Amounts of carbon mineralised and leached as DOC during decomposition of Norway spruce needles and fine roots

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Abstract

Changes in climate or forest management practices leading to increased litter production will most likely cause increased leaching rates of dissolved organic carbon (DOC) from the O horizon. The rhizosphere is often assumed to have a large carbon flux associated with root turnover and exudation. However, little has been done to quantify the amount of DOC originating from root litter. We studied decomposition of fine root and needle litter of Norway spruce (*Picea abies*) through a combined incubation and leaching experiment in the laboratory using five different litter types: fresh needle litter, aged needles from the litter layer, fresh and dead roots from mineral soil samples, and seven-year-old roots from a previous litter bag study. After respiration measurements, the samples were percolated with artificial throughfall water and DOC and UV absorbance were measured in the leachate. Mineralisation of dissolved organic matter in the leachate and sorption of DOC to ferrihydrite were determined as a measure of DOC ability to be stabilised by iron (hydr)oxide surfaces.

The mineralisation rate and DOC production rate of root samples were always lower than that of needle samples. However, root and needle derived dissolved organic matter (DOM) were similar in terms of aromaticity, as indicated by their specific UV absorbance, and ability to be sorbed by ferrihydrite. For seven-year-old roots, a significantly higher fraction of carbon was lost as DOC (30%) than for younger roots (20%). Furthermore, DOM from old roots bound more strongly to ferrihydrite and is mineralised at a lower rate than DOC from younger roots, suggesting that roots at late stages of decomposition, although a small fraction of total litter, significantly contribute to carbon build-up in mineral soils. The slower decomposition rate of roots compared with needles must be taken into account when modelling litter decomposition.

40 *Key words:* Dissolved organic carbon, mineralisation, fine roots, needles, litter
41 decomposition, Norway spruce, carbon dioxide
42

1. Introduction

Temperate forest ecosystems account for about 25% of the carbon stock in global terrestrial ecosystems, of which about half is stored in soil organic matter (King et al. 1997). Thus, a small change in the carbon balance of soils in these ecosystems might affect atmospheric CO_2 concentration. Compared to the processes controlling soil organic matter (SOM) turnover in the forest floor, our understanding of the chemical and microbial processes controlling turnover of SOM in mineral soil layers is poor. This is a problem when assessing the potential of forest soils to act as carbon sinks or sources since a major fraction of carbon is normally found in the mineral soil. According to an inventory of soil organic carbon (SOC) pools in boreal forest soils in Scandinavia, 70-80% of the organic carbon in the upper 100 cm is found in the mineral soil (Callesen et al. 2003). The major carbon inputs to mineral soil layers are from fine root litter and dissolved organic carbon (DOC) leached from the forest floor. Thus, the carbon pool and its dynamics in the mineral soil are determined by the input rates of root litter and retained DOC, as well as their decomposition rates.

In a recent study, fluxes of carbon into the mineral soil in the form of DOC and fine root litter were measured in three Norway spruce ecosystems situated along a climate gradient in Sweden (Kleja et al. 2008). The annual inputs of carbon as fine root (<1 mm) litter to the mineral soil (0-50 cm) ranged between 73 to 78 g m^{-2} yr^{-1}, whereas the corresponding range for DOC was 9 to 26 g m^{-2} yr^{-1}. Thus, root litter clearly dominates the carbon input. However, the net contribution of root litter to the steady state carbon pool is less clear, because detailed information on the decomposition rates of root litter and DOC in the mineral soil – and their determining factors – is not yet available.

Michalzik et al. (2003) used the Dynamic DOC model (DyDOC) to estimate the contribution of DOC input and root litter to the steady-state carbon pool. According to their

