorland ecosystems. The Grassland 3 aquatic sample (see Fig. 1a) had the highest TOC (13.86 ± 0.41 mg/l), whereas water from the Grassland 2 site had the lowest TOC (1.50 ± 0.75 mg/l, Fig. 1a), which were significantly different ($P < 0.001$). The Grassland 2 aquatic OC concentration (1.50 ± 0.75 mg/l) was also significantly different from the Grassland 1 sample (9.41 ± 2.64 mg/l), whereas water from the Woodland 1, Woodland 2 and mixed land-use River sites had

of SPE isolated phenols detected using THM for Grassland 2, Grassland 3, Woodland 1, Woodland 2 and River sites here were comparable with total lignin phenol (Λ_8) concentration determined from freshwaters [Penobscot River, Maine (Spencer et al., 2010) and the Big Pine Creek watershed (Dalzell et al., 2005)] using CuO oxidation. Following SPE extraction, GC-FID only allowed phenols to be detected at 4 of the 6 sites and in lower abundance than those released during THM, with concentration ranging between 0 mg/100 mg OC in Grassland 2 and River to 0.05 mg/100 mg OC in Grassland 1 (Fig. 2).

THM in the presence of TMAH released an increase of more than one order of magnitude in the amount of phenols relative to the amount detected with GC-FID for all the samples.

Table 3
Mean (± standard error of mean, n = 3) water-transportable benzoic acids concentrations, as TMS derivatives, from six sites (see Table 1), extracted using SPE and detection using GC-FID (PA, protocatechuic acid; n.d., not detected).

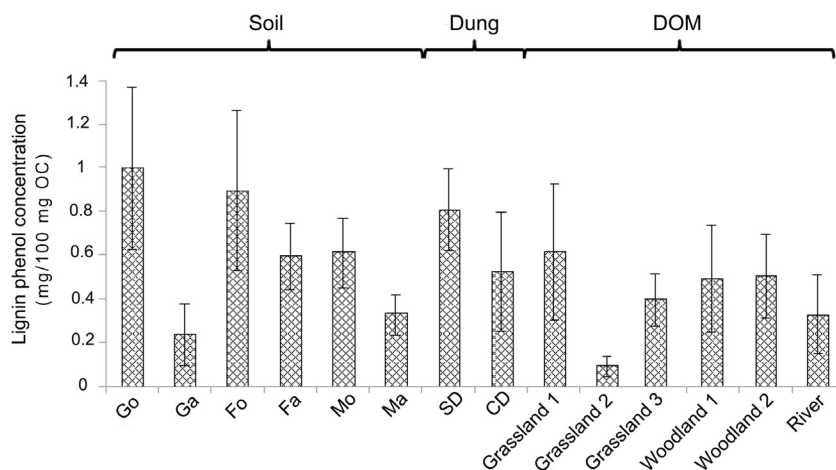| TMS derivative	Abbr	Concentration (mg/100 mg OC)					
		Grassland 1	Grassland 2	Grassland 3	Woodland 1	Woodland 2	River
4-[(TMS)oxy]-benzoic acid TMS ester	P6	38.75 (21.51)	n.d.	0.28 (0.28)	n.d.	0.08 (0.08)	n.d.
3-Methoxy-4-[(TMS)oxy]-benzoic acid TMS ester	G6	3.40 (2.82)	n.d.	0.57 (0.57)	0.3 (0.3)	n.d.	n.d.
3,4-Bis[(TMS)oxy]-benzoic acid TMS ester	PA	3.61 (0.70)	n.d.	0.36 (0.36)	0.27 (0.27)	0.73 (0.73)	n.d.
3,4,5-Tris(TMSoxy)-benzoic acid TMS ester	S6	n.d.	n.d.	n.d.	1.63 (1.63)	n.d.	n.d.

Fig. 3. Mean (± standard error of mean, n = 3) total lignin phenol concentrations in soils, dung and DOM extracts (G, grazed grassland; F, woodland; M, moorland; subscripts o and a denote the soil organic and A horizons respectively; SD, sheep dung; C, cattle dung).

statistically similar TOC concentrations (5.45 ± 1.60, 3.42 ± 0.46 and 2.32 ± 0.72 mg/l, respectively in Fig. 1a). Increased TOC content was measured in the Grassland 1 and 3 water as compared with both woodland samples. Grassland 2 was probably diluted by a contribution of underground spring water with low OM content (Fig. 1a). The TOC content of SPE extracted WBM was also greater in grazed grassland than in woodland water samples (Table 2). In another study, increased TOC values were also detected in the leachates from grass litter-amended soil lysimeters compared with ash and oak leaf litter-amended lysimeters, also revealing that grass litter lost more OC as DOC than oak and ash litter (Williams et al., 2016).

Mean water pH (± standard error, n = 3) decreased as follows: River (7.35 ± 0.18) > Grassland 1 (7.33 ± 0.20) > Woodland 1 (7.14 ± 0.09) > Woodland 2 (6.54 ± 0.01) > Grassland 3 (6.50 ± 0.21) > Grassland 2 (6.39 ± 0.06, Table 2).

Since SPE followed by THM was the better approach for detecting and identifying lignin phenol biomarkers, this combination was used to characterise the phenolic thermochemolysis product distributions with the aim of investigating a relationship with land use. Fig. 2 shows that total dissolved phenols represented a relatively minor component (0.09 ± 0.05% to 0.82 ± 0.37%) of DOC from all ecosystems, comparable with total phenol concentrations in leachates from lysimeter grass and woody leaf litter degradation studies also detected with THM (Williams et al., 2016) as well as in leaf litter itself (Klotzbücher et al., 2011; Williams et al., 2016). This indicates little or no net additional soil contribution to total phenolic concentration lost in the dissolved phase at the lysimeter or ecosystem scale. Negligible amounts of phenols were detected in the River sample with SPE followed by GC-FID (Table 3), although SPE followed by THM of the same sample detected similar total phenol concentration to the Grassland 3 sample. This indicates that the phenols in the River sample were in oligomeric form, so not identifiable with GC-FID.

G2 (3,4-dimethoxytoluene), G3 (3,4-dimethoxystyrene), P3 (4-methoxystyrene) and P4 (4-methoxybenzaldehyde) were only present in grassland-sourced water. These phenols have been detected in grassland litter and soils (Huang et al., 1998; Mason et al., 2012). G3 and P3 are also associated with non-woody lignin (Clifford et al., 1995; Chefetz et al., 2000). S2 (3,4,5-trimethoxytoluene) and S18 [trans-3-(3,4,5-trimethoxyphenyl)-propenoic acid methyl ester) were present only in the Woodland 1 water sample.

Total lignin phenol concentration values in soil O and A horizons, as well as animal dung and DOM from grazed grassland, woodland and moorland are presented in Fig. 3. This comparison shows that OC-normalised total lignin concentration for DOM in the freshwater samples was similar to total lignin concentration in soil O and A horizons, for their respective land uses (Fig. 3). This suggests that all the different land uses export a similar amount of lignin per unit weight of OC into the drainage water.

4. Conclusions

The amounts of vascular plant-derived phenols from THM were more than an order of magnitude higher than those measured using GC-FID in SPE extracted WBM, so SPE followed by THM was recognised as the approach to recover and identify water-transportable phenols. However, SPE is not able to recover phenols in particulate form, so there may be an underestimation of the flux of total waterborne lignin phenols lost from soils and ecosystems using this method. All the different land uses investigated export similar amounts of lignin per unit weight of OC into the drainage waters.

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