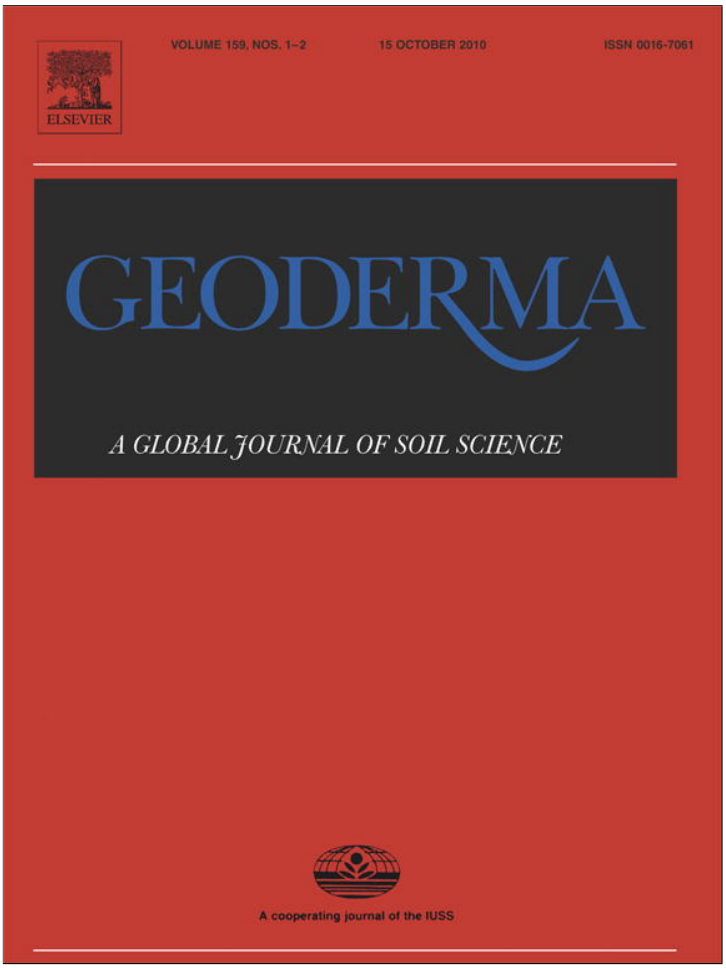

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Spectroscopic characterization of hot-water extractable organic matter from soils under four different vegetation types along an elevation gradient in the Wuyi Mountains

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ABSTRACT

Water extractable organic carbon (WEOC) is the most active component in global carbon cycle and its chemical and structural characteristics most likely influence its biodegradation. Our objective was to investigate the structural characteristics of hot-water extractable organic matter (HWEOM) from soils collected at various depths (S1, S2, S3) under four different vegetation types, i.e. evergreen broad-leaved forest (EBF), coniferous forest (CF), dwarf forest (DF) and alpine meadow (AM) along an elevation gradient in the Wuyi Mountains, and to relate its structural characteristics to soil properties and biological properties. Here we examine the structural characteristics of HWEOM by UV, fluorescence and Fourier-transform infrared (FTIR) spectroscopic techniques. In the synchronous fluorescence spectra of all HWEOM samples, the main emission peaks were aromatic amino acid-like fluorophores, fulvic acid-like fluorophores and polycyclic aromatic structures with a high degree of conjugation. FTIR spectroscopy showed that HWEOM featured aliphatic C–H, aromatic C=C and carbohydrate C–O functional groups. The aromaticity, the humification indices (HIXem, HIXsyn) and the fluorescence efficiency of HWEOM from the S1 layer were significantly higher in CF than those in the other three vegetation types and, in all cases they decreased with depth in the soil profile. Soil C/N ratio was highly correlated with UV absorption, humification indices and fluorescence efficiency of HWEOM. Soil metabolic quotient (qCO_2) was highly negatively correlated with UV absorption and humification indices of HWEOM. The ratio of microbial biomass C to total organic C was significantly correlated with humification indices and fluorescence efficiency of HWEOM. Our results indicate that the HWEOM from CF soils contain more highly condensed aromatic and/or heterocyclic compounds and, at the same time, the high molecular weight and complex molecules are less mobile, leading to being preferentially retained in the upper soil layer.

1. Introduction

Dissolved organic matter (DOM) in soil may be the most important C source, since most microbial metabolism depends on water transport of resources (Metting, 1993). Approximate 10–20% of the above-ground C input from litter is released as dissolved organic carbon (Currie et al., 1996; Michalzik et al., 2001). Some of this C is utilized by microbes, some is retained in the soil by abiotic mechanisms, and some is transported into aquatic systems (McCracken et al., 2002).

The quantity and biological nature of DOM are affected by extraction temperatures. The amount of hot-water (at 80 °C) extractable organic carbon (HWEOC) exceeds cold-water extractable one by a factor of two (Gregorich et al., 2003). HWEOC was strongly positively correlated with microbial biomass C, microbial biomass N, total carbohydrates, and therefore it is one of the most sensitive indicators among the soil biochemical measurements considered to reflect the changes in the SOM caused by different soil management practices (Ghani et al., 2003).