67 simulations, 73-89% of the mineral soil carbon originated from DOC. However, in producing
68 this estimate they assumed that root litter behaved as needle litter in terms of carbon
69 mineralisation and DOC production rates. Furthermore, they assumed that the quality of
70 dissolved organic matter (DOM) produced from the two substrates was identical. These
71 assumptions are critical and might not be valid. For example, a litter bag study by Majdi
72 (2004) found that the mass loss of fine root litter of Norway spruce was half that of needle
73 litter after one year of decomposition. Palviainen et al (2004) reported similar values, with
74 34% mass loss of fine root litter compared with 59% mass loss of needle litter after three
75 years of decomposition in a Norway spruce stand. However, litter bag studies provide no
76 information on the relative losses as CO_2 and DOC. Needle litter is known to produce
77 substantial amounts of DOC during the decomposition process (Fröberg et al. 2005). The
78 extent to which this occurs for fine root litter is less well known. In a recent study, Uselman et
79 al. (2007) incubated ^{14}C-labelled fine root material and leaf litter in 50-cm soil microcosm
80 columns and measured the production of CO_2 and DOC. Their experiment showed that roots
81 decomposed more slowly than leaf litter and that DOC made a significant contribution
82 (~60%) to total carbon losses during the 47-day experimental period. The experimental setup
83 did not allow for any qualitative characterisation of the DOM produced by the two substrates.
84 The quality of DOM formed during decomposition of root litter is probably crucial for its
85 contribution to the build-up of soil carbon stocks in mineral soil layers because the sorption of
86 DOM to mineral surfaces such as ferrihydrate is influenced by its chemical composition.
87 Constituents with higher molecular weight have been shown to adsorb preferentially, and
88 fractions rich in aromatic structures such as lignin-derived hydrophobic compounds, fulvic
89 and humic acids show stronger sorption than compounds rich in carbohydrates (Chorover and
90 Amistadi 2001, Kaiser 2003). As shown by Mikutta et al. (2007), the binding mode of DOM
91 to mineral surfaces is decisive for its bioavailability.

The decomposition rate and DOC production of a substrate changes with time, due to changes in substrate quality during decomposition (Moore and Dalva 2001, Don and Kalbitz 2005). In a study of the fine root dynamics of black spruce (*Picea mariana* L.), decomposition was found to be rapid soon after the roots were identified as being dead, but decreased with time (Ruess et al. 2003). Berg (2000) suggests that the decomposition rate of plant litter at late decomposition stages is very slow and approaches zero. Different stages of decomposition should therefore be considered when estimating DOC originating from litter. To our knowledge, there is no previous study on DOC production from roots in different stages of decomposition. In the present study we focused on determining leached DOC and respired carbon for Norway spruce fine roots and needles at different stages of decomposition. Our specific objectives were (i) to investigate the extent to which the fraction of DOC lost during decomposition depended on the stage of decomposition of the substrate; (ii) to make a brief qualitative comparison of DOM leached from root and needle litter at different stages of decomposition; (iii) to determine the ability of DOM originating from root and needle litter to be sorbed by ferrihydrite; and (iv) to determine the mineralisation of DOM derived from roots and needles.

2. Materials and methods

2.1 Site description

All litter samples were taken from Asa Experimental Forest (57º08'N, 14º45'E), in southern Sweden. The site is one of three Norway spruce (*Picea abies* (L.) Karst.) stands used within the LUSTRA research programme (Berggren et al. 2004, Kleja et al. 2008). Asa is located 190-200 m above sea level in the boreonemoral vegetation zone. Mean annual air temperature is 5.5 ºC and mean annual precipitation 688 mm. The duration of the growing season (temperature > 5 ºC) is 190 days. Field samples were collected in LUSTRA plots with a mesic moisture regime. Stand age was 44-47 years in 2007. Site productivity ranges from 10.1 to 11.3 $m^3*ha^{-1}*yr^{-1}$ and the field and ground vegetation is grass or no vegetation. According to FAO (1990) the soil is classified as a Haplic Podzol, developed on a glacial till. The texture is a stony sandy loam with a medium boulder frequency. Site productivity ranges from 10.1 to 11.3 m^3 ha^{-1} yr^{-1} and the field and ground vegetation is grass or no vegetation (Berggren et al. 2004).

2.2 Root and needle litter samples

Five different litter types were sampled: fresh needle litter, aged needles from litter layer, fresh roots from mineral soil, dead roots from mineral soil and seven-year-old roots from a previous litterbag study. Each litter type was a mix of several subsamples. Needle litter was collected in December 2006 and stored in the freezer. Fresh needle litter samples were obtained by shaking trees and collecting the falling needles. Green needles were excluded. Aged, slightly decomposed needles were taken from the litter (Oi) layer. The turnover time of this layer is about 5 years (Fröberg et al. 2005). Mineral soil samples (0-10 cm soil depth) were collected in October and November 2007. Roots were carefully removed from the soil,

placed in deionised water and gently stirred to remove soil particles. They were carefully cleaned and sorted using forceps under 10× magnification into living and dead roots, based on visual criteria described by Vogt and Persson (1991). Grass roots were excluded. All roots used in the incubation experiment had a diameter of < 2 mm. Strongly decomposed roots were obtained from a previous litterbag study. Fresh roots with a diameter < 2 mm were cut into 1-4-cm-long pieces and placed in litterbags in 1999. These litterbags were buried in the mineral soil at 10 cm depth and recovered in December 2006 and stored in the freezer. All roots used in the experiment were cut into pieces of approximately needle length, 1-2 cm. Water content in the material was determined by weighing litter samples, drying them at 105 ºC for 24 h and calculating the weight loss. Total carbon and nitrogen (N) content in the dried samples were analysed by dry combustion (CN2000, LECO Corporation). Samples used in incubation were not dried.

