Phytotoxic effects of the high molecular weight fraction of an aqueous leaf litter extract on barley root development

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Abstract

The root development of barley seedlings grown for one week in an aerated nutrient solution was studied in the
presence of dissolved organic matter from an aqueous chestnut leaf litter extract. In particular, the different effects
of low and high molecular weight fractions (small molecules: molecular weight <1000; large molecules: >10,000)
of the leaf litter extract were examined. In the presence of large molecules root growth was inhibited, an irregular
root tip morphology was observed, and Ca and Mg concentrations in the shoots were lower than in control plants.
These phytotoxic effects were not caused by the formation of an impermeable layer of large molecules on the root
surfaces that lower accessibility for nutrient cations as inferred from voltammetric experiments. A germination
assay using spruce seeds, however, indicated allelochemical effects of large molecules, which exhibit a higher
aromaticity than the small molecules as indicated by spectroscopic characterisation. In the growth experiments
with small molecules, no influence on the root development of barley was evident, but an increase of Ca and Mg in
the shoots was detected. During these growth experiments, a large amount of the small molecules, mainly simple
phenols and amino acids, disappeared from the nutrient solution. The loss of small molecules was most likely the
effect of mineralisation.

Abbreviations: DOC - dissolved organic carbon, DOM - dissolved organic matter, LLE - leaf litter extract, MW -
molecular weight, HMDE - hanging mercury drop electrode

Introduction

Dissolved organic matter (DOM) in the soil solution
has a major influence on soil formation and plant nutri-
tion because of its chemical reactivity and mobility
in the soil profile (Blaser, 1994; Bloomfield, 1964;
Bruckert, 1970). The sources of chemically active
DOM in the soil solution are excretions from microor-
ganisms and plant roots, leaching from the canopy as
well as the litter mat, and, to a minor extent, humus
mineralisation (Blaser, 1994; DeLong and Schnitzer,
1955; Lutwick and DeLong, 1954).

Dissolved organic substances originating from leaf
litter or humus can influence the development of plants.
Humic substances mostly have a stimulatory effect
on plant growth (Chen and Aviad, 1990; Vaughan et
al., 1985). In contrast, substances originating from
aqueous litter extracts from trees, shrubs, grasses, or
ferns, mostly negatively affect plant growth (Einhellig,
1986; Fisher, 1987; Mallik, 1992; Nilsson et al., 1993;
Rice, 1984). In a detailed investigation, Grimvall et al.
(1991) attributed the inhibitory effect on root growth
by the runoff of a forested catchment mainly to the
high molecular weight fraction of DOM.

The aim of the present study was to investigate the
influence of low and high molecular weight fractions

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of DOM on plant root development, using barley as the model plant. As model for DOM in the soil solution, an aqueous chestnut leaf litter extract (LLE) was chosen because of its well characterised structural- and complexation-chemical properties (Blaser et al., 1984; Blaser and Sposito, 1987; Luster, 1990; Luster et al., 1994). Spectroscopic, analytical-chemical and biological approaches were used to elucidate possible interactions between organic molecules and root surfaces. The formation of a layer of organic molecules on root surfaces may lower the permeability for nutrient elements (Blaser, 1994). In addition, organic molecules can react specifically with roots which is known as allelochemical interference (Fischer, 1987; Rice, 1984). Both mechanisms can have an inhibitory effect on plant root development.

Materials and methods

Chemicals, labware, elemental analysis and spectroscopy

All chemicals were reagent grade. Water used for seed germination, preparation of nutrient solutions and extraction of leaf litter was purified by a Millipore Milli-Q plus water system. Before each use, extraction bottles and growth buckets were cleaned in four steps with 0.1 M NaOH, purified water, 1 M HCl, and purified water. Filters for the preparation of LLE were washed with 1 M HCl followed by thorough rinsing with purified water. Membranes used for molecular filtration were cleaned and stored according to the manufacturers recommendations (Amicon, Scientific Systems Division, Danvers, MA, USA).

Elemental concentrations were measured as follows: dissolved organic carbon (DOC) on a Shimadzu TOC-500, total carbon and nitrogen in solid samples on a Carlo Erba Instruments NA-1500 Series 2, other elements in LLE and fractions of it or in digests of roots and shoots by inductively-coupled plasma optical emission spectrometry (ARL 3580). The digests were obtained by dissolving the ash from dry combustion at 500 °C in 0.1 M HNO$_3$.

Molecular absorption and fluorescence spectra were recorded on a Shimadzu UV-40 and a Shimadzu RF-5000 spectrometer, respectively, using quartz cells (path length 1 cm). Prior to recording the spectra, sample solutions were diluted to a DOC concentration of 20 mg kg^{-1} or less and adjusted to pH 4. Molar absorption coefficients, ε_c, were calculated by dividing the absorbance by the molar carbon concentration in solution.

For ^{1}H-nuclear magnetic resonance (NMR) spectroscopy, 10 mg of freeze-dried sample were dissolved in 600 μL D$_2$O to which a drop of 1 M NaOD was added. Spectra were recorded on a Bruker AC200 spectrometer operating at 200 MHz frequency. About 1000 scans with an acquisition time of 4.1 s were accumulated. Chemical shifts were referenced to external tetramethyl-silane.

Preparation and molecular filtration of leaf litter extracts (LLE)

Leaf litter was collected from beneath a chestnut tree (*Castanea sativa* Mill.) at the WSL test site Copera in southern Switzerland. The fresh material was dried at 105 °C and ground using an ultracentrifugal mill with a W-coated rotor and a 1 mm sieve (Blaser et al., 1984). The extraction was performed by purging a suspension of 2.5 g dried powder and 100 g water in a polypropylene bottle with nitrogen for 15 min and shaking it on an end-over-end shaker for about 15 h. The suspension was then filtered through a sequence of Schleicher and Schuell 589/1, 589/3 and ME25 (0.45 μm) filters.

Molecular filtration was carried out in the diafiltration mode (washing technique, cf. Buffle et al., 1978) using Amicon TCF-10A thin channel systems. Aliquots of 200 g LLE were filtered at an excess pressure of 10 to 15 kPa through an Amicon YM10 filter (nominal molecular weight cut-off MWCO =10,000) using 1 L of 10^{-4} M HClO$_4$ (pH 4) as washing solution. The filtrate was concentrated on a rotary evaporator to 200 g and then filtered at an excess pressure of 15 to 25 kPa through an Amicon YM1 (MWCO 1000) filter, again using 1 L of 10^{-4} M HClO$_4$ (pH 4) as washing solution. Thus, three fractions were obtained with molecular weight (MW) >10,000 (large molecules), 1000 < MW < 10,000 (medium molecules), and MW <1000 (small molecules), respectively. For all experimental work, only the fractions with MW <1000 and >10,000 were used.

Growth of barley roots

Barley seeds (*Hordeum vulgare* L. var. Flica) were spread on a square polyester net (side length 9 cm, mesh size 3 mm, styrofoam swimmers on each side) floating on water. For germination, this set-up was placed in a growth chamber with a constant temper-

ature of 20 °C, 70% humidity and 16 h illumination (PAR:100 μmol m^{-2} s^{-1}) per day. After 4 days, ungerminated seeds were removed and the number of seedlings reduced to twenty. These selected seedlings were placed together with the polyester raft into a 3 L polycarbonate bucket containing 2 L aerated nutrient solution (7.5 mM KNO$_3$, 3.25 mM Mg(NO$_3$)$_2$, 5 mM Ca(NO$_3$)$_2$) and LLE or a molecular weight fraction at a DOC concentration between 0 and 40 mg kg^{-1}. The solution pH was set to 4 and adjusted daily. After a growth period of 7 days under the same conditions as during germination, the plants were harvested. In general, treatments were carried out in duplicate.

For each treatment, the total root lengths of ten randomly selected plants were averaged. Selected root tips were examined with a dissecting microscope and by low-temperature scanning electron microscopy using a Balzers SCU 020 scanning cryopreparation unit and a Philips SEM 515 according to Müller et al. (1991) and Scheidegger et al. (1991). Roots and shoots of all twenty plants per treatment were dried at 60 °C for 3 days and ground. Samples from mixtures of all powdered shoots were then used for elemental analysis.

Adsorption of LLE at a Hg electrode and interaction with Cd^{2+}

A hanging Hg drop electrode (HMDE) was used as a model surface to study adsorption of organic compounds by root surfaces. Although the two surfaces cannot be compared directly, the major processes responsible for adsorption of organic compounds (hydrophobic expulsion from solution and attraction by electrostatic forces) are assumed to be comparable. The surface charge of roots is supposed to be mainly negative due to the presence of pectin-like structures of the mucigel (Rougier, 1981), but minor contributions of positively charged functional groups cannot totally be excluded. Thus, conditional adsorption isotherms for the adsorption of small and large molecules of the LLE by the HMDE were recorded at negatively and positively charged electrodes. In addition, the influence of a layer of these small or large molecules adsorbed at the Hg electrode on the reduction and oxidation wave of Cd was studied. This experiment served to assess the permeability of an adsorbed layer of organic molecules for Cd^{2+} (e.g. Batina and Cosovic, 1987; Brezonik et al., 1976; Plavsic et al., 1991). This permeability may influence the diffusive transport of cations to the root surface which is a first step in nutrient uptake. Cadmium^{2+} was used as an electrochemically acces-

sible model ion for the macronutrient Ca^{2+}. Cadmium exhibits a well-defined, reversible two-electron reduction wave at a half-wave potential of -0.6 V which is close to the potential of maximum adsorption of most natural organic compounds (Cosovic, 1985), whereas the redox potential of Ca^{2+} lies outside the range that is accessible with a Hg-electrode. The two cations, Ca^{2+} and Cd^{2+}, are chemically similar in terms of effective ionic radii and stability constants for complexes with O-donor ligands (Martin, 1984). In addition, it has been observed that the affinity of proteins for Ca^{2+} and Cd^{2+} is similar (Behra and Gall, 1991; Cox et al., 1984).

Experiments were performed with a Metrohm E 506 polarograph connected to a Metrohm VA 663 electrode stand. The latter was equipped with (1) a multimode Hg working electrode used as a HMDE, (2) a Ag/AgCl/3 M KCl reference electrode connected to the electrolyte solution through a salt bridge (0.1 M NaNO$_3$), and (3) a Pt counter electrode. Adsorption of LLE by the Hg surface was studied using phase selective alternating current voltammetry (for details see Jehring, 1974, or Bond, 1980). Experiments were carried out with stirred electrolyte solutions (0.1 M NaNO$_3$) adjusted to pH 2.1 or 4.0 containing molecular weight fractions of LLE in DOC concentrations of up to 20 mg kg^{-1}. A constant electrode potential of -0.4 V (vs. Ag/AgCl) resulted in a hydrophobic Hg surface with a slightly positive charge, whereas a potential of -0.8 V led to a slightly negative surface. Amplitude and frequency of the superimposed alternating voltage were 10 mV and 75 Hz, respectively, and the phase angle φ was 90°. The surface coverage Θ of the Hg–electrode after an accumulation time of 60 s was calculated as follows: $\Theta = (i_{c,0} - i_{c,\Theta})/(i_{c,0} - i_{c,LLE})$. Here, $i_{c,0}$, $i_{c,\Theta}$, and $i_{c,LLE}$ refer to the capacitive current, i_c, measured with an electrode surface that is uncovered, partially covered, and completely covered by LLE, respectively. The latter was defined as i_c measured after an accumulation time of 30 min with DOC = 20 mg kg^{-1}. The influence of LLE fractions adsorbed by the Hg surface on the reduction (cathodic wave) and oxidation (anodic wave) of Cd^{2+} was investigated under the same experimental conditions as described above except pH, which was 2.1 to avoid possible competition by complexation in solution by dissolved organic molecules. The concentration of Cd in the electrolyte solution was 1×10^{-4} M. To study the reduction process, the organic molecules were accumulated for 60 s at the slightly positive Hg surface by applying a constant potential of -0.4 V. Then, Cd^{2+}

-ions in the electrolyte solution were reduced at the modified electrode surface by scanning the potential towards more negative values (-0.4 V → -0.8 V). In order to investigate the oxidation process, the initial potential was set to -0.8 V. In this case, the reduction of Cd^{2+} occurred simultaneously with the adsorption of the organic material, and, during a scan towards positive potentials (-0.8 V → -0.4 V), Cd^0 was oxidised at the modified electrode surface. Differential pulse voltammetry and differential pulse anodic stripping voltammetry, both with a scan rate of 20 mV s^{-1} and a pulse amplitude of 10 mV, were used to quantify the resulting cathodic and anodic waves. The results are given in terms of normalised peak heights (i_p/i_{p0}, where i_{p0} and i_p stand for the peak current of the Cd^{2+}-wave in the absence and presence of LLE, respectively).

Seed germination bioassay

The methodology was adapted from Leather and Einhellig (1986) and Pellissier (1990). Molecular weight fractions of the LLE were sterilised by 0.2 μm filtration and added to hot autoclaved water agar in DOC concentrations of 5 or 40 mg kg^{-1}. Twenty mL aliquots of these or DOC-free agar suspensions were poured into Petri dishes. After cooling, 20 seeds of Norway spruce (*Picea abies* (L.) Karst.) were placed randomly on the solid surface under aseptic conditions. Seeds were collected in 1977 from a single tree at Tägerwilen in the eastern part of Switzerland and surface sterilised for 20 min with 30% H_2O_2. Dishes were sealed with parafilm and stored at room temperature. Germination of seeds, defined by radicle length exceeding seed length, was recorded daily. Germinated seeds were marked on the dishes and counted cumulatively for 27 days. Statistical analyses for each treatment consisting of 10 replicates were carried out using standard analysis of variance. Least significant differences were calculated at $p \leq 0.05$ using Fisher's PLSD test.

Results

Development of barley roots in the growth experiments

Mean plant root length after 4 days of germination was 9.0±0.9 cm, and reached a value of 71.3±9.6 cm after 7 days of exposure in the nutrient solution (Fig. 1). Increasing additions of chestnut LLE to the nutri-
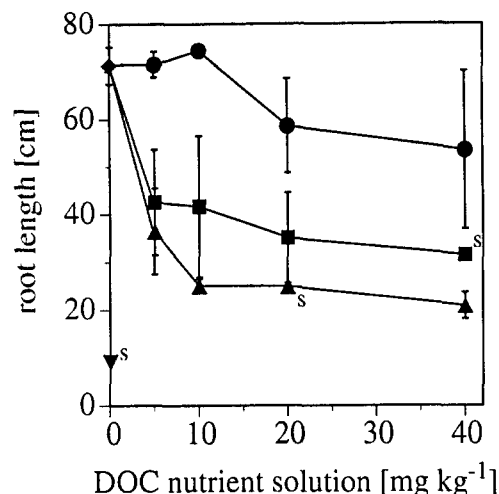

Fig. 1. Mean total root length of 10 plants at the end of growth experiments vs. concentration of DOC from chestnut LLE (■) or its molecular weight (MW) fractions (MW<1000 (●) and MW> 10,000 (▲)) added at the beginning. Root length measured at the end of the control experiment (◆) and at the end of the germination phase (▼) are shown. Error bars indicate the standard deviation of two repetitions, an (s) marks single values.

ent solution led to a reduction of the root length (Fig. 1). Large LLE molecules (MW>10,000) in concentrations of 5 mg kg^{-1} DOC or more led to a drastic reduction of root length (Fig. 1), whereas the addition of small molecules (MW<1000) only in concentrations of 20 mg kg^{-1} or more led to a slight reduction of root length.

Root tips developed in nutrient solutions containing low or high concentrations of the small molecules showed a morphology similar to those of control plants, with regular root caps and root cap cells (Figs. 2a, b). Root tips from plants grown in nutrient solutions containing the large molecules at various concentrations were thickened and brown coloured, and displayed a disordered arrangement of root cap cells (Figs. 2c, d). In addition, root hairs were collapsed or missing. In contrast to the roots, the development of shoots was not affected by the addition of LLE to the nutrient solutions (data not shown).

Element contents of shoots at the end of the growth experiments

Increasing concentrations of small molecules of the LLE in the nutrient solution led to increased Ca, Mg, and Mn concentrations in shoots (Fig. 3). Higher concentrations of large molecules led to slightly lower
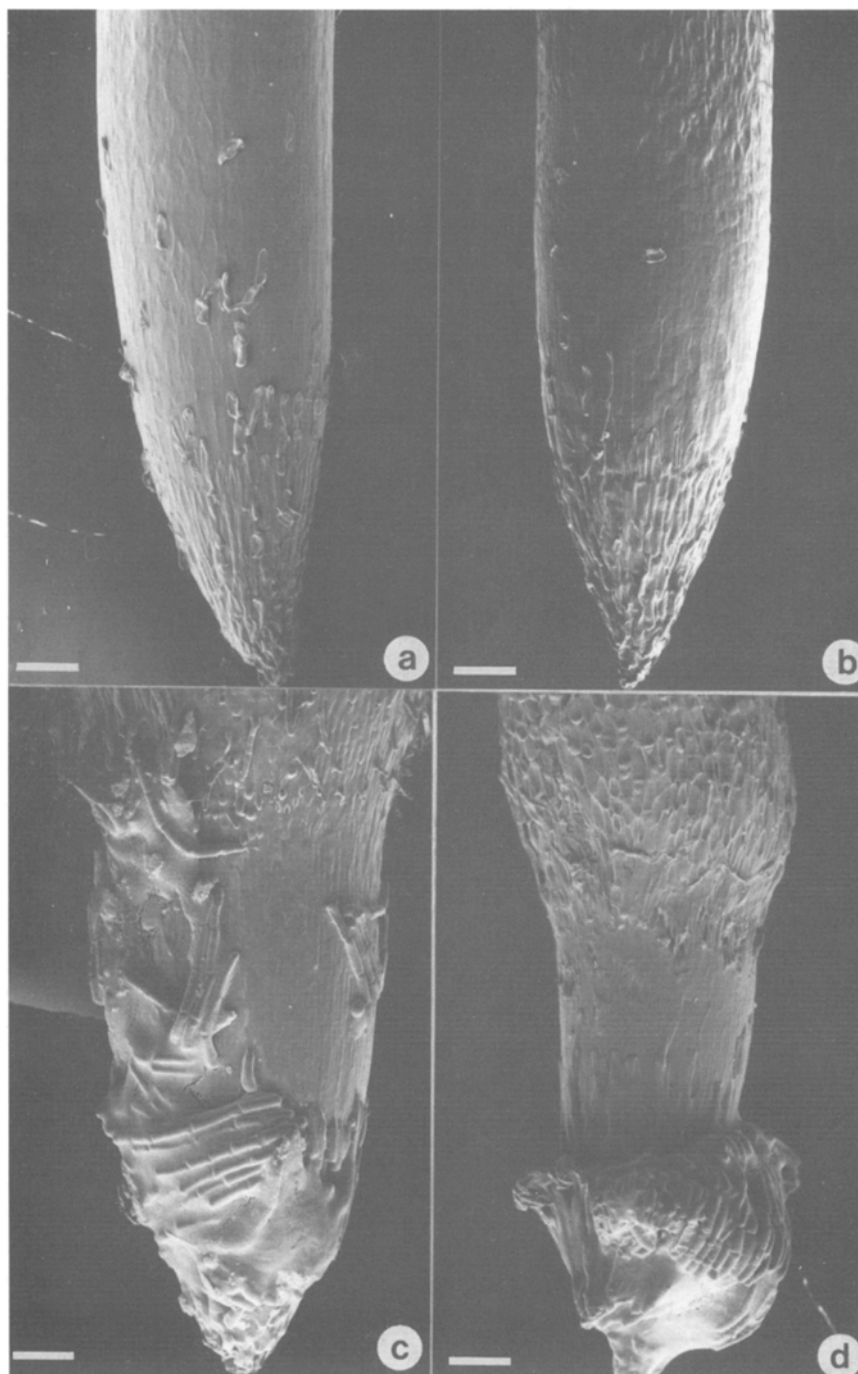
Fig. 2. Low-temperature scanning electron micrographs of barley root tips grown in nutrient solutions with chestnut LLE or its molecular weight (MW) fractions at various DOC concentrations (Scale = 0.1 mm). **a**). Root tip grown without LLE added. Note the regular appearance of the root cap and the root cap cells. **b**). Root tip grown at 40 mg kg^{-1} DOC of small molecules (MW>1000). Note the regular appearance of the root cap and the root cap cells. **c**). Root tip grown at 10 mg kg^{-1} DOC of large molecules (MW>10,000). Note disordered arrangement of the root cap and the root cap cells. **d**). Root tip grown at 40 mg kg^{-1} DOC of large molecules (MW>10,000). Note disordered arrangement of the root cap and the root cap cells.

Table 1. Molecular characteristics of DOM in the chestnut LLE and its molecular weight (MW) fractions. (n.d.: not determined)

	DOC conc.	C	N	C/N	Distribution of organic structures (¹H NMR spectroscopy)				Molar absorption coefficient	
					Alkyl groups	Alkyl groups α to carbonyl or aromatic structures	Carbo-hydrates	Aromatic structures	ε_c (280 nm)	ε_c (400 nm)
		(%)	(%)		(%)	(%)	(%)	(%)	(kg (mol cm)$^{-1}$)	(kg (mol cm)$^{-1}$))
Total extract	1750±170 (mg kg^{-1})	42.6±0.9	n.d.	–	14	16	63	8	331±48	39±6
MW <1000	35±4(%)	39.5±1.2	0.61±0.05	65	14	18	61	8	313± 37	20±2
MW >10,000	44±4(%)	44.7±0.5	0.55±0.01	81	14	11	65	11	323±64	41±12

Table 2. Contents of selected elements (g kg^{-1} C; ± SD) in the chestnut LLE and its molecular weight (MW) fractions

	Ca	Mg	Mn	P
Total extract	19±8	14±3	2.6±0.6	2.6±0.7
MW <1000	28±8	23±6	4.0±1.1	7.4±1.9
MW >10,000	18±6	13±2	2.5±0.1	0.4±0.1

concentrations of Ca and Mg, and to slightly increased concentrations of Mn (Fig. 3). In the presence of total LLE, concentrations of selected elements in shoots in general were in between the concentrations measured in the corresponding experiments with small and large molecules (Fig. 3).

LLE characteristics and changes during the growth experiments

The chestnut LLE had a pH of 4.11±0.01. The DOC concentration of the total extract was 1750 mg kg^{-1} with 44% corresponding to large and 35% to small molecules (Table 1). The C content of the low molecular fraction was distinctly lower than that of the high molecular fraction, whereas the N content of both fractions was similar (Table 1). According to ¹H-NMR spectroscopy, small and large molecules were composed of about two-thirds of carbohydrates. The ratio of the content of alkyl groups to that of aromatic structures was 4.0 for small and 2.3 for large molecules (Table 1). The molar absorption coefficient, ε_c, at 280 nm was about 300 for small and large molecules (Table

1). In contrast, at 400 nm the large molecules showed a higher molar absorption coefficient than the small molecules (Table 1). Concentrations of all elements in the LLE were higher in the low than in the high molecular weight fraction (Table 2). In addition, the P concentration in the high molecular weight fraction was much lower than in the low molecular weight fraction, whereas for Ca, Mg, and Mn the differences were less pronounced (Table 2).

Figure 4 shows the DOC concentration in the nutrient solution measured at the end of the growth experiment vs. the DOC concentration of LLE or fractions of it added at the beginning of the experiment. At the end of the control experiment (without LLE) a DOC concentration of 3.2±0.3 mg kg^{-1} was found, which could be due to the presence of root exudates. Considering the presence of root exudates, in the experiments with small molecules, distinctly less DOC was measured at the end of the experiments than was added at the beginning. In experiments with large molecules or the total LLE, a distinctly smaller decrease of DOC was observed (Fig. 4). Figure 5 shows the change of the absorption spectra of the nutrient solutions during the growth experiments for the control without LLE added and for the experiments at an initial concentration of LLE fractions of 10 mg kg^{-1} DOC. In order to demonstrate the change of the LLE spectra alone, the spectrum at the end of the control experiment was subtracted from the spectra at the end of the experiments with LLE fractions. The spectra of the small and the large molecules at the beginning of the experiments were very similar. A change during the growth experiments, when considering the corrected spectra at the end of the experiments, consisted in both cases mainly of a decrease at shorter wavelengths, where simple
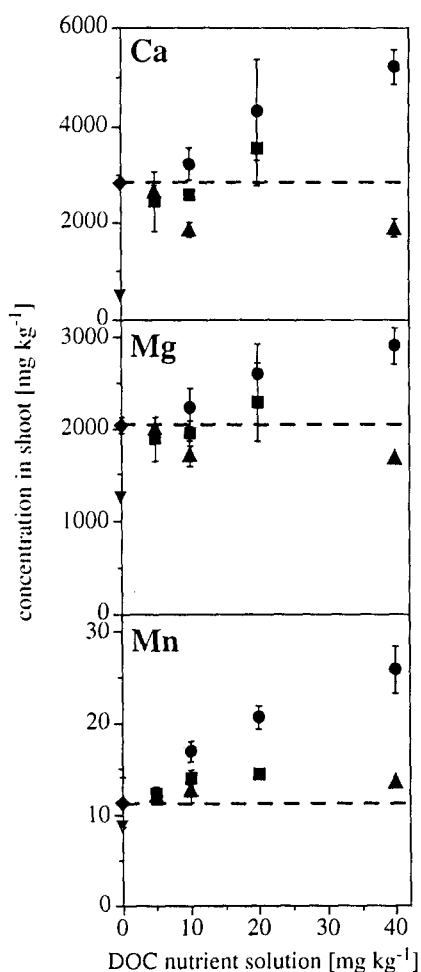
Fig. 3. Concentration of selected elements in shoots of barley at the end of the growth experiment vs. concentration of DOC from chestnut LLE (■) or its molecular weight (MW) fractions (MW<1000 (●) and MW>10.000 (▲)) added at the beginning. Element concentrations measured at the end of the control experiment (♦) and at the end of the germination phase (▼) are shown. Error bars indicate the standard deviation of two repetitions.

Fig. 4. DOC concentration in the nutrient solution measured at the end of the growth experiment vs. DOC added at the beginning for the control experiment (♦) and the experiments with LLE (■) and its molecular weight (MW) fractions (MW<1000 (●) and MW>10,000 (▲)). Error bars indicate the standard deviation of two repetitions, an (s) marks single values.

Fig. 5. Molecular absorption spectra of the nutrient solution measured at the beginning (——) and at the end (——) of the growth experiments with 10 mg kg⁻¹ DOC of molecular weight (MW) fractions of the LLE added. In addition, the spectrum at the end of the growth experiment (- - -) is shown that was corrected for the one at the end of the control experiment (- - - -).

phenols and small conjugated ketons absorb (Pretsch et al., 1981). This decrease was much more pronounced for the small molecules than for the large molecules. In Figure 6 the change of the contour fluorescence spectra of the nutrient solution from the growth experiment with small molecules at a DOC concentration of 20 mg kg^{-1} is shown. The spectrum of the small molecules exhibited three major peaks with maxima at excitation/emission wavelength pairs (EEWP) of 275/325, 330/440 and 250/440 (in nm). The first peak is characteristic for the amino acids tyrosine and tryptophane, the second one can mainly be attributed to phenolic substances like simple phenols, coumarins,
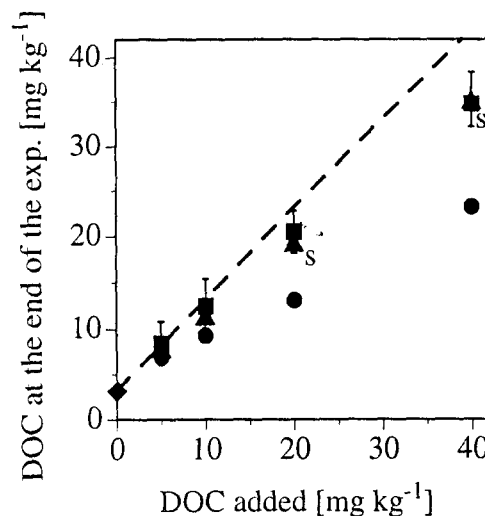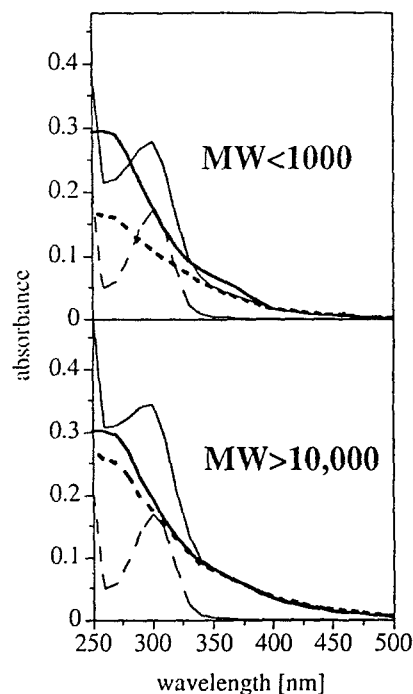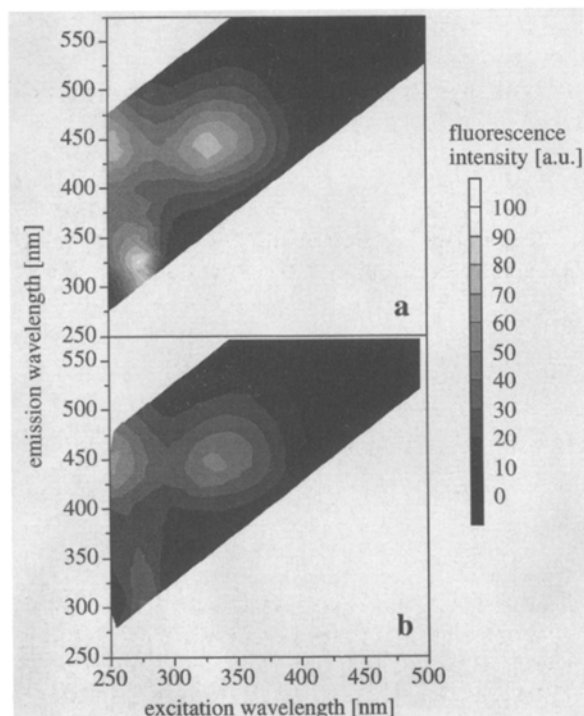
Fig. 6. Contour fluorescence spectra of the nutrient solution from the growth experiment with small molecules of the LLE (molecular weight <1000) added at a DOC concentration of 20 mg kg^{-1}. a) Spectrum at the beginning of the experiment. b) Spectrum at the end of the experiment, corrected for the spectrum at the end of the control experiment without LLE.

Fig. 7. Behaviour of the chestnut LLE at a hanging Hg drop electrode at a deposition potential of -0.8 V (a, c) and -0.4 V (b, d). a,b) Adsorption of small (molecular weight <1000, ●) and large (molecular weight >10,000, ▲) molecules expressed as relative surface coverage of the Hg drop (Θ). c, d. Effect of an adsorbed layer of small (●) and large (▲) molecules on the anodic (oxidation, deposition potential = -0.8 V) and cathodic (reduction, deposition potential = -0.4 V) wave of 10^{-4} M Cd^{2+}, expressed as peak current of Cd^{2+} in the presence of DOC (i_p) relative to peak current in the absence of DOC ($i_{p,0}$).

or flavonoids (Senesi et al., 1991; Wolfbeis, 1985), whereas the nature of the substances responsible for the third peak is unknown. The comparison of this spectrum with the corrected spectrum at the end of the experiment showed a distinct decrease of the peak at EEWP 275/325. The decrease of the peak at EEWP 330/440 was less pronounced, and the third one did not decrease at all. In contrast to the small molecules, the large molecules exhibited very little fluorescence (data not shown).

An almost equal loss of DOC and very similar changes of absorption and fluorescence spectra were observed in a control experiment with LLE but without plants (data not shown).

Adsorption of LLE fractions by a HMDE

For both the negatively (potential -0.8 V) and the positively (potential -0.4 V) charged electrode, a similar adsorption behavior of small and large molecules was found (Fig. 7a, b). A small difference was observed in
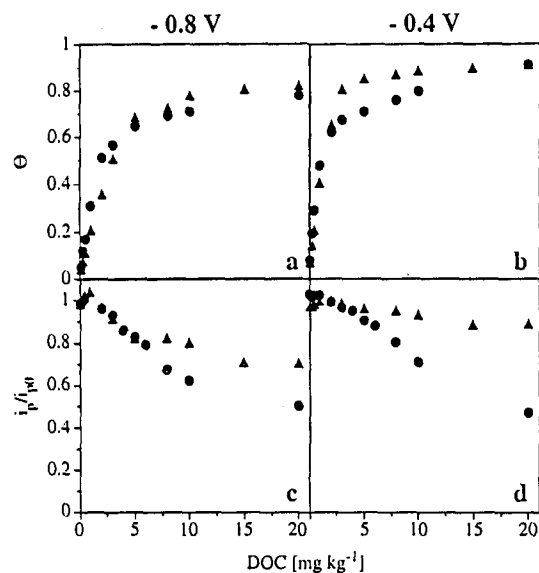
the case of the positively charged electrode in the DOC range 3-10 mg kg^{-1} (Fig. 7b). These results obtained at pH 2.1 were similar to those at pH 4.0 (data not shown). Both small and large molecules suppressed the cathodic (following adsorption at a positively charged electrode), as well as the anodic (following adsorption at a negatively charged electrode) wave of Cd^{2+} (Fig. 7c, d). At DOC concentrations >5 mg kg^{-1}, at which surface coverage by the organic molecules was 70% or higher, the suppression of both waves was stronger by the small than by the large molecules. The influence of the small molecules on the cathodic and anodic process was similar, whereas the large molecules showed a more pronounced inhibition of the anodic process.

Seed germination bioassay on the influence of LLE fractions

Small molecules of the LLE had no significant influence on the germination of Norway spruce seeds (Table 3). The presence of large molecules, however, led to a reduction of the number of germinated seeds

Table 3. Number of germinated spruce seeds (from a total of 20 seeds; ± SE) after 7, 14, and 21 days on agar plates (n=10) containing various molecular weight (MW) fractions of a chestnut LLE in various DOC concentrations. Different letters within columns indicate a significant difference ($p \leq 0.05$)

	DOC conc. (mg kg^{-1})	Days		
		7	14	21
Control	0	3.2±0.4b	18.5±0.4a	19.1±0.3a
MW < 1000	5	2.4±0.5b	18.8±0.3a	19.3±0.3a
MW < 1000	40	2.0±0.5b	17.4±0.5a	18.6±0.5a
MW >10,000	5	5.0±0.7a	17.3±0.6a	17.8±0.4ab
MW >10,000	40	4.3±0.6ab	15.1±0.8b	16.7±0.6b

after 14 and 21 days which was significant at high DOC concentrations.

Discussion

The presence of organic molecules from an aqueous extract of chestnut leaf litter had a negative influence on the development of barley roots in terms of length and morphology. The use of different molecular weight fractions of the LLE revealed that small molecules had only little effect on the root length and no effect on root tip morphology whereas large molecules strongly suppressed root growth and led to an irregular root tip morphology. The phytotoxic effects of the large molecules were visible at DOC concentrations as low as 5 mg kg^{-1}. Similar observations were made by Grimvall et al. (1991), who investigated the influence of the runoff of an old spruce stand on the root development of cucumber. The observed inhibitory effect was mainly attributed to molecules with a molecular weight >1000. For humic substances extracted from a composted sewage sludge a contrary observation was made (Dell'Amico et al., 1994). An inhibitory effect on the shoot and root dry weight of barley was shown for small molecules (MW<1000), whereas a stimulation of growth was detected for larger molecules (MW>1000) and for the total extract. These contradictory observations suggest that the behavior of the MW fractions, of DOM on plant development depends on the source of the DOM. Furthermore, authors reporting on phytotoxic effects of water-extractable organic leaf litter substances give little information on the mechanisms involved (Fisher, 1987; Grimvall et al., 1991; Mallik, 1992; Nilsson et al., 1993).

The inhibitory effect of large molecules on root development can be the result of either physical-chemical or structural-chemical characteristics, which can be expressed in two hypotheses. Hypothesis a) states that, in contrast to small molecules, large molecules form an impermeable layer on root surfaces, and, therefore, reduce the physical accessibility of the root surface for nutrient cations. This is based on the concept that adsorption of DOM increases with increasing polyfunctionality of the organic molecules (Blaser, 1994; Ochs et al., 1993; Tipping, 1990). Hypothesis b) states that specific chemical interactions between large molecules of the LLE and the roots lead to growth inhibition and irregular root morphology. Such phytotoxic effects of plant produced chemical inhibitors have widely been observed and defined as allelochemical interactions (Fisher, 1987; Rice, 1984; Rizvi et al., 1992).

Voltammetric data give direct information on the tendency of molecules to form an impermeable layer on a surface (Cosovic and Vojvodic, 1989; Plavsic and Cosovic, 1991), and thus, they allow to test hypothesis a). In general, the LLE fractions displayed the same qualitative pattern of adsorption to the HMDE and inhibition of the Cd wave as humic substances (Plavsic and Cosovic, 1991). With respect to the affinity of the molecules to the Hg-surface, the conditional adsorption isotherms showed a similar behaviour for both small and large molecules irrespective of the surface charge. The relative peak currents measured at a surface coverage of 70%, or higher indicate a better Cd^{2+} permeability of an adsorbed layer of large molecules than of a layer of small molecules, independent of the electrode surface charge. Thus, the results of the voltammetric experiments contradict hypothesis a) that large molecules form a more impermeable layer on root surfaces than small molecules. Considering elemental concentrations in the shoots, the increase of Mn in the presence of large molecules supports the results of the voltammetric permeability experiment, whereas the decrease of Ca and Mg is in contradiction. In summary, both the voltammetric data and the results of the element concentrations in the shoots are partly contradictory and mainly argue against hypothesis a).

The results of the seed germination bioassay and the microscopic evaluation of the root morphology give clues on specific chemical interactions and thus allow to test hypothesis b). The significant inhibitory effect of the high molecular weight LLE fraction suggests an allelochemical effect of certain large

molecules, and thus, argues in favour of this hypothesis. Spectroscopic characterisation revealed that large molecules in the LLE exhibit a higher aromaticity than the small molecules. This higher aromaticity is mainly due to highly-conjugated aromatic structures such as coumarins, tannins, or flavonoids (Pretsch et al., 1981; Siqueira et al., 1991), as indicated by the comparison of the molar absorption coefficients at 280 and 400 nm. It is known that such compounds exhibit allelochemical effects on plant processes (Rice, 1984; Siqueira et al., 1991). In particular, cell division and elongation can be affected (Einhellig, 1986) and result in an abnormal radical growth (Mallik, 1992).

Besides the inhibitory effect of large molecules on the root development, an increased uptake of elements into shoots in the presence of small molecules was observed. Because Mn was not included in the basic nutrient solution, the presence of this element can be attributed to uptake of residual Mn of the LLE, either in organically complexed form together with the small organic molecules or after release from these molecules following mineralisation. Both processes are consistent with the loss of small molecules detected by DOC-balance. The change of the molecular absorption spectra indicated a preferential loss of simple phenols and small conjugated ketones, and, in addition, the change of the fluorescence spectrum showed a marked loss of amino acids. The almost equal loss of DOC and the similar changes of absorption and fluorescence spectra in the control experiment without plants strongly suggest that the loss of small molecules of the LLE can be attributed mainly to mineralisation and not to uptake by roots. This conclusion is supported by much higher P contents of the low molecular weight fraction. Phosphorus is a key element for microbial growth that has not been added in the basic nutrient solution. However, the increase of the Ca and Mg concentrations in the shoots cannot be explained simply by release from LLE molecules following mineralisation, because it is not proportional to the enhancement of the concentration of these elements in the nutrient solution by the residual contents of the LLE. Different mechanisms have been proposed for a comparable stimulatory effect on the uptake of nutrient cations by humic substances. They either form complexes with the cations that are taken up by plants, or they directly influence uptake mechanisms at root surfaces, plant metabolic processes, or enzyme activities (Chen and Aviad, 1990; Vaughan et al., 1985).

In conclusion, small and large molecules of LLE adsorb at root surfaces to a similar extent. The interaction between the adsorbed large molecules and the roots leads to an inhibition of root growth, to an irregular root tip morphology, and to a slight decrease of Ca and Mg concentrations in the shoots. These effects cannot be attributed to inhibition of nutrient uptake due to formation of an impermeable layer by adsorbed large molecules, but rather to a specific phytotoxic influence of highly conjugated phenolic substances. Furthermore, small molecules stimulate uptake of the macronutrients Ca and Mg.

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