The biodegradability of DOM is controlled by its intrinsic characteristics such as its molecular structure, functional group content or molecular size (Marschner and Kalbitz, 2003). Chemical characteristics of DOM/WEOM are usually analyzed by techniques such as UV, fluorescence, FTIR and NMR spectroscopy (Chin et al., 1994; McKnight et al., 1997; Zsolnay et al., 1999; Parlanti et al., 2000; Kalbitz et al., 2003). So far, few studies have focused on the structural characteristics of HWEOM from soils under different vegetation types along an elevation gradient.

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To obtain deeper insights into the dynamics of carbon in soils under different vegetation types in the Wuyi Mountains in southeastern China, we have studied the structural characteristics of HWEOM most likely to influence its biodegradability. The objectives of this study were to (1) compare the quantities and the structural characteristics of HWEOM from soils under four different vegetation types along an elevation gradient by means of chemical and spectroscopic analysis, (2) investigate the changes in the structural characteristics of HWEOM down the soil profile, and (3) relate its structural characteristics to soil properties and biological properties.

2. Materials and methods

2.1. Site description

The selected site, Wuyi Mountain National Nature Reserve (27°33′–27°54′N, 117°27′–117°51′E) with distinct vertical zones of vegetation along the elevation gradient, is located in the subtropics in southeastern China. Four sites with different vegetations were established along an elevation gradient. Subtropical evergreen broad-leaved forest (EBF), sampled at an elevation of 500 m above sea level (masl), with annual mean rainfall of 1700 mm and annual average temperature of 17–19 °C (He et al., 1994; Zheng and Fang, 2004), is mainly composed of *Castanopsis carlesii*. Conifer forest (CF), sampled at an elevation of 1150 m masl, with annual average temperature of approximately 14.5 °C and annual mean rainfall of 2000 mm (He et al., 1994; Zheng and Fang, 2004), is dominated by *Pinus tanwanensis* and *Oligostachyum oedogonatum*. Subalpine dwarf forest (DF), sampled at an elevation of 1750 m masl, with annual average temperature of 11.2 °C and annual mean rainfall of 2200 mm (He et al., 1994; Zheng and Fang, 2004), is dominated by deciduous *Symplocos paniculata* and *Stewartia sinensis* with an average stature of 4.5 m. Alpine meadow (AM), sampled at an elevation of 2150 m masl, is covered by herbaceous vegetation with a mean height of 25 cm, i.e. *Calamagrostis brachytricha, Miscanthus sinensis* and *Lycopodium clavatum*. The annual average rainfall and temperature are about 3100 mm and 9.1 °C, respectively (He et al., 1994; Zheng and Fang, 2004).

2.2. Soil sampling and analysis

We set three 3×3 m soil sampling plots in each of the four vegetation types in May 2008. Soil samples were collected at three different soil depths, 0–10 cm (S1), 10–25 cm (S2) and 25–40 cm (S3). The soil samples were sieved (2 mm) and at the same time visible roots and animals were removed. Soil samples were stored at 4 °C until analysis. Soil pH was measured in a 1:2.5 soil–water suspension using a glass electrode. The particle size was determined by wet sieving and sedimentation using the pipette sampling technique. Soil total organic C (TOC) was measured by wet digestion with potassium dichromate (Nelson and Sommers, 1982). Total N was measured with dry combustion and thermal conductivity detection using a C/N/S-Analyser (Vario EL III, Elementar, Germany).

2.3. Hot-water soluble organic matter extraction

Soil samples were extracted with de-ionized water (water/soil ratio of 2:1) and shaken at 80 °C for 20 min. The suspension was centrifuged for 20 min at 4000 rpm, and then the supernatant was filtered through 0.45 μm cellulose acetate membrane filters (Schleicher & Schuell, OE 67). This filtered HWEOM was then stored in a −20 °C freezer until analysis. The concentration of HWEOC was measured with a Shimadzu 5050 TOC Analyzer.

2.4. Soil microbial biomass C

Microbial biomass carbon (MBC) was measured using fumigation-extraction method (Vance et al., 1987). Each sample (20 g fresh soil) was fumigated for 24 h at 25 °C with $CHCl_3$ (ethanol-free). After removal of the $CHCl_3$, the soil was extracted with 60 ml of 0.5 M K_2SO_4 by oscillating for 30 min at 200 rpm and filtered (Whatman no. 42). Organic C in the filtrate was analyzed with a Shimadzu 5050 TOC Analyzer. The non-fumigated samples were extracted simultaneously. Microbial biomass C was calculated as follows: $MBC = E_C/k_{EC}$, where E_C is the difference between organic C extracted from fumigated and non-fumigated soils and $k_{EC} = 0.38$.

2.5. Soil basal respiration and metabolic quotient (qCO_2)

Basal respiration was measured by CO_2 evolution. Briefly, the field moist soils (50 g oven-dry equivalent) was placed in a 500 ml sealed glass jar and incubated at 25 °C for 10 days. Glass vial holding 10 ml of 1 M NaOH to trap the evolved CO_2 was also placed in the glass jar. The excess alkali was back titrated with standard 0.5 M HCl to phenolphthalein end point after precipitating the carbonate with a 1.0 M $BaCl_2$ solution. The metabolic quotient (qCO_2) was calculated as the ratio of basal respiration to microbial biomass C and was expressed as μg CO_2–C mg^{-1} MBC day^{-1}.

2.6. Spectroscopic analysis

UV absorbance was measured at 254 nm to estimate the aromaticity of HWEOM (UV-2450 Spectrophotometer, Shimadzu) (Zsolnay, 2003).