2.3 Column incubation and measurements

Litter samples were incubated in glass columns (35 cm long with an inner diameter of 2.4 cm) using a method adapted from Sjöberg et al. (2003). Each column had a bottom plug made of silicone, containing a glass drain pipe connected to a silicone tube closed with a clip. A glass fibre filter (1.0 µm pore size, Whatman GF/B) was placed in the bottom of the column to avoid leaching of particles. The columns were filled with litter (equivalent to 1 g dry weight) mixed with 25 g quartz sand (washed with acid and heated to 600 ºC to remove carbon), and a second glass fibre filter (0.7 µm pore size, Pall Corporation) was placed on top. During incubation, plastic films were placed on the opening of the column to allow gas exchange but prevent evaporation of water. Four replicates of each litter type (in total 20 columns) were incubated. Prior to each percolation, column outlets were connected by silicone tubes to vacuum chambers in which borosilicate glass bottles were placed to collect the leachate. A suction of approximately -0.2 bar was set to create unsaturated flow

157 conditions. The chemical composition of the leaching solution resembled throughfall water at
158 the site. The solution consisted of deionised water with addition of ions to give a
159 concentration of Na^+: 0.066 mM, K^+: 0.054 mM, Ca^{2+}: 0.014 mM, Mg^{2+}: 0.01 mM, NH_4^+:
160 0.014mM, NO_3^-: 0.014, SO_4^{2-}: 0.027 mM and Cl^-: 0.114 mM.
161 Prior to the start of the experiment, litter samples were inoculated with a litter extract from
162 the site. Needle litter (14.55 g) was ground and mixed with 1 L deionised water. After 30 min
163 of sedimentation, 5 mL of the solution, with large particles removed, were added to each
164 column. Columns were incubated in a dark room at a constant temperature of 15 °C.
165 Production of carbon dioxide (CO_2) was measured after 1, 2, 3, 6, 9, 12, 15, 19 and 28
166 weeks of incubation. The columns were left uncovered for 30 min and to assist with
167 circulation, air was blown into the columns using a rubber air pump. Columns were then
168 closed with silicone plugs and samples extracted after 10 min (t_0) using a syringe. Total air
169 volume in the columns was 0.09 L. The columns were incubated for 3 to 7 h, dependent on
170 mineralisation rate of substrate, after which a second set of samples was extracted (t_1). The
171 samples were analysed on a gas chromatograph (Hewlett Packard 5890A with Thermal
172 Conductivity Detector, helium in 2 m Porapak T-column and a carrier flow of 25 mL min^{-1}).
173 The CO_2 production rate was calculated as the difference between the total amount of
174 inorganic carbon in gas and pore water phases at t_1 and t_0, divided by the length of the
175 incubation period (t_1- t_0). The amount of dissolved inorganic carbon (DIC) in the pore water
176 was calculated using thermodynamic equilibrium calculations. Before each respiration
177 measurement, the columns were weighed to calculate water content.
178 On the day after CO_2 measurements, the columns were percolated with 50 mL throughfall
179 water solution at a rate of 0.55 mL/min. A preliminary study with spruce needles showed that
180 >90% of accumulated DOC was leached with the first 50 mL of solution (results not shown).
181 Leachate was filtered using a 0.2 µm Acrodisc PF-filter to remove microorganisms and then

182 analysed for DOC concentration (Shimadzu TOC-5000A analyser), pH (Radiometer
183 Copenhagen PHM93 reference pH meter) and UV absorbance at 285 nm (Jasco V-530
184 spectrophotometer). Specific ultraviolet absorbance at 285nm ($SUVA_{285}$) was calculated as
185 absorbance divided by DOC concentration. SUVA has been shown to be strongly positively
186 correlated to the aromaticity of the compound tested (Traina et al. 1990, Chin et al. 1994,
187 Aitkenhead-Peterson and Kalbitz 2005). Kalbitz et al. (2003) used UV absorbance at $\lambda = 280$
188 nm as a parameter to investigate the aromaticity of DOM, whereas Weishaar et al. (2003)
189 used UV absorbance at $\lambda = 254$ nm. In the near UV spectrum ($\lambda = 200$-380 nm), conjugated
190 systems such as those present in aromatic molecules have the highest absorptivities, whereas
191 other electronic structures do not absorb in this spectrum (Weishaar et al. 2003). Samples
192 from percolation after 1, 2, 6, 12 and 19 weeks were analysed for ammonium (NH_4) and
193 nitrate (NO_3) using a colorimetric method (FIASTAR 5000, FOSS AS).

194 *2.4 DOC biodegradation*

195 To determine carbon mineralisation of leachate obtained after 12 weeks of the column
196 experiment, samples were frozen directly after percolation and kept frozen until further usage
197 in the experiment. All replicate samples (20) were incubated in 60-mL sealed flasks at 20 °C
198 in the dark for 8 weeks, with 3 replications. 20 mL of sample was added to each flask.
199 Aqueous samples with more than 10 mg C L^{-1} were diluted before incubation to avoid
200 overgrowth of microorganisms (Hongve et al. 2000) and to minimise concentration effects on
201 DOM biodegradation (Zsolnay 2003). Nutrients (equal weights of NH_4NO_3 and K_2HPO_4)
202 were added in order to adjust the C:N ratio to about 10:1 and facilitate DOM biodegradation
203 (McDowell et al. 2006). All incubation solutions were adjusted to pH 5.5 by adding HCl or
204 NaOH. The flasks were gently shaken by hand every day.
205 For inoculation, a uniform microbial community was chosen for all samples so that any
206 variation measured would only be the result of variation in DOM properties. Before extraction

of the inoculum, the air-dried Oh horizon of a Norway spruce soil was rewetted to a water capacity of 60% and incubated for two weeks at 20 °C to reactivate the microorganisms. The soil was then shaken for 30 min with a 5 mM $CaCl_2$ solution (soil:solution ratio 1:2) and filtered through a 5-µm filter (SMWP 4700, Millipore, Bedford, MA, USA). This inoculum was added to the sample in a sample/inoculum volume ratio of 100:1.

Air in the head space of the flasks was sampled using a syringe and analysed for CO_2 using a gas chromatograph with flame ionisation detector/methanizer (SRI 8610C, SRI Instruments, Schambeck, Bad Honnef, Germany). CO_2 was measured 7 times during the incubation period, at short intervals at the beginning of the experiment and at long intervals at the end. Before starting the incubation, air was applied to each flask to maintain a pressure of about 30 kPa for proper sampling of the headspace. The CO_2 in the flasks was calculated using the general gas equation for the gas phase concentration, and from solubility constants and the pH measured at the end of incubation for the liquid phase concentration.

The CO_2 evolution by mineralisation of the inoculum carbon was determined in control samples (ultrapure water, inoculum and nutrients) and subtracted from the values obtained for the other samples. A glucose solution was used as a second control to test the functioning of the microbial community. After 8 weeks, more than 80% of the glucose-C had been mineralised.

2.5 Sorption of DOM to iron hydroxide

Sorption of dissolved organic matter (DOM) in the mineral soil horizons is probably the main process by which DOM is retained in forest soils (Kalbitz et al. 2005). In Sweden, 60 % of all forest soils are podsolic, with most soil organic matter concentrated in the iron-rich B-horizon (Olsson et al. 2009). The ability of DOM to be sorbed by ferrihydrite was investigated on leachate obtained after 6, 9 and 19 weeks. Due to the limited sample volume obtained, it

was not possible to conduct DOC biodegradation and sorption experiments using leachate from the same percolation.

Ferrihydrite was synthesised using a method adapted from Swedlund and Webster (1999) and Schwertmann and Cornell (2000). NaOH (4 M) was added drop-wise under stirring to a solution containing 36 mM $Fe(NO_3)_3$ and 12 mM $NaNO_3$ until pH 8 was reached. The resulting suspension was aged for 18 h at 20 °C and then back-titrated to pH 4.69 with 0.1 M HNO_3. To release CO_2 the suspension was stirred for 1 h and then 1.40 mL of ferrihydrite suspension was added to 15 mL of sample, corresponding to 0.33 g $Fe(OH)_3$ per litre. Samples for each litter type and time were pooled. Sorption was investigated in the pH range 4.0-6.5, adjusted with NaOH or HNO_3. The samples were shaken end-over-end for 5 h in darkness at 20 °C, and then centrifuged for 20 min at 2000 rpm and 20 °C (Beckman Coulter J6-MI Centrifuge). The supernatant was extracted and pH was measured. The residual supernatant was filtered using a 0.2 µm Acrodisc PF filter and UV absorbance and DOC concentration were analysed. To confirm that there were no ferrihydrite particles in the filtered supernatant, original solutions and filtered supernatant were analysed for iron (using ICP Optima 3000 DV) on the first occasion the sorption test was performed. The iron concentration in filtered solutions was found to be low (<0.5 mg/L), suggesting that all DOC in the filtrate was fully dissolved. Original solutions were analysed for iron, sodium, potassium, calcium, magnesium, and aluminium.

2.6 Statistical analysis

One-way analysis of variance (ANOVA) with pair-wise comparisons was carried out on the accumulated values of DOC and respired C and on C mineralisation values, all using SAS software. Analysis on Fe, Na, K, Ca, Mg, and Al content was carried out using the same analysis, treating values from different weeks as replicates. The correlations between

255 $SUVA_{285}$ and mineralisation rate and between $SUVA_{285}$ and sorption to ferrihydrite were

256 determined using regression analysis in Minitab 15 software.

257

3. Results

3.1 Total carbon and nitrogen content in litter

Carbon content was similar in different litter types and ranged between 44-48%. However, nitrogen content differed between litter types, ranging from 0.6% N in fresh needle litter to 1.2% N in seven-year-old roots (Table 1). Consequently, the C:N ratio was highest (82) for fresh needle litter and lowest (40) for seven-year-old roots. For both needles and roots, the C:N ratio decreased with increasing degree of decomposition.

3.2 Respiration and DOC production

Respiration decreased with time for all litter types except the seven-year-old roots from the litter bag study, for which respiration was very low and almost constant over time (Figure 1a). The mineralisation rate of needle samples was always higher than that of root samples. Accordingly, the accumulated mass of respired carbon was significantly higher for both needle types than for all root types (Figure 2). There were also significant differences in accumulated mass of respired carbon in fresh and older material, both between fresh and old needle litter and between fresh and seven-year-old root litter.

Leaching rate of DOC tended to decrease with time for all litter types and was most pronounced for fresh roots from mineral soil and aged needles from the litter layer, which had the highest DOC leaching on the first measurement occasion (Figure 1b). After the first two months, changes over time became small. As for mineralisation rates, DOC production rates for needles were higher than those for roots. The accumulated mass of leached DOC per gram (dry weight) of litter was significantly higher ($P<0.005$) for needles than for roots (Figure 2).

Fresh litter types showed increasing $SUVA_{285}$, i.e. an increasing aromaticity of DOM, through the first four measurements, after which values stabilised, whereas $SUVA_{285}$ values for other litter types remained almost constant over time (Figure 3). Seven-year-old roots had the highest $SUVA_{285}$ throughout the study, indicating DOM with a high aromaticity.

The fraction of carbon leached as DOC decreased with time for all litter types except fresh needle litter (Figure 4). After the first three percolations, all litter types except seven-year-old roots stabilised at around 20% (Figure 4). The seven-year-old roots had the highest proportion of carbon lost as DOC, ranging from 60% initially to 30% during the later phase of the experiment.

3.3 Water chemistry

For fresh and dead roots, pH in leachate decreased with time, from 6.8 to 5.9 and 6.5 to 5.8 respectively, whereas the pH for other litter types remained fairly constant over time (Figure 5). Leachate from seven-year-old roots had the lowest pH, varying between 5.0 and 5.6.

Ammonium and nitrate concentration in leachate remained low for all litter types except seven-year-old roots (Figure 5). Ammonium concentration decreased and nitrate concentration increased with time in leachate from the seven-year-old roots, indicating onset of nitrification. The relatively high concentration of inorganic nitrogen in leachate from the seven-year-old roots is probably due to a combination of low litter quality and low C:N ratio compared with the other litter types.

3.4 DOC biodegradation

Most mineralisation of DOC in leachate took place in the first three days, when 7-45% of the DOC was mineralised (Table 2). DOM originating from fresh and dead roots had significantly higher mineralisation than that originating from needles ($P<0.005$), whereas

DOM from seven-year-old roots did not significantly differ from DOM from needles. For the root litters, DOM produced during decomposition became more recalcitrant with increasing degree of decomposition, i.e. the fraction mineralised decreased in the following order: fresh roots > dead roots > seven-year-old roots. No correlation was found between the fraction mineralised and $SUVA_{285}$ (P=0.715).

3.5 Sorption to ferrihydrite

Initially, DOM from more decomposed litter types sorbed more strongly to ferrihydrite than DOM from fresh litter types (Figure 6a). Later, the fraction of sorbed DOM did not differ markedly between litter types and ranged between 80 and 95%. As indicated by the small standard error bars in Figure 6, the pH dependency of DOM sorption was low for all litter types in the pH range 4.5-6.5.

We found no significant correlation between percentage DOC sorbed and $SUVA_{285}$ (P=0.078), even though such a relationship between aromaticity and ability to be sorbed by the Ferrihydrite may still exist. The ratio between $SUVA_{285}$ before and after contact with the ferrihydrite was highest at the first test for all litter types, indicating initial preferential sorption of aromatic compounds (Figure 6b). Fresh roots had the highest ratio (2.9), whereas dead roots from mineral soil had a ratio close to one and consequently no preferential sorption. In the experiment carried out on leachate obtained after 19 weeks of incubation, all litter types had a ratio between 1.0 and 1.4, indicating only weak preferential sorption of aromatic compounds.

Leachate from both needle litter types had significantly higher Mg and Ca concentrations than leachate from all root litter types (P<0.05), (Table 3). For Fe, Na, K, and Al there were no clear differences between litter types.

4. Discussion

Respiration rates were highest for fresh litter types (needles and roots), indicating that they contained a relatively large amount of easily degradable substances (Figure 1a). After a few weeks of incubation the easily degradable substances in the fresh litter had decomposed and fresh and aged litter no longer differed. However, the significant difference between needles and roots persisted, suggesting that roots decompose more slowly than needles. This is consistent with other studies on root and foliage litter decomposition (Taylor et al. 1991, Heim and Frey 2004, Palviainen et al. 2004, Bird et al. 2008). Litter quality is important for predicting litter decomposition rates in forest soils (Silver and Miya 2001). Bird et al. (2008) attribute the lower decomposition rate of roots (compared with needles) to lower litter quality, with less labile constituents. Taylor et al. (1991) reported differences in initial chemical quality of litter for different litter types, with 35-37% lignin and 17-29% labile compounds for coniferous roots, compared with 15% lignin and 49% labile compounds for spruce needles. They concluded that lignin content is the most reliable indicator of decomposition rate, followed by nutrient content. In a litter bag study, Palviainen et al. (2004) suggest that lower initial N and P concentrations in roots compared with needles can explain the lower mass loss in roots, in combination with higher lignin content and a smaller fraction of soluble compounds. In our study, however, initial N concentration was higher in roots than in needles, with a lower C:N ratio for roots (Table 1). The difference in mass loss is therefore more probably explained by differences in the structure of carbon compounds.

For root litter, leached DOC followed a similar pattern to respired C, with initially high DOC leaching for fresh roots, in agreement with findings by Uselman et al. (2007). The fresh needle litter differed from this pattern, with lower initial DOC leaching (Figure 1b, Figure 4). A possible explanation for the lower initial DOC leaching in fresh needles compared with

roots is that the needles contained more easily degradable substances (carbohydrates, sugars, amino acids), resulting in losses as CO_2 rather than DOC. Another explanation is that the fresh needles still had a protective wax layer that prevented leaching of DOC.

The $SUVA_{285}$ was initially lower for DOM from fresh litter types, indicating a larger proportion of organic compounds with low aromaticity. This is in agreement with results reported in a study on DOM leaching from forest litter, where UV absorbance of initially leached DOM was very low (Hagedorn and Machwitz 2007). In the present study, seven-year-old roots had the highest $SUVA_{285}$ and the lowest pH and respiration. In this litter type, most of the easily degradable compounds were already decomposed, resulting in DOM with a high degree of aromaticity. The $SUVA_{285}$ for seven-year-old roots ranged between 0.030 and 0.034 L mg^{-1} cm^{-1}, which could be compared with the average $SUVA_{285}$ value for O horizon leachates at the site of 0.023 L mg^{-1} cm^{-1} (Fröberg et al. 2005). The latter value represents a mixture of DOM originating from a range of different substrates and decomposition stages; in our study varying from 0.010 L mg^{-1} cm^{-1} (fresh needles) to 0.034 L mg^{-1} cm^{-1} (seven-year-old roots) (Figure 3).

In general, the fraction of carbon lost from the columns as DOC decreased over time (Figure 4). After the first three weeks of decreasing percentage DOC, all litter types seemed to stabilise, although seven-year-old roots stabilised at a higher fraction of DOC than the other litter types. To our knowledge, there are no previous studies of DOC production from such old fine roots. In a study on the release of DOC by plant tissues (Moore and Dalva 2001), fresh maple leaves were found to release 58% of lost carbon as DOC, whereas old (over-wintered) maple leaves only released 28% as DOC. Those authors concluded that chemical composition and degree of decomposition of the substrate are important in controlling DOC production, with less DOC released from more decomposed materials. However, Kalbitz et al. (2006) reported that DOC production from decaying needles decreases in the first phase of

litter decomposition, whereas an increase takes place with further ongoing litter decomposition because of degradation of lignin. Therefore, lignin-derived compounds can comprise a large proportion of total DOC. The large proportion of DOC from seven-year-old roots in our study indicates that roots follow the same pattern. Seven-year-old roots had the lowest C:N ratio, the highest total nitrogen content in litter and the highest aromaticity ($SUVA_{285}$) of DOC. Nitrate and ammonium content in leachate were also highest for seven-year-old roots. These are all signs of strong decomposition, with all carbon bound in complex structures, leading to carbon deficiency.

DOM biodegradation and the ability of DOM to bind to iron (hydr)oxide surfaces in the soil are important factors affecting the contribution of fine roots to carbon sequestration in mineral soils. Biodegradation of DOM and sorption capacity are both closely related to chemical properties of DOM (Kalbitz et al. 2003, Kalbitz et al. 2005). In our study, DOM from roots had a higher mineralisation rate than DOM from needles (Table 2), which was unexpected since needles had higher respiration and DOC production. However, as decomposition of roots proceeds, the recalcitrance of root-derived DOM seems to become similar to that of needle litter, as indicated by the low fraction of DOM mineralised in the seven-year-old root leachate. There were no significant differences in mineralisation rates between fresh and older litter types except for seven-year-old root leachate, which differed significantly from fresh root leachate but not from needle leachate. However, the mineralisation study was carried out on water samples from week 12, when differences in $SUVA_{285}$ and consequently in DOC quality were small (Figure 3). In such cases, a close relationship between SUVA and degradability cannot be expected. Most mineralisation of DOC in leachate took place in the first three days of incubation (Table 2). This is in agreement with previous studies. Kalbitz et al. (2003) reports a half-life of the labile DOC pool of between 2.6 and 5.0 days for DOC originating from the O horizon in spruce forest,

402 while in a study by Don and Kalbitz (2005) a half-life of 0.9 and 4.0 days is reported for DOC
403 leached from decomposed and fresh spruce litter.
404 In a recent study, Mikutta et al. (2007) showed that binding of organic matter to mineral
405 surfaces generally decreased its biodegradability. Consequently, compounds capable of
406 sorbing strongly to mineral surfaces are more likely to be preserved for a long time in the soil
407 than other compounds. In our study, DOM from older litter types was initially strongly sorbed
408 to ferrihydrite (Figure 6a), whereas DOM from fresh litter types, with easily degradable
409 substances with a low aromaticity, was not as strongly sorbed. At the second and third
410 adsorption test, after 9 and 19 weeks of incubation, most easily degradable, hydrophilic
411 compounds were degraded, even in the fresh litter types, as indicated by the strong sorption to
412 ferrihydrite for all litter types. At the first adsorption test, the high ratio between $SUVA_{285}$
413 before and after contact with the ferrihydrite showed preferential sorption of hydrophobic
414 compounds (Figure 6b), but at the third adsorption test such preferential sorption was very
415 weak. This was expected, since most easily degraded, hydrophilic substances were
416 decomposed by then and differences in $SUVA_{285}$ between litter types were small (Figure 3).
417 When estimating the contribution of DOC and root litter input to the steady-state carbon
418 pool, using DyDOC, Michalzik et al. (2003) assumed that root litter had similar DOC
419 production rates to needle litter. They also assumed the DOC quality of roots and needles to
420 be similar. Our results suggest that the first assumption probably not holds, whereas the
421 second does. Berg et al. (2000) suggest that substrate quality can be the main controlling
422 factor for litter decomposition rates. However, they ignore the contribution of root litter in the
423 forest floor when trying to explain the present carbon stocks and dynamics in the forest floor
424 of Swedish spruce forests and they only include above-ground litter, mainly needles. Our
425 results show that it is important to take litter origin (i.e. above- vs. below-ground litter) into
426 account when estimating DOC production and its contribution to soil carbon sequestration. At

our study site, Asa in southern Sweden, above-ground litter production is estimated to be 118 g C m^{-2} yr^{-1} and fine root litter production in the O horizon 27 g C m^{-2} yr^{-1}(Kleja et al. 2008). If we assume the mass loss rate, i.e. carbon losses as CO_2 and DOC, to be twice as large for needles as for roots throughout the decomposition, then the relative contribution from fine roots to carbon sequestration in the O horizon is 31% (27/(27+(118/2))). Regarding the role of root litter-derived DOC compared with DOC originating from forest floor leachate to carbon build-up in mineral soil layers, our results suggest that root litter-derived DOC makes a significant contribution. During root decomposition about 20% will be lost as DOC. In the mineral soil at Asa, fine root (<1 mm) litter input is 74 g C m^{-2} yr^{-1}, resulting in an approximate input of DOC to the mineral soil of 15 g C m^{-2} yr^{-1}. Compared to 28 g C m^{-2} yr^{-1}, which is the DOC input originating from the O horizon, this means that 35% of the DOC in the mineral soil comes from root litter decomposition. Even though this figure is a rough estimate, it clearly suggests that root litter-derived DOC will make a significant contribution to the carbon build-up in mineral soil layers.

5 Conclusions

Root and needle litter differ in both CO_2 and DOC production. Our results suggest that root-derived DOC can significantly contribute to carbon sequestration in mineral soil layers. Roots had a lower total mass loss than needles, increasing their relative contribution to carbon build-up in the soil. Old roots had a significantly higher fraction of carbon lost as DOC than fresher litter types. Furthermore, DOM from roots in later stages of decomposition bound more strongly to ferrihydrite than DOC from fresh litter types and was mineralized at a lower rate, suggesting that roots at late stages of decomposition, although a small fraction of total litter, significantly contribute to carbon build-up in mineral soils.

Even though roots and needles seem to follow the same decomposition pathways, roots have a slower decomposition rate. This needs to be taken into account when modelling litter decomposition.

Acknowledgements

This work is dedicated to Professor Hooshang Majdi, who passed away in the initial phase of this study. He contributed to initiate this study and with old roots from a litterbag study. Thanks to Göran Ågren and Tryggve Persson for valuable comments. Thanks to Tomas Grönqvist for laboratory assistance, and to personnel at Asa Research Station for helping collect litter. This research was supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning.

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Tables

Table 1. Total carbon (Tot-C) and nitrogen (Tot-N) content (%) and C:N ratio in the five different litter types.

Substrate	Tot-C (%)	Tot-N (%)	C:N
Fresh needle litter	48.0	0.59	82
Aged needles, litter layer	47.9	1.15	42
Fresh roots, mineral soil	44.8	0.88	51
Dead roots, mineral soil	43.6	0.75	58
Seven-year-old roots	46.4	1.17	40

575 **Table 2.** Carbon mineralisation (% of initial C) after 3 and 57 days from DOM obtained after
576 12 weeks of column incubation (n=4, mean, SE in brackets). Means with different letters
577 differ significantly ($p<0.05$).
578

Substrate	Day 3		Day 57	
Fresh needle litter	6.8 (5.5)	a	14 (3.4)	a
Aged needles	21 (11)	ab	24 (9.5)	ab
Fresh roots from mineral soil	45 (5.3)	c	50 (5.4)	c
Dead roots from mineral soil	31 (9.7)	bc	36 (8.3)	bc
Seven-year-old dead roots	11 (5.2)	ab	21 (1.2)	ab

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580

581 **Table 3.** Fe, Na, K, Ca, Mg and Al concentration (mg L^{-1}) in pooled samples of leachate
582 obtained after 6, 9 and 19 weeks of column incubation.
583

Week	Fe	Na	K	Ca	Mg	Al
	Fresh needle litter					
6	0.02	6.62	5.80	2.14	1.04	0.52
9	0.42	4.58	4.16	3.58	1.21	1.05
19	0.02	5.80	5.36	4.74	1.54	1.04
	Aged needles, litter layer					
6	0.05	5.56	9.82	9.04	2.36	0.76
9	0.09	2.82	1.78	4.14	0.83	0.36
19	0.02	5.06	4.90	4.34	1.00	0.39
	Dead roots, mineral soil					
6	0.18	10.04	5.26	0.43	0.24	0.49
9	0.19	4.84	4.46	0.48	0.37	0.38
19	0.18	5.90	6.88	0.64	0.80	0.80
	Fresh roots, mineral soil					
6	0.24	13.00	9.28	0.58	0.49	0.59
9	0.58	4.34	3.16	0.29	0.15	0.29
19	0.20	5.70	7.50	0.33	0.51	0.55
	Seven-year-old roots					
6	0.09	6.54	5.92	0.32	0.14	0.25
9	0.14	7.20	5.58	0.47	0.08	0.39
19	0.11	7.28	6.94	0.47	0.13	0.57

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585

586 Figure captions

587 **Figure 1.** Losses of carbon (mg C g^{-1} substrate day^{-1}) in the form of a) respired C and b)
588 dissolved organic C (DOC) leached from columns (n=4, mean ± SE).

589

590 **Figure 2.** Accumulated losses of respired C (CO_2-C) and leached dissolved organic C (DOC)
591 during 19 weeks of incubation (n=4, mean ± SE). Bars with different letters differ
592 significantly ($p<0.05$).

593

594 **Figure 3.** $SUVA_{285}$ (ultraviolet absorbance at 285 nm divided by DOC concentration)
595 measured in leachate (n=4, mean ± SE). