Biogeochemistry **47:** 219–238, 1999.
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Spectroscopic properties of dissolved humic substances – a reflection of land use history in a fen area

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Received 26 May 1998; accepted 14 December 1998

Key words: dissolved humic substances, FTIR spectroscopy, land use history, peat, synchronous fluorescence spectroscopy, UV/VIS spectroscopy

Abstract. The elemental composition and spectroscopic properties of dissolved fulvic acids isolated from different sampling media (topsoil, ground and surface water) of a natural fen area (high portion of organic soils) were examined to reveal the effects of land use history. These effects need to be known if dissolved humic substances are to be a major factor in identifying the impact of present and future changes in land use. Dissolved fulvic acids (topsoil, groundwater) from highly degraded peatlands (due to a long-term agricultural use) exhibit lower C/N ratios, higher absorption in the UV spectra, and higher absorption at 1,620 cm^{-1} in the FTIR spectra compared with fulvic acids from relatively intact peatlands. These properties illustrate that long-term agricultural use with high inputs results in increased aromatic structures and a further humification of dissolved fulvic acids due to very strong peat decomposition compared with relatively intact peatlands. Synchronous fluorescence spectra also indicate the higher level of aromatic structures within fulvic acids isolated from sites with long-term agricultural use (high peat decomposition) compared with a land use history resulting in a lower peat decomposition. The different sources of fulvic acids in surface water (precipitation, runoff, interflow, groundwater) are the main reason for these effects not being detected in fulvic acids isolated from surface water. Short-term changes in land use characterized by a transition from crop farming to an unimproved grassland were found not to affect the spectroscopic properties of dissolved fulvic acids. A humification index deduced from the synchronous fluorescence spectra is proposed. We have strong evidence that dissolved humic substances indicate changes in the environmental conditions (both anthropogenic and natural) of wetlands with a high proportion of organic soils.

Introduction

The great significance of dissolved humic substances for the mobilization and transport of nutrients and pollutants in terrestrial and aquatic ecosystems

(Zsolnay 1996; Tegen & Dörr 1996; Kalbitz et al. 1997; Li & Shuman 1997), especially in fen areas, calls for information concerning the environmental impact on the quantity and composition of these humic substances. Much information exists concerning the effects of natural conditions on the levels and partly also the composition of dissolved organic matter in soil solution (Dalva & Moore 1991; Homann & Grigal 1992; Chittleborough et al. 1992; Hunchak-Kariouk & Suffet 1994; Liechty et al. 1995; Mulholland 1997). The results of some management practices are known, such as liming on dissolved organic matter (Cronan et al. 1992; Homann & Grigal 1992; Kreutzer 1995; Erich & Trusty 1997). However, much less is known regarding the impact of different land use systems on dissolved humic substances. This is particularly critical if major changes in land use take place, such as those occurring in eastern Germany (Meissner et al. 1993). One example is the Droemling natural fen area, an important drinking water supply area, which has undergone significant land use changes since the early 1990s (Meissner et al. 1995). These changes are characterized by the extensification of grassland farming (reduced input of fertilizer and reduced number of cuttings), changes from intensive crop production to unimproved pasture, and the abandonment of agriculture on some sites located within nature reserves. In future this extensification will be greatly expanded, resulting in the majority of this area being used extensively (Meissner et al. 1995; Anonymus 1996). The objective of these actions is to stop further peat decomposition. Nothing is known concerning the impact of these practices on dissolved humic substances, despite the fact that water from this catchment area is used for drinking water production and high concentrations of dissolved organic carbon occur in the soil solution, groundwater and surface water of this catchment area (Kalbitz et al. 1997).

In order to identify the effects of present and future land use changes, the historical influences of land use on dissolved humic substances must be studied. Large parts of this catchment area were intensively used for 200 years resulting in strong peat degradation. Consequently, many soils nowadays only contain relict amounts of peat. In contrast to the intensively used sites, a relatively small part of the catchment area was used extensively or unused for agriculture. These relatively intact peatlands contain peat layers up to 40–60 cm thick.

It is known, that long-term differences in land use (crop production, grassland, forest) result in changes in soil organic matter quantity and composition (Guggenberger et al. 1994; Capriel 1997; Parfitt et al. 1997; Koerschens et al. 1998). So, Capriel (1997) reported differences in IR spectra of arable soils caused by a different agricultural management. Such differences should occur and could be detected much faster in the dissolved fraction because it represents the most mobile part of soil organic matter (Zsolnay 1996). Zsolnay (1996) used dissolved organic matter as an indicator for the ability of a given ecological process to take place in that soil. If we want to use dissolved humic substances as an indicator for land use effects on soil and water, we need an rapid, easy to handle, and not expensive method to characterize some aspects of DOM composition.

The two objectives of our study therefore were:

- to identify differences in dissolved humic substances caused by different land use intensity in the past;
- to test the suitability of spectroscopic properties (UV, Fluorescence, IR) to distinguish between dissolved humic substances originating from sites with long-term intensive land use and sites with extensive land use or no agricultural use.

Once the historical effects of land use on dissolved humic substances are known, they could serve as indicators for environmental stresses affecting carbon cycling in soil and water over different time scales (past, present).

Material and methods

Site description

With a size of 28,000 ha, the Droemling research region is the largest fen area in the Federal State of Saxony-Anhalt (Germany) (Figure 1). Peat drainage followed by agricultural usage were carried out as of the late 18th century. The result of this agricultural use was peat degradation from 2–3 m in 1750 to 0.4–0.6 m in 1995 (Altermann & Refior 1997). Today only 36% of the area is covered by organic soils (Histosols). Other significant soil types are Mollic Gleysols surrounding the Histosols and dry sandy soils (Cambisols).

We selected 6 sites with different usages (Figure 1, Table 1). The soils of the long-term intensively used sites (sites 1–3) contain only relict amounts of peat as a result of agricultural usage stretching back 200 years. These sites are described as degraded peatlands. According to FAO (1990), these soils can be classified as Mollic Gleysols. Whereas sites 1 and 3 were long used as intensive arable land, site 2 was used for many years as intensive grassland. The land use pattern of site 3 was changed in 1996 to extensive grassland. This site was included in our studies to determine whether a short-term change in land use results in prompt changes to dissolved humic substances as compared with a constant crop farming (site 1). The soils from a long-term extensive grassland farming (site 4) or with no agricultural use (site 5: former grassland in natural succession; site 6: alder swamp forest) can be classified as Histosols (FAO 1990) and described as relatively intact peatlands. At these sites (sites

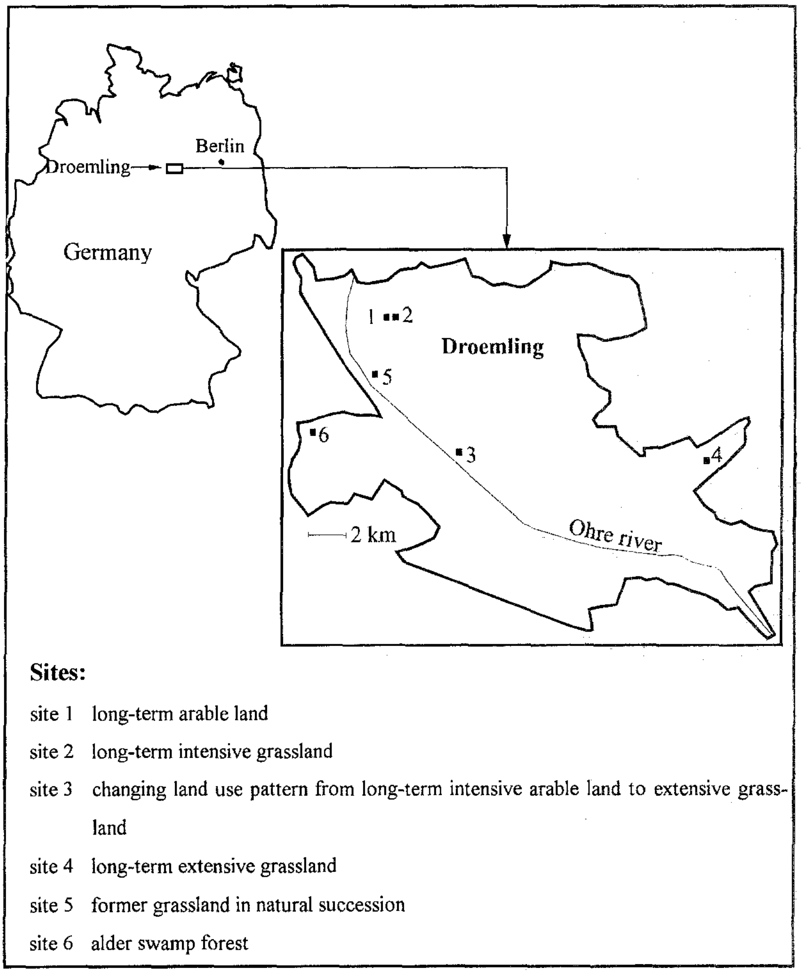


Figure 1. Study area (Droemling fen area with the main river Ohre) and sampling sites.

4–6) peat degradation was much lower than at the long-term intensively used sites.

Sample collection and isolation of humic substances

In the summer of 1996, the autumn of 1996 and the spring of 1997 groundwater, surface water, and aqueous extracts from the topsoil, respectively, were sampled and extracted. Groundwater samples were collected from wells (1–2 m depth) using a 25 L Teflon container. Immediately after sampling, the groundwater was filtered (0.45 μm, BALSTON type AAH, borsilica filter with anorganic binder) and acidified to pH 2 with concentrated HCl. Surface water was directly pumped from the adjacent draining ditch into the Teflon

Table 1. Site description and some properties of the organic soil horizons.

Site/soil[1]	Intensity of land use	Depth (cm)	TOC[2] (g kg^{-1})	TON[3] (g kg^{-1})	TOC/TON	pH[4]
Site 1/mineral	Intensive arable land	0–18	41.8	3.60	11.6	5.49
		18–32	43.7	3.01	14.5	5.47
Site 2/mineral	Intensive grassland	0–32	47.2	3.65	12.9	5.32
Site 3/mineral	Changing land use[5]	0–26	46.2	2.58	17.9	5.97
Site 4/organic	Extensive grassland	0–37	205.6	11.8	17.4	5.33
		37–52	150.2	7.65	19.6	5.56
Site 5/organic	Former grassland in	0–28	86.8	8.76	9.9	5.74
	Natural succession	28–55	452.4	23.09	19.6	5.67
Site 6/organic	Almost natural	0–30	452.7	30.7	14.7	5.21
	Woodland	30–42	447.7	22.84	19.6	6.32

[1] Mineral soils (strongly degraded fen soils with a organic matter content <15%; only relict amounts of peat); organic soils (organic matter content >30%).

[2] Total organic carbon content of the soil.

[3] Total organic nitrogen content of the soil.

[4] pH of the soil (0.01 M $CaCl_2$).

[5] Changing land use from long-term intensive arable land to extensive grassland.

container and treated in the same way as the groundwater. However, the ditches were dry at some sites in summer 1996 (Table 2). Aqueous extracts of the topsoil were prepared as described based on the method presented by Kalbitz and Wennrich (1998).

We used 25 kg field moist soil, added approximately 10 L distilled water (water was added up to saturation point, then 0.25 L distilled water per kg soil was supplemented) and stirred thoroughly three times over a period of 24 h at a temperature of about 10 °C. A 25 L HDPE barrel was used for this extraction. After 24 h the extract was centrifuged with a large self-made centrifuge (adapted spin drier with an installed nylon filter fabric holding up to 5 L soil extract) at 1,000 r.p.m. The supernatants were centrifuged a second time at 10,000 g. This second supernatant was then filtrated through 0.45 μm and acidified to pH 2 as described above. The aim of this procedure was to obtain dissolved humic substances that resemble humic substances in soil solution.

The groundwater (20 L; except groundwater of site 6 in spring 1997 – we used only 10 L because of a high DOC concentration; Table 2), surface water (20 L) and aqueous extracts of the topsoil (10 L) were treated analogously. Humic substances were extracted by liquid chromatography using adsorp-

Table 2. DOC content of the samples used for the extraction of fulvic acids (mg C L^{-1}).

	Aqueous extract of the topsoil			Groundwater			Surface water		
Sampling date	8/96	11/96	4/97	8/96	11/96	4/97	8/96	11/96	4/97
Degraded peatlands (mineral soils)									
Site 1 – arable land	41.7	41.0	28.9	28.1	27.8	29.2	8.9	12.6	12.2
Site 2 – intensively used grassland	45.4	31.6	45.4	16.2	15.8	29.0	n.v.	41.2	29.1
Site 3 – changing land use pattern	37.4	30.4	29.2	29.4	23.6	21.8	n.v.	18.6	22.0
Relatively intact peatland (organic soils)									
Site 4 – extensively used grassland	62.3	59.2	35.2	9.0	25.6	24.6	16.6	18.7	23.5
Site 5 – former grassland in natural succession	82.0	39.4	71.1	13.5	48.6	60.8	n.v.	41.5	65.0
Site 6 – alder swamp forest	161.0	75.5	89.5	30.2	30.2	119.0	n.v.	72.5	48.6

n.v.: no value (no water in the ditch)

tion chromatography on XAD-8 resin (Thurman & Malcolm 1981). We used a column with 1 L XAD-8 resin. XAD-8 resin was prepared according to the procedure presented by Thurman and Malcolm (1981). Between the samples the resin was thoroughly cleaned with NaOH, methanol, and ultra purified water. DOC concentrations vary between 9 and 119 mg C L^{-1} in groundwater, 8.9 and 72.5 mg C L^{-1} in surface water, 29.2 and 161 mg C L^{-1} in aqueous extracts of the topsoil (Table 2). After passing through an XAD-8 filled column, humic substances were eluted with 0.1 N NaOH. These eluted humic substances are equivalent to hydrophobic acids in accordance with the procedure described by Leenheer (1981). Hydrophobic acids mainly comprise fulvic acid macromolecules (Thurman et al. 1982; Wassenaar 1990). They are described as fulvic acids (FA). The dissolved FA in the supernatant was purified by double adsorption on XAD-8, then H^+-saturated using AG MP-50 cation exchange resin (Biorad) and freeze-dried (Aiken et al. 1996).

Characterization methods

Dissolved organic carbon concentration in the aqueous soil extracts, the groundwater and the surface water was determined by an autoanalyzer (a Shimadzu TOC 5050) using the difference method (total organic carbon = total carbon − inorganic carbon). The detection limit was 0.3 mg C L^{-1}. Measurement was performed using three point calibration curves. Total C standards were prepared from potassium hydrogen phthalate and inorganic carbon from a mixture of Na_2CO_3 and $NaHCO_3$. We analyzed each sample in triplicates. Five replicates were analyzed, if the variation coefficient exceeded 2 per cent.

The elemental composition of the humic substances was determined by a C, H, N analyzer (LECO CHN932). We analyzed each sample in triplicates (analytical error: C: ±0.2%, N: ±0.03%, H: ±0.14%). The ash content was determined after ashing at 750 °C for 4 h and the oxygen content (including sulfur and other elements) was calculated by determining the difference (oxygen = 100 − (ash + carbon + nitrogen + hydrogen).

UV/VIS spectra were performed with a Varian Cary 3 spectrophotometer in the wavelength range 200–620 nm. For this purpose 2.5 mg freeze-dried FA was redissolved in 125 mL 0.05 M $NaHCO_3$. DOC concentration in this solution was about 10 mg L^{-1}. All spectra were normalized using the measured C content of the solid humic substances (L 10 mg^{-1} cm^{-1}). Synchronous fluorescence spectra were recorded in these solutions using a Perkin Elmer LS 50B luminescence spectrometer in the excitation wavelength range 260–560 nm with a scan speed of 240 nm s^{-1} and a $\Delta\lambda$ of 18 nm (Miano & Senesi

1992). All spectra were normalized using the measured C content of the FA in order to compare the relative fluorescence units of the different samples.

The humic substances samples were prepared as KBr pellets (about 1 mg of FA [depending on the C content; about 0.5 mg C] was mixed with about 300 mg of coarse disperse KBr by milling for 2 min, the resulting powder pressed being under a vacuum for 4 min to form a thin pellet with a diameter of 13 mm) to record Fourier-transformed infrared (FTIR) spectra. Absorbance spectra were obtained using a FTIR spectrometer (System 2000 FTIR Perkin Elmer) in the frequency region from 3,800 to 450 cm^{-1} using a resolution of 2 cm^{-1} and a data interval of 0.2 cm^{-1}. 128 scans were accumulated. The spectra were scaled to 1 mg sample/cm and normalized to the carbon content of the compound.

Results and discussion

Elemental composition of FA

The element content and atomic ratios of the FA (Table 3) are typical of this humic fraction according to the C/N ratio (e.g. Senesi et al. 1996). The measured O/C ratios are in the upper range of FA (Senesi et al. 1996; Peuravuori & Pihlaja 1997; McKnight et al. 1997; Geyer, W. et al. 1998). This is possibly related to the young age of our dissolved humic substances (Geyer, S. et al. 1998). The effects of different long-term land use are only reflected by FA isolated from the topsoil. FA from the degraded peatlands (intensively used sites) contains more nitrogen and exhibits a lower C/N ratio than FA of the relatively intact peatlands (long-term extensively used or unused sites). Long-term intensive land use results in strong peat decomposition. This peat decomposition is measurable by means of a low C content and a low C/N ratio in the topsoil (Table 1). Fertilization stimulates this peat decomposition, which includes a relative enrichment of nitrogen, while N addition further reduces the C/N ratio. Thus, the composition of solid organic matter (C/N ratio) is reflected in the water-soluble fulvic acid of the topsoil. However, the relatively high N content and low C/N ratio of the FA from the intensively used sites are not reflected in the elemental composition of the FA in groundwater and surface water.

UV-visible spectra of fulvic acids

All the fulvic acid samples exhibited the familiar increase in absorbance with decreasing wavelength (Figure 2) (Chin et al. 1994; McKnight et al. 1997). The spectra of the FA isolated from topsoil and groundwater show a higher

Table 3. Elemental composition (calculated on an ash-free basis) and atomic ratios of dissolved fulvic acids isolated from topsoil, groundwater, and surface water of differently used sites in a fen area (mean ± standard deviation of 3 sampling dates).

	Ash	C	N	H	O	C/N[1]	C/H[1]	O/C[1]
	%	%	%	%	%	Atomic ratio	Atomic ratio	Atomic ratio
Degraded peatlands (mineral soils)								
Site 1 – arable land								
Soil extract	1.99	42.7	2.1	4.3	50.9	23.6 ± 1.9	0.8 ± 0.04	0.9 ± 0.09
Groundwater	1.56	45.4	1.3	4.8	48.5	40.5 ± 3.0	0.8 ± 0.03	0.8 ± 0.04
Surface water	2.96	42.3	1.2	4.4	52.1	41.8 ± 2.2	0.8 ± 0.01	1.0 ± 0.33
Site 2 – intensively used grassland								
Soil extract	3.99	44.2	2.2	4.3	49.3	23.3 ± 2.6	0.9 ± 0.10	0.8 ± 0.18
Groundwater	6.43	37.8	1.3	3.8	57.1	35.1 ± 0.5	0.8 ± 0.01	1.2 ± 0.60
Surface water	0.40	44.2	1.4	4.2	50.2	37.9 ± 1.6	0.9 ± 0.01	0.9 ± 0.18
Site 3 – changing land use pattern								
Soil extract	4.40	39.0	2.3	3.9	54.8	20.0 ± 1.2	0.8 ± 0.03	1.1 ± 0.24
Groundwater	0.10	46.5	1.8	4.7	47.0	29.7 ± 0.6	0.8 ± 0.01	0.8 ± 0.05
Surface water	1.79	42.5	1.4	4.4	51.7	34.5 ± 2.0	0.8 ± 0.02	0.9 ± 0.09
Relatively intact peatland (organic soils)								
Site 4 – extensively used grassland								
Soil extract	0.40	43.2	1.8	4.4	50.6	27.9 ± 1.4	0.8 ± 0.03	0.9 ± 0.06
Groundwater	2.15	41.5	1.3	4.3	52.9	36.1 ± 0.2	0.8 ± 0.01	1.0 ± 0.46
Surface water	5.42	49.2	1.8	4.7	44.3	31.9 ± 1.4	0.9 ± 0.03	0.7 ± 0.20
Site 5 – former grassland in natural succession								
Soil extract	5.60	43.5	2.0	4.4	50.1	26.2 ± 3.1	0.8 ± 0.08	0.9 ± 0.33
Groundwater	1.53	46.4	1.4	4.6	47.6	38.8 ± 9.2	0.8 ± 0.02	0.8 ± 0.03
Surface water	5.28	43.3	1.6	4.3	50.8	31.6 ± 1.9	0.8 ± 0.02	0.9 ± 0.16
Site 6 – alder swamp forest								
Soil extract	0.86	44.5	1.7	4.3	49.5	30.9 ± 3.6	0.9 ± 0.04	0.8 ± 0.06
Groundwater	1.11	48.6	1.2	4.9	45.3	48.3 ± 9.4	0.8 ± 0.03	0.7 ± 0.07
Surface water	0.10	44.4	1.5	4.3	49.8	33.4 ± 0.3	0.8 ± 0.05	0.8 ± 0.08

[1] Means were calculated on the basis of each (3) sample.

absorbance at the degraded peatlands (long-term intensively used; sites 1–3) than at the relatively intact peatlands (long-term extensively used or unused; sites 4–6) over a broad wavelength range (e.g. topsoil FA are shown in Figure 2). A wavelength of 285 nm was chosen to compare the specific absorption in UV light (absorption per mass unit DOC) as absorption around 280 nm is a measure of the aromatic character of dissolved humic substances (Chin et al. 1994; McKnight et al. 1997) and the fulvic portion in dissolved organic matter (Buffle et al. 1982).

Specific absorption at 285 nm highlights the greater aromatic character of FA (groundwater, aqueous extracts of the topsoil) after long-term intensive

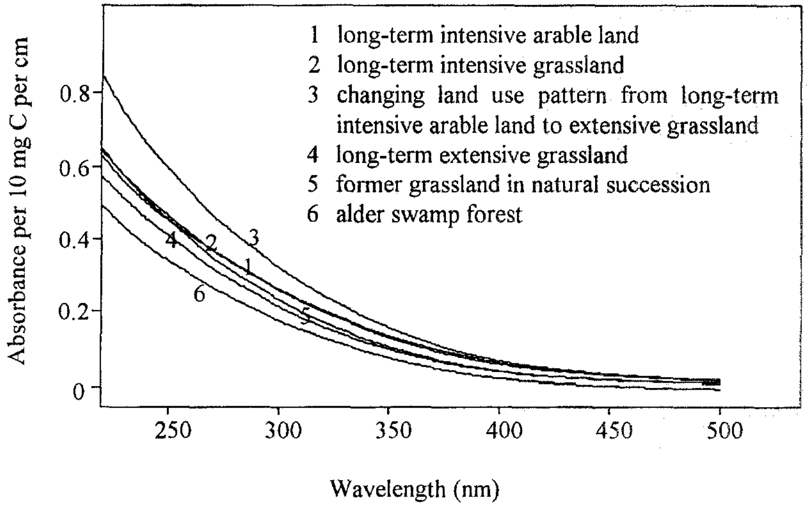


Figure 2. UV spectra of typical water-soluble fulvic acids isolated from the topsoil of long-term differently used sites.

land use (sites 1–3) than FA extracted from sites with a lower intensity of land use (site 4) or with no agricultural use (sites 5–6) (Table 4). According to Zech et al. (1997), humification is linked to an enrichment in recalcitrant, nonlignin aromatic structures. In the same way Guggenberger et al. (1994) explainded a decreased content of lignin-derived phenols and a decreased C/N ratio with a decreased particel size by an enhanced humification. According to Kögel-Knabner et al. (1988), decreasing yields of CuO oxidation products are characteristic of decomposition and humification processes. Thus, a very strong peat decomposition due to long-term intensive land use (only relict amounts of peat) is associated with the further humification of the soluble FA in the topsoil and in groundwater compared with extensive or non-agricultural land use and lower peat degradation. Correlations between absorption at 285 nm and the C/N ratio of the FA fraction ($r = -0.75$, $n = 46$) are additional signs that that UV spectroscopy provides an initial estimate of the degree of humification because peat decomposition and humification tend to result in the decay of carbon and thus the relative enrichment of nitrogen.

However, no significant differences exist between the FA of the different surface waters in the UV absorption. Surface water at these sites is a mixture of precipitation, runoff, interflow and groundwater. Similarities in the composition of the FA are evidently accounted for by this mixture of different sources.

Table 4. Selected spectroscopic data (mean ± standard deviation of 3 sampling dates) of dissolved fulvic acids isolated from topsoil, groundwater, and surface water of differently used sites in a fen area.

	A 285[1] (L g C cm^{-1})	A 1620[2]	A 1725/1620[2]	400/360[3]	470/360[3]
Degraded peatlands (mineral soils)					
Site 1 – arable land					
Soil extract	37 ± 4	1.16 ± 0.09	1.88 ± 0.21	1.35 ± 0.07	0.98 ± 0.24
Groundwater	26 ± 3	0.99 ± 0.20	2.86 ± 0.38	1.12 ± 0.11	0.41 ± 0.09
Surface water	25 ± 2	0.90 ± 0.15	2.76 ± 0.01	1.10 ± 0.11	0.42 ± 0.11
Site 2 – intensively used grassland					
Soil extract	31 ± 5	1.19 ± 0.07	2.16 ± 0.35	1.20 ± 0.07	0.68 ± 0.14
Groundwater	27 ± 2	1.03 ± 0.12	2.29 ± 0.12	1.18 ± 0.05	0.47 ± 0.04
Surface water	28 ± 2	1.04 ± 0.01	2.17 ± 0.02	1.15 ± 0.02	0.48 ± 0.01
Site 3 – changing land use pattern					
Soil extract	34 ± 2	1.18 ± 0.12	2.02 ± 0.18	1.29 ± 0.01	0.74 ± 0.01
Groundwater	25 ± 1	0.88 ± 0.04	2.31 ± 0.11	1.11 ± 0.04	0.42 ± 0.04
Surface water	25 ± 2	0.93 ± 0.06	2.46 ± 0.04	1.01 ± 0.01	0.33 ± 0.01
Relatively intact peatland (organic soils)					
Site 4 – extensively used grassland					
Soil extract	28 ± 3	0.91 ± 0.04	2.41 ± 0.27	1.15 ± 0.08	0.51 ± 0.11
Groundwater	21 ± 1	0.82 ± 0.01	2.99 ± 0.21	0.99 ± 0.04	0.30 ± 0.02
Surface water	26 ± 1	0.88 ± 0.14	2.50 ± 0.15	1.04 ± 0.03	0.39 ± 0.02
Site 5 – former grassland in natural succession					
Soil extract	29 ± 2	1.00 ± 0.03	2.15 ± 0.14	1.11 ± 0.02	0.47 ± 0.02
Groundwater	22 ± 3	0.86 ± 0.06	2.64 ± 0.03	1.04 ± 0.04	0.34 ± 0.01
Surface water	28 ± 2	0.97 ± 0.02	2.24 ± 0.11	1.05 ± 0.05	0.37 ± 0.01
Site 6 – alder swamp forest					
Soil extract	26 ± 2	0.96 ± 0.07	2.33 ± 0.40	1.20 ± 0.03	0.54 ± 0.06
Groundwater	18 ± 1	0.84 ± 0.14	2.74 ± 0.07	1.06 ± 0.03	0.35 ± 0.04
Surface water	24 ± 3	0.92	2.30	1.14 ± 0.04	0.47 ± 0.01

[1] Specific absorption at 285 nm (UV spectra).

[2] Absorption at 1620 cm^{-1} and quotient 1725 cm^{-1}/1620 cm^{-1}, respectively (FTIR spectra).

[3] Quotients of relative fluorescence intensity (synchronous mode).

FTIR spectra of FA

The IR spectra of the FA examined are very similar to each other in the position of principal absorption bands and shoulders, and comparable to spectra of other FA isolated from soil or water (Figure 3) (Stevenson & Goh 1971; Senesi et al. 1989; Kaiser & Zech 1997; Geyer et al. 1998). All the spectra are characterized by strong bands at around 3,400 and 2,925 cm^{-1} (H-bounded OH groups, aliphatic C-H stretching), 1,725 cm^{-1} (COO^{-}),

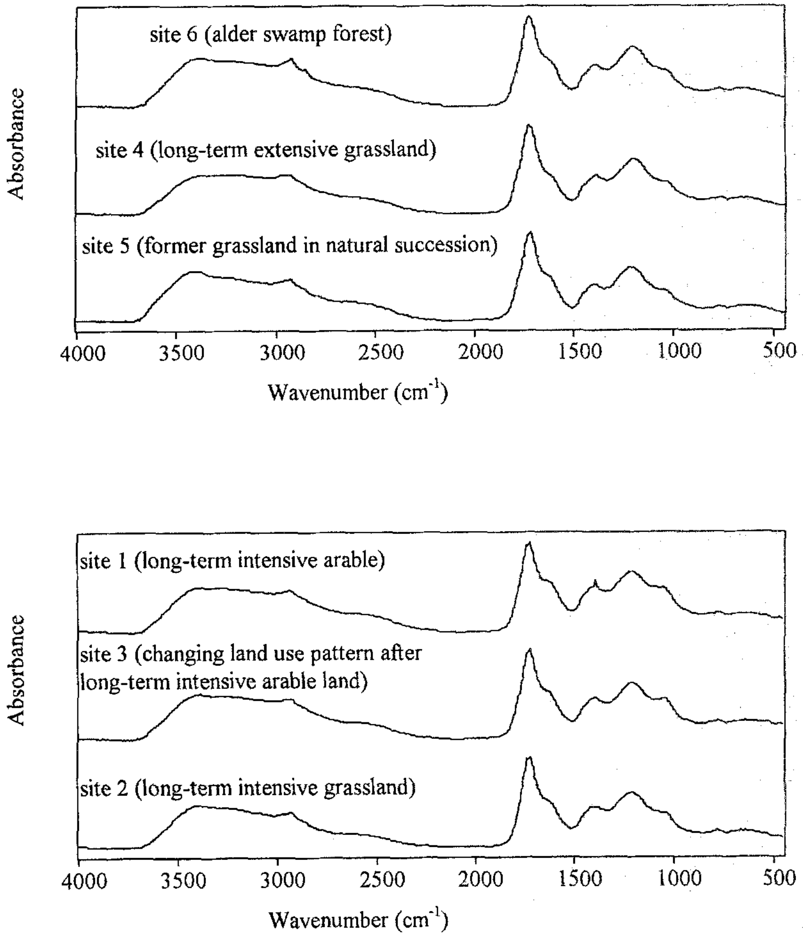


Figure 3. FTIR spectra of typical water-soluble fulvic acids isolated from the topsoil of long-term differently used sites.

1,400 cm^{-1} (OH deformation and C-O stretching of phenolic OH groups), 1,200 cm^{-1} (C-O stretching and O-H deformation of COOH groups), and 1,050 cm^{-1} (C-O stretching of polysaccharides). In addition, a shoulder was detected at 1,620 cm^{-1} (mainly aromatic C = C). Band and shoulder assignments were performed after Stevenson and Goh (1971) and Senesi et al. (1996).

Although the FTIR spectra seem very similar, differences between the spectra are apparent in the relative intensity of some bands depending on the sampling site and the sampling media (topsoil, groundwater, surface water). Absorption at 1,620 cm^{-1} of the FA is higher at the degraded peatlands (long-term intensive land use) than at the relatively intact peatlands (extensive or no agricultural use) (Figure 3, Table 4). This is especially evident when considering the FA isolated from the topsoil. However, this is also the case for most of the FA isolated from groundwater. If the band at 1,620 cm^{-1} mainly refers to aromatic structures (Senesi et al. 1996), FTIR spectra prove the hypothesis derived from UV absorption of the increasing aromatic character of FA due to long-term intensive agricultural use resulting in sharp peat degradation. This is confirmed by significant correlation between IR absorption at 1,620 cm^{-1} and specific absorption at 285 nm ($r = 0.68$, $n = 44$).

When using absorption at 1,725 cm^{-1} it is not possible to clearly distinguish between the different FA. However, the ratio between 1,725 and 1,620 cm^{-1} showed the same pattern as described for 1,620 cm^{-1} with lower values for the degraded peatlands than the relatively intact peatlands (Table 4). A decreasing ratio between 1,725 and 1,620 cm^{-1} means that the portion of aromatic structures is on the increase compared to the protonated carboxyl groups. Therefore, this decreasing ratio of IR absorption indicates the increasing humification of the FA fraction after long-term intensive land use. This is verified by correlations between the ratio of IR absorption (1725/1620 cm^{-1}) and specific absorption at 285 nm ($r = -0.77$, $n = 44$), as well as between the ratio of IR absorption and the C/N ratio of the FA ($r = 0.71$, $n = 44$).

If the absorption of water-soluble FA from topsoil and groundwater at 1,620 cm^{-1} and the ratio at 1,725 and 1,620 cm^{-1} are compared, a decrease is observed in absorption at 1,620 cm^{-1} along with an increase in the absorption ratio (1,725/1,620 cm^{-1}) from topsoil to groundwater (Table 4). These results confirm the preferred adsorption of aromatic compounds during the soil passage of FA from topsoil to groundwater (Jardine et al. 1989; McKnight et al. 1992; Gu et al. 1995) and the suitability of this analytical method for detecting aromatic structures within the FA.

Other strong bands of the IR spectra do not seem to reflect the history of land use. As with UV absorption, the IR spectra of the FA isolated from surface water do not display any significant effects of land use history.

Synchronous fluorescence spectra of FA

Fluorescence spectroscopy has been recently used as a powerful means for classifying and distinguishing among humic substances of various origin and nature (Senesi et al. 1991; Miano & Senesi 1992; Senesi et al. 1996). Basic principles, instrumentation, procedures, methodology and limitations of the fluorescence technique are summerized by Senesi (1990). Miano and Senesi (1992) report that "The most efficient fluorophores in humic polymers are indicated to be variously substituted, condensed aromatic rings, and/or highly unsaturated aliphatic chains". Gehring et al. (1997) used fluorescence spectroscopy besides ^{13}C NMR to indicate differences in the amount of aromatic compounds within a soil profile. Mobed et al. (1996) emphasize a significant discrimination among most humic substances by fluorescence spectroscopy. Therefore it should be possible to recognize the effects of long-term differences in land use pattern by fluorescence spectroscopy if such differences affect the composition of water-soluble FA. The synchronous mode was chosen to obtain the best spectral information (Miano & Senesi 1992).

Common to the synchronous fluorescence spectra of the FA are two peaks at around 360 and 400 nm, and a shoulder at around 470 nm (Figure 4). These spectra are typical of soil fulvic acids (Senesi et al. 1991). However, there are differences between the samples in fluorescence peak ratios (400/360 nm, 470/360 nm, 470/400 nm). The shift in the maximum fluorescence intensity from shorter to longer wavelengths is associated with an increasing number of highly substituted aromatic nuclei and/or with conjugated unsaturated systems capable of a high degree of resonance (Senesi et al. 1989; Miano & Senesi 1992). Thus, the quotient of fluorescence intensity at 400 and 360 nm or at 470 and 360 nm ought to be a measure of the degree of polycondensation of the DOM. Increasing quotients of fluorescence intensity indicate a higher degree of polycondensation and of humification. Therefore, we propose using this quotient as a humification index. For calculation, the peaks (or shoulders) at a high (high degree of polycondensation) and a short wavelength (simple structural components, i.e., low degree of aromatic polycondensation; Senesi et al. 1989) should be used. As this index is independent of the relative fluorescence intensity, it can be reproducibly measured. The two humification indices calculated (400/360 nm and 470/360 nm) correlate closely with each other ($r = 0.91$, $n = 46$). Thus, only two clearly distinguishable peaks (at a shorter and a longer wavelength) are necessary to calculate the humification index. Study of the humification indices of the water-soluble FA (topsoil, groundwater) of the degraded peatlands (long-term intensively used; sites 1–3) revealed a higher degree of humification (higher degree of polycondensation) than the FA of the relatively intact peatlands (long-term extensively used or unused; (sites 4–6) (Table 4). However, FA isolated from surface water did not show any effects of long-term differences in land use. In addition to UV and IR absorption, the humification index closely reflects the decreasing aromatic content of FA after soil passage. This was seen from the decreasing indices values from topsoil to groundwater (Table 4).

Synchronous fluorescence spectra are the third proof that water-soluble FA isolated from topsoil and groundwater reflects long-term differences in land use. Good correlations between the humification index (470/360 nm) and parameters derived from elemental analysis, UV and FTIR spectroscopy were calculated ($n = 44$; C/N: $r = -0.63$; A 285 nm: $r = 0.85$; IR 1,620

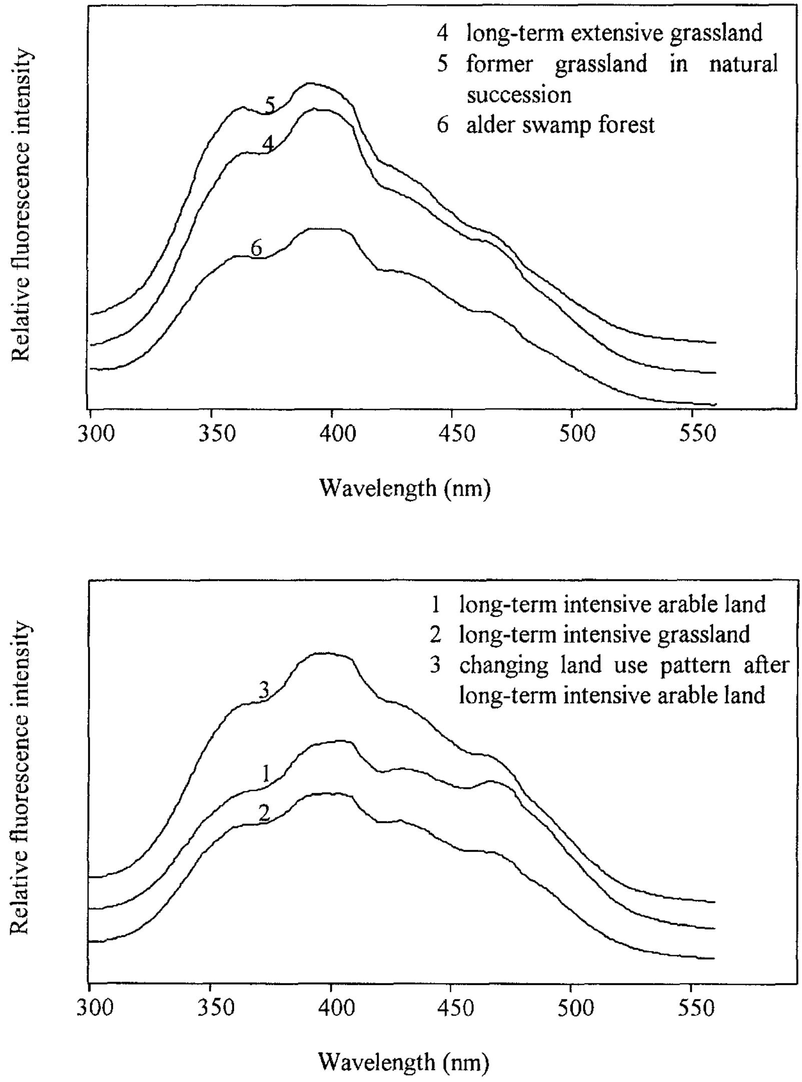
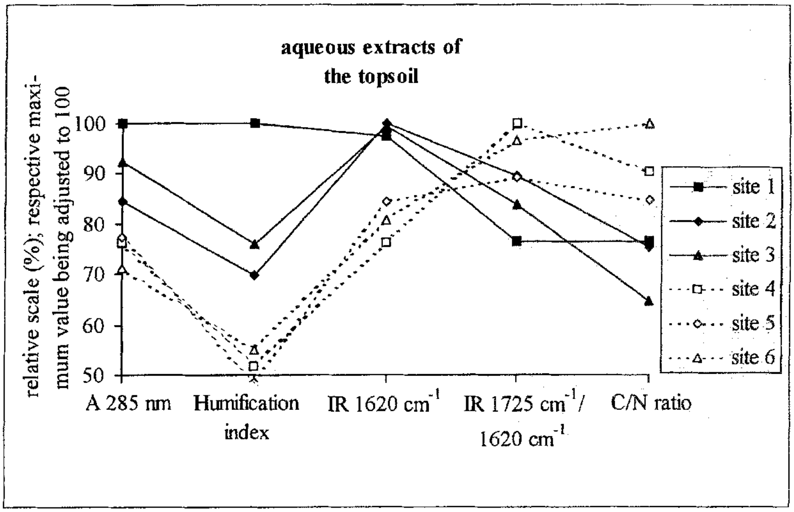


Figure 4. Synchronous fluorescence spectra of typical water-soluble fulvic acids isolated from the topsoil of long-term differently used sites.

cm^{-1}: $r = 0.71$; IR 1725/1620 cm^{-1}: $r = -0.69$). However, no changes in FA composition after a short-term change of the land use pattern (site 3) could be detected by fluorescence spectroscopy.

Comparison of the methods used

The results of the various methods used are summarized in Figure 5. Looking at topsoil water-soluble FA, it is possible by using all the methods to distin-



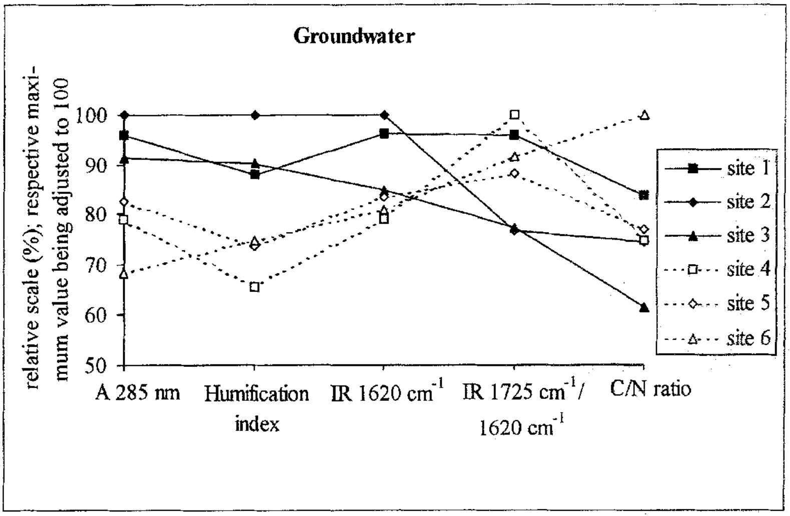


Figure 5. C/N ratio, specific absorption at 285 nm, humification index (quotient of relativ fluorescence intensity at 470 nm/360 nm), IR absorption at 1620 cm^{-1}, and quotient of IR absorption (1725 cm^{-1}/1620 cm^{-1}) of water-soluble fulvic acids isolated from topsoil (top figure) and groundwater (lower figure) of long-term differently used sites (site 1–3 long-term intensively used – unbroken lines, site 4–6 long-term extensively or unused – dashed lines; means of 3 sampling dates); The results are shown over a relative scale (the respective maximum value being adjusted to 100).

guish FA from the degraded peatlands (long-term intensively used) as one group compared to FA from the relatively intact peatlands (extensively used or unused) as a second group. The distinction of these two groups is not as clear for groundwater FA. Mainly groundwater FA of the arable land (site 1) showed a C/N ratio and a ratio between 1,725 and 1,620 cm^{-1}, tending towards values characterizing FA from the relatively intact peatlands. This partly unclear distinction between groundwater FA from the two types of land use studied is probably the result of adsorption and decomposition processes during water percolation through the soil into the groundwater.

However, synchronous fluorescence spectroscopy and the specific absorption at 285 nm were useful to distinguish between all the investigated dissolved humic substances (topsoil and groundwater) originating from sites with a different land use history in terms of their degree of humification and aromatic content. These methods are rapid, not expensive and accessible for a lot of laboratories compared with other procedures to characterize the structure of humic substances (e.g. NMR methods). The advantage of fluorescence spectroscopy over UV spectra is the better resolution of the synchronous fluorescence spectra and usually better distinction between different samples. Compared with FTIR spectroscopy, fluorescence spectroscopy is much less time-consuming. However, FTIR spectroscopy was powerful enough to verify that high absorption at 285 nm and high humification indices are mainly caused by aromatic structures.

Conclusions

The combination of the methods used clearly showed that long-term intensive land use results in increasing aromatic structures within water-soluble FA (topsoil and groundwater) due to very strong peat decomposition (with nowadays merely relict amounts of peat remaining) compared with a land use history resulting in lower peat decomposition (extensive land use or non-agricultural use). Different FA sources (runoff, interflow, soil percolation and groundwater) are the main reasons why these effects could not be detected in FA isolated from surface water. Short-term changes in land use did not affect the spectroscopic properties of FA in topsoil, groundwater or surface water. As a result of our study there is clear indication that dissolved humic substances indicate changes in the environmental conditions (both anthropogenic and natural) of wetlands with a high proportion of organic soils. Moreover, it ought to be possible to transfer this conclusion to other types of landscapes.

Acknowledgements

The authors would like to acknowledge the financial support of the Ministry for Regional Planning, Agriculture and Environment of Saxony-Anhalt (Germany). We would also like to thank three unanimous referees for their helpful comments.

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