Fluorescence emission spectra were obtained with a Varian Cary Eclipse Fluorescence Spectrophotometer (λ_{ex} 254 nm, slit 10 nm, λ_{em} 300–480 nm, slit 10 nm and scan speed 4800 nm min^{-1}). Humification index (HIX_{em}) was defined as the ratio of the peak area in the upper quarter (Σ435–480 nm) of the usable fluorescence emission spectrum to the one in the lower usable quarter (Σ300–345 nm) (Zsolnay et al., 1999) and it represented the degree of condensation of WEOM (Zsolnay, 2003). The fluorescence efficiency index (F_{eff}) is considered to be proportional to the quantum efficiency of fluorophores in the sample. It was calculated as the ratio of the maximum fluorescence intensity (F_{max}) of each emission spectrum over the relative UV absorption at 254 nm (F_{max}/Abs) (Zsolnay, 2003).

Synchronous fluorescence spectra were recorded with a constant offset Δλ ($\lambda_{em} - \lambda_{ex}$) at 18 nm and slit width at 10 nm, wavelength range from 250 to 500 nm. The scan speed was 1200 nm min^{-1}. On the basis of synchronous fluorescence spectra, humification index (HIX_{syn}) was calculated by dividing the intensity at a band of a longer wavelength (460 nm) by that of a shorter wavelength (345 nm) (Kalbitz et al., 1999). For the UV and fluorescence spectroscopy measurements, the HWEOC concentration was diluted to 20 mg C l^{-1} and pH was adjusted to 7 to ensure comparability of all HWEOM solutions.

Freeze-dried HWEOM samples, mixed with 97 wt.% KBr, were homogenized in an agate mortar and then pressed into a tablet. FTIR spectra were recorded in a range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} by a Bruker-70 infrared spectrophotometer (Bruker Optics Inc., Germany).

2.7. Statistical analysis

One-way ANOVA followed by an LSD test was used to determine whether the differences between the means in the corresponding soil depth for different vegetation types were significant. Statistical significance for all tests was set at $P<0.05$. We calculated Pearson correlation coefficients (r-value) between soil properties, biological

Table 1
Vegetation and soil type, bulk density, texture, pH, total organic carbon (TOC) and C-to-N ratios.

Site		Depth (cm)	Bulk density (g cm^{-3})	pH (H_2O)	TOC (mg g^{-1})	C/N ratio	Soil texture (%)		
Vegetation type	Soil type						Sand	Silt	Clay
Evergreen broad-leaved forest (EBF)	Red soil	0–10	0.87	4.54	57.97	14.1	40.3	45.5	14.2
		10–25	0.89	4.82	21.00	13.3	38.1	48.9	13.0
		25–40	0.96	4.94	11.91	12.1	41.7	37.1	21.2
Coniferous forest (CF)	Upland yellow soil	0–10	0.64	4.64	50.52	17.1	50.3	37.1	12.6
		10–25	0.77	4.79	23.35	16.0	46.9	38.3	14.8
		25–40	0.87	4.88	13.29	14.1	43.1	42.0	14.9
Dwarf forest (DF)	Upland yellow soil	0–10	0.61	4.59	77.43	12.7	45.9	40.7	13.4
		10–25	0.79	4.84	38.13	12.5	44.1	42.3	13.6
		25–40	0.82	4.91	23.69	11.6	41.5	45.4	13.1
Alpine meadow (AM)	Alpine meadow soil	0–10	0.54	4.86	149.80	15.6	34.7	48.6	16.7
		10–25	0.65	5.13	100.66	14.6	30.3	54.4	15.3
		25–40	0.80	5.22	34.24	12.3	35.4	38.8	25.8

properties and spectroscopic properties of HWEOM. These statistical analyses were conducted with SPSS13.0.

3. Results and discussion

3.1. Soil properties

Some physical and chemical properties of soil samples are reported in Table 1. Soil bulk density was greater under EBF than that under the other three vegetation types, and increased gradually with depth. The pH values of all soil samples ranged from 4.54 to 5.22 and increased slightly with depth. The particle size distribution showed relatively higher sand and silt contents for all soil samples. Across soil depth, the amount of TOC was the highest in AM soils, while the C/N ratio was the highest in CF soils. The decrease of TOC contents with depth in the soil profile (Table 1) may be attributed to the decay products of the organic residues from plants, animals or microbes supplied to the soil surface.

3.2. HWEOC, MBC and qCO_2

The amounts of HWEOC from the S1 layer were significantly ($P<0.05$) higher in EBF (454.0 ± 25.7 mg l^{-1}, mean ± SE, n = 3) and AM (451.9 ± 36.6 mg l^{-1}), and the HWEOC/TOC ratio was the highest in EBF soils (Table 2). The amounts of MBC in the whole soils were in the order: AM>DF>CF>EBF (Table 2). In all cases, the amounts of HWEOC decreased sharply with increasing depth, which were approximately 2–4 times greater in the S1 layer than those in the S3 layer. Similar trend was found in the amounts of MBC (Table 2). The metabolic quotient (qCO_2), defined as the community respiration rate per unit of biomass, indicates the efficiency of soil microbial populations in utilizing organic C compounds (Dilly and Munch, 1998). If community respiration is low, more carbon will be available for biomass production which should be reflected in a high percentage MBC to TOC (Anderson and Domsch, 1986). Higher soil qCO_2 and lower MBC/TOC ratio in the EBF than those in the other three vegetation types (Table 2) reflects more available organic substrates and less efficient use of them by soil microbes for biosynthesis in the EBF soils (Anderson, 2003).

3.3. UV absorption of HWEOM

Specific UV absorption at 254 nm (A254) of HWEOM from the S1 layer was the highest in CF (1.79 ± 0.10 l mg C^{-1} m^{-1}) and the lowest in EBF (1.09 ± 0.07 l mg C^{-1} m^{-1}) (Fig. 1A). A254 values of HWEOM from soils under the four vegetation types decreased approximately 18–35% from the S1 layer to the S3 layer (Fig. 1A). Special UV absorption can reflect the content of aromatic compounds and it was positively related to the portion of aromatic H measured by liquid-phase ^{1}H NMR spectroscopy (Kalbitz et al., 2003). Corvasce et al. (2006) also showed that A254 values of WEOM were related to aromaticity and degree of poly-condensation

Table 2
Soil hot-water extractable organic C (HWEOC), basal respiration, microbial biomass C (MBC), metabolic quotient (qCO_2), HWEOC/TOC ratio and MBC/TOC ratio by depth from soils under four different vegetation types[a].

Vegetation type[b]	HWEOC (mg l^{-1})	MBC (mg g^{-1})	Basal respiration (μg CO_2–C g^{-1} day^{-1})	qCO_2 (μg CO_2–C mg^{-1} MBC day^{-1})	HWEOC/TOC (%)	MBC/TOC (%)
0–10 cm						
EBF	454.0 ± 25.7a	0.82 ± 0.07c	45.5 ± 2.0b	55.5 ± 3.1a	1.57 ± 0.12	1.41 ± 0.15
CF	388.8 ± 39.0b	1.09 ± 0.03bc	38.6 ± 2.5b	35.4 ± 1.8c	1.22 ± 0.13	2.16 ± 0.20
DF	266.4 ± 21.7c	1.33 ± 0.16b	62.9 ± 4.3a	47.3 ± 2.3b	0.70 ± 0.05	1.71 ± 0.14
AM	451.9 ± 36.6a	2.37 ± 0.15a	71.2 ± 3.1a	30.0 ± 1.9c	0.60 ± 0.05	1.58 ± 0.08
10–25 cm						
EBF	135.4 ± 13.3e	0.35 ± 0.02f	19.1 ± 1.6e	54.6 ± 3.7e	1.29 ± 0.14	1.66 ± 0.13
CF	141.2 ± 15.7e	0.55 ± 0.06ef	17.8 ± 1.9e	32.4 ± 2.8g	1.20 ± 0.09	2.48 ± 0.17
DF	132.7 ± 12.0e	0.70 ± 0.10e	33.3 ± 1.6d	47.6 ± 2.5f	0.71 ± 0.04	1.84 ± 0.12
AM	311.4 ± 22.7d	1.68 ± 0.17d	51.7 ± 2.5c	30.8 ± 3.9g	0.62 ± 0.05	1.67 ± 0.10
25–40 cm						
EBF	78.8 ± 6.5k	0.16 ± 0.01h	10.9 ± 1.2m	68.1 ± 3.6j	1.34 ± 0.07	1.34 ± 0.09
CF	87.1 ± 7.6g	0.20 ± 0.03hj	11.6 ± 0.6m	58.0 ± 2.5k	1.28 ± 0.08	1.50 ± 0.11
DF	88.9 ± 6.1g	0.26 ± 0.02j	16.5 ± 1.5f	63.5 ± 2.2jk	0.75 ± 0.04	1.10 ± 0.09
AM	112.0 ± 13.2f	0.45 ± 0.02g	20.6 ± 1.9f	45.8 ± 1.7m	0.64 ± 0.04	1.31 ± 0.07

[a] Data are means (n = 3); mean ± standard error. Means within a column of the corresponding depth followed by different letters are significantly different at α = 0.05.
[b] EBF: evergreen broad-leaved forest; CF: coniferous forest; DF: dwarf forest; AM: alpine meadow.

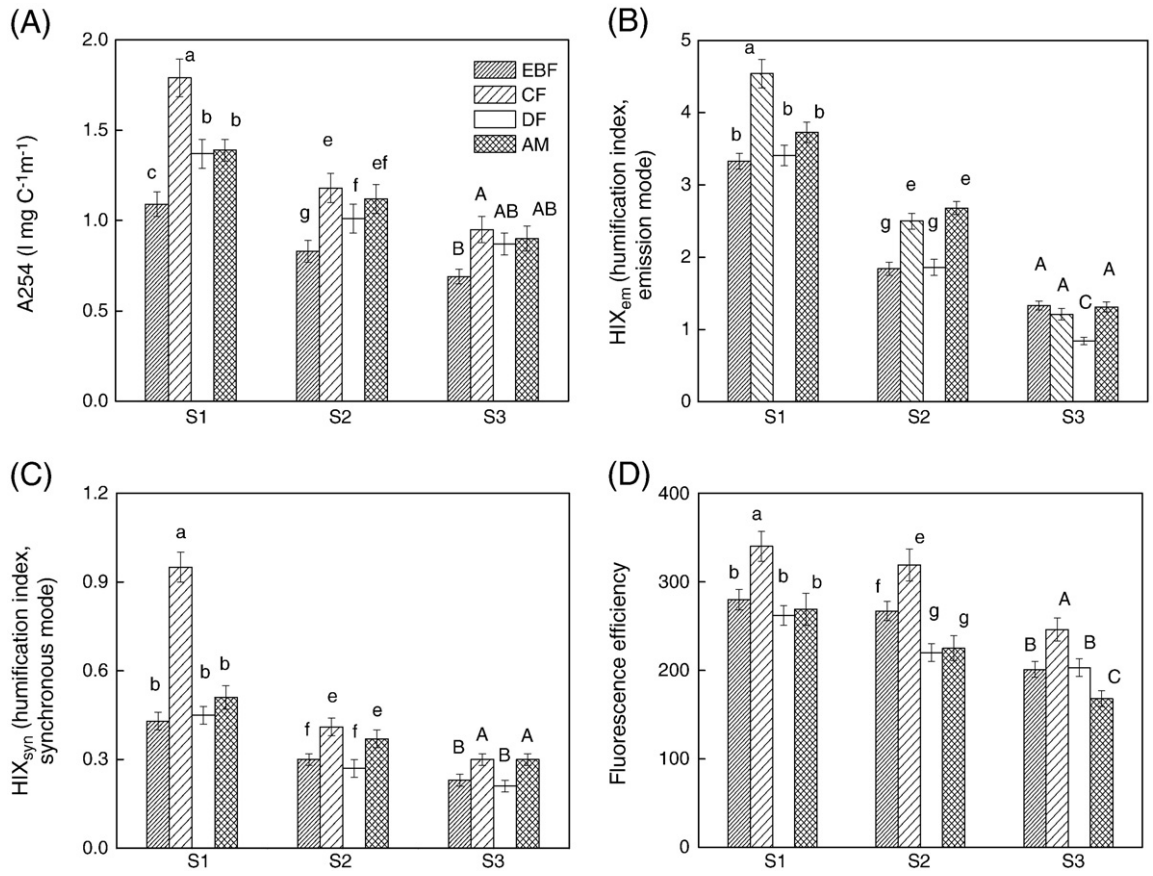

Fig. 1. Spectroscopic characteristics of the HWEOM from soils under four different vegetation types (EBF: evergreen broad-leaved forest; CF: coniferous forest; DF: dwarf forest; AM: alpine meadow) and corresponding soil depth (S1 = 0–10 cm; S2 = 10–25 cm; S3 = 25–40 cm). Bars represent standard error of the mean (n = 3). Significant differences ($P<0.05$) between the means are marked with different letters in the corresponding soil depth.

in WEOM samples. The result suggests that the HWEOM from the S1 layer of CF with higher A254 value contains abundant aromatic compounds such as lignin-derived compounds (Kiem and Kögel-Knabner, 2003), while the HWEOM from the S1 layer of EBF with lower A254 value has less aromatic compounds.

3.4. Fluorescence spectroscopic analysis

The emission fluorescence spectra of all HWEOM samples (Fig. 2) typically consisted of a broad band with the peak value centered at about 440–450 nm. The fluorescence intensity values were normalized to the relative fluorescence intensity (RFI) values by the HWEOC contents. The RFI values of HWEOM samples along the soil profile decreased with increasing soil depth (Fig. 2).

Synchronous fluorescence (SF) can provide better sensitivity and improved peak resolution in comparison with the conventional emission fluorescence, and thus can be used to explore structural information of DOM/WEOM (Chen et al., 2003; Świetlik and Sikorska, 2004). The synchronous-scan fluorescence spectra were similar for all HWEOM samples, but they showed differences in relative fluorescence intensity (RFI) of four characteristic peaks at about λ_{ex} 278–283 nm, λ_{ex} 340–350 nm, λ_{ex} 395–405 nm and λ_{ex} 460–472 nm, respectively (Fig. 3). This was most likely caused by different contents of fluorophores in HWEOM solution. The RFI values of HWEOM in synchronous fluorescence spectra also decreased with increasing soil depth (Fig. 3). Some tentative interpretations for Fig. 3 are:

i) Peak 1, located around $\lambda_{ex}/\lambda_{em}$ of 278–283/296–301 nm, could be ascribed to protein-like fluorophore (i.e. aromatic amino acids of tryptophan-like) (Baker, 2001; Yamashita and Tanoue, 2003; Baker et al., 2007; Janhom et al., 2009).
ii) Peak 2, located around $\lambda_{ex}/\lambda_{em}$ of 340–350/358–368 nm, could be attributed to aromatic and aliphatic groups and commonly labeled as fulvic acid-like fluorophore (Coble, 1996; Marhaba and Kochar, 2000).
iii) Peak 3, with λex/λem of 395–405/413–423 nm, could be attributed to polycyclic aromatic structures with approximately five fused benzene rings (Peuravuori et al., 2002).
iv) Peak 4, with $\lambda_{ex}/\lambda_{em}$ of 460–472/478–490 nm, is the most common lignin fluorescence region, which could be probably reflecting the influence of polycyclic aromatics consisting of about seven fused benzene rings (Peuravuori et al., 2002; Chen et al., 2003).

The shift in the maximum fluorescence intensity from shorter to longer wavelengths may reflect an increasing number of highly substituted aromatic nuclei or conjugated systems showing a high degree of resonance (Haken and Wolf, 1995). In addition, the electron-donating substituents such as hydroxyl, methoxyl, and amino groups of aromatic compounds can enhance the fluorescence intensity by increasing the transition probability between the singlet state and ground state (Senesi et al., 1991).

The humification indices derived from emission fluorescence spectra (HIX_{em}) and synchronous fluorescence spectra (HIX_{syn}) are well-suited for determining the extent of humification of DOM (Ohno, 2002), and describe not only the content of aromatic compounds like special UV absorption, but also the molecular structure of DOM (Michel et al., 2006). The HIX_{em} value of HWEOM in the S1 layer was significantly ($P<0.05$) higher under CF (4.54 ± 0.20) than that under

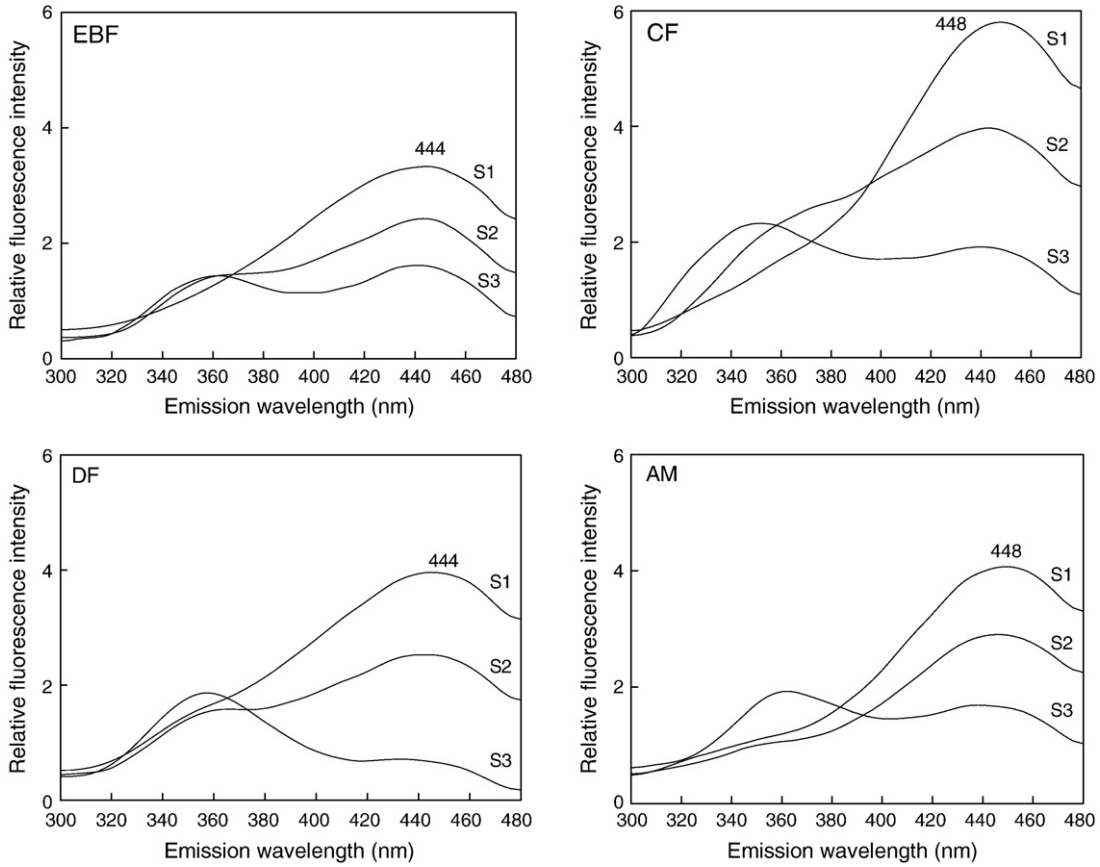

Fig. 2. Fluorescence emission spectra of the HWEOM from soils under four different vegetation types (EBF: evergreen broad-leaved forest; CF: coniferous forest; DF: dwarf forest; AM: alpine meadow) and corresponding soil depth (S1 = 0–10 cm; S2 = 10–25 cm; S3 = 25–40 cm).

EBF (3.33 ± 0.11), DF (3.41 ± 0.14) and AM (3.73 ± 0.14) (Fig. 1B). The HIX_{syn} value of HWEOM in the S1 layer followed the same trend (Fig. 1C). The higher HIX_{em} values are associated with the presence of more complex molecules such as condensed aromatic rings and compounds with high molecular weight which are typical properties for humic materials (Senesi et al., 1991; Miano and Senesi, 1992). The HIX_{syn} value was positively related to the portion of aromatic C measured by liquid-phase ^{13}C NMR spectroscopy (Michel et al., 2006). The results indicated that the HWEOM from the S1 layer of CF with higher HIX_{em} and HIX_{syn} values contains more recalcitrant components with highly condensed aromatic rings.

The fluorescence efficiency value of HWEOM from the S1 layer was in the order: CF>EBF>AM>DF (Fig. 1D). The fluorescence efficiency value of DOM is related to its molecular structure, which means that with more π electronic conjugation the fluorophore would be excited more easily and it would have higher fluorescence efficiency. In general, the overwhelming majority of fluorophores have aromatic or heterocyclic structures. Therefore, the HWEOM from the S1 layer of CF with higher fluorescence efficiency should contain more aromatic and/or heterocyclic compounds, and less readily degradable components such as carbohydrates, amino acids, and carboxylic acids (Kiem and Kögel-Knabner, 2003; Fischer et al., 2007).

The humification indices and the fluorescence efficiency values of HWEOM decreased with depth in the soil profile in all cases (Fig. 1). This is because that plant tissues contain various aromatic compounds which could be released into the upper soil layer together with the products of microbial origin (Qualls and Haines, 1992), and these aromatic and hydrophobic components of WEOM were less mobile, so that they were preferentially adsorbed onto soil mineral surfaces, instead of migrating towards deeper soil layers (McKnight et al., 1992). Corvasce et al. (2006) reported that the aromaticity and the humification index of WEOM decreased dramatically down the soil profile, and the fluorescence efficiency index tended to decrease from 0 to 85 cm depth, while increase markedly in the deeper soil layers.

3.5. Fourier-transform infrared spectroscopic analysis

Infrared spectroscopy has been widely used for gross characterization of DOM constituents and can provide valuable information on the structural functional properties of DOM, i.e. oxygen- containing functional groups, carbohydrate moieties and relative proportions of aromatic versus aliphatic moieties (Peuravuori et al., 2005). The FTIR spectra of HWEOM from the S1 layer of four vegetation types (Fig. 4) showed the following absorption bands:

a) A broad band around 3300–3400 cm^{-1}, due to −OH groups of carboxyl and alcohols;
b) The range of 2950–2850 cm^{-1}, due to aliphatic C–H stretching of CH_3/CH_2 groups;
c) At 1733 cm^{-1}, due to C=O stretching of protonated carboxyl groups;
d) Around 1630–1670 cm^{-1}, due to a mixture of C=O stretching of carboxylates/amides and C=C stretching of aromatic;
e) Around 1400 cm^{-1}, describing several functionalities, aliphatic C–H stretching of methyl groups, C–H bending, O–H deformation and C–O stretching of phenolic groups, COO^- antisymmetrical stretch of carboxylates (Peuravuori et al., 2005);
f) The range of 1100–1000 cm^{-1}, due to C–O stretching of hydroxyl and ether bonds (typical of carbohydrate linkages); and
g) Small bands of 910–730 cm^{-1}, due to aromatic C–H vibrations.

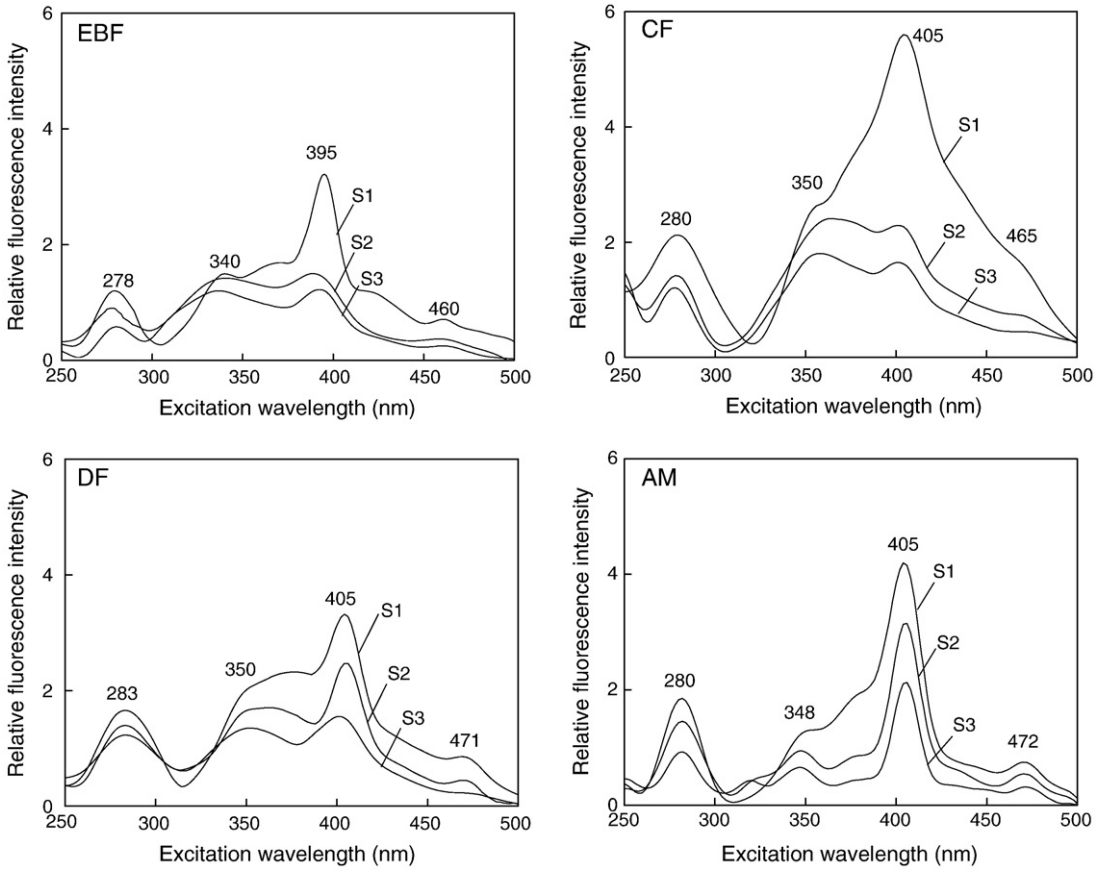

Fig. 3. Synchronous fluorescence spectra of the HWEOM from soils under four different vegetation types (EBF: evergreen broad-leaved forest; CF: coniferous forest; DF: dwarf forest; AM: alpine meadow) and corresponding soil depth (S1 = 0–10 cm; S2 = 10–25 cm; S3 = 25–40 cm).

The ratio of the relative intensity of C–O to C–H of HWEOM from the S1 layer was the highest in AM (2.56) and the lowest in CF (1.33), whereas the ratio of the relative intensity of C=C to C–H of HWEOM from the S1 layer was the highest in CF (2.17) and the lowest in EBF (1.31) (Fig. 4). This result reveals that HWEOM from the S1 layer of AM contains abundant carbohydrates, while HWEOM from the S1 layer of CF is dominated by aromatic compounds.

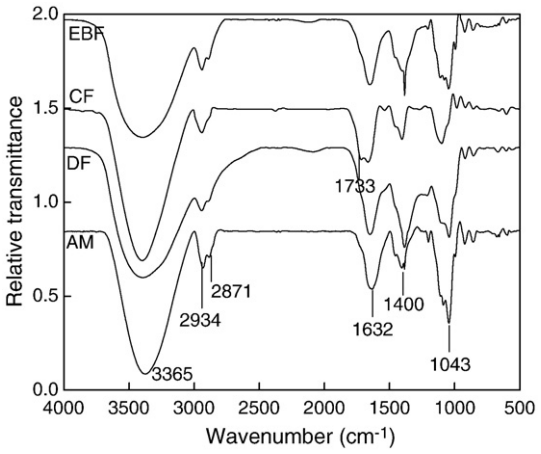

Fig. 4. FTIR spectra of the HWEOM from the S1 layer under four different vegetation types (EBF: evergreen broad-leaved forest; CF: coniferous forest; DF: dwarf forest; AM: alpine meadow).

3.6. HWEOM spectroscopic properties in relation to soil properties and biological properties

Soil C/N ratio was highly ($P<0.01$) correlated with A254, humification indices and fluorescence efficiency of HWEOM (Table 3). Michel et al. (2006) reported that soil C-to-N ratio was significantly correlated with UV absorption and HIX_{syn}. Lower soil C/N ratio suggested lower degree of humification of the sample and more protein-derived moieties in that (Claus et al., 1999). There was no correlation between soil clay content and any spectroscopic results (Table 3). The qCO_2 provides a measure of the specific metabolic activity that varies according to the composition and physiological state of the microbial community, the availability of substrates, and various abiotic factors (Anderson, 1994). Soil qCO_2 was highly ($P<0.01$) negatively correlated with A254, HIX_{em} and

Table 3
Correlation coefficients (*r*-values) between soil properties, biological properties and HWEOM spectroscopic properties.

	A254 (l mg C^{-1} m^{-1})	HIX_{em}	HIX_{syn}	Fluorescence efficiency
Soil C/N ratio	0.692**	0.663**	0.716**	0.754**
Clay content (%)	−0.270	−0.310	−0.219	−0.318
qCO_2 (μg CO_2–C mg^{-1} MBC day^{-1})	−0.484**	−0.533**	−0.451**	−0.265
HWEOC/TOC (%)	−0.194	−0.012	0.075	0.296
MBC/TOC (%)	0.297	0.365*	0.377*	0.424*

n = 36; for abbreviations see Tables 1, 2 and Fig. 3.
** Represents $P<0.01$.
* Represents $P<0.05$.

HIX_{syn} (r = −0.484, −0.533, −0.451, respectively). Soil MBC/TOC ratio was significant ($P<0.05$) correlated with HIX_{em}, HIX_{syn} and fluorescence efficiency (r = 0.365, 0.377, 0.424, respectively) (Table 3). Low qCO_2 and high MBC/TOC ratio reflected a low microbial metabolic activity and more efficient use of organic substrates by soil microbes (Anderson, 2003; Joergensen and Emmerling, 2006). Tree species and litter quality affect the availability of C sources and nutrients, and thus control soil microbial properties (Borken et al., 2002). The HWEOM from CF soils with higher aromaticity and degree of humification (Fig. 3) indicates that CF soils contain more polymeric aromatic compounds that may be resistant to microbial degradation, leading to decreasing microbial metabolic activity. Therefore, we found that the aromaticity and degree of humification of HWEOM were positively correlated with Soil MBC/TOC ratio and negatively with qCO_2 (Table 3). Dilly and Munch (1996) also reported that a lack of easily-degradable carbon sources may be responsible for the decline in qCO_2.

4. Conclusions

Structural characteristics of HWEOM revealed by spectroscopy measurement are related to its chemical composition. Our results indicate that CF soils have a greater C/N ratio, a higher MBC/TOC ratio and a lower qCO_2, and at the same time, HWEOM leached from soils under CF is more complex and aromatic than that under the other three vegetation types (EBF, DF and AM), and thus we presume that the C mineralization in CF soils may be more slowly. The recalcitrant components of HWEOM with highly conjugated aromatic structures were less mobile, so that they were preferentially adsorbed onto soil mineral surfaces, instead of migrating towards deeper soil layers.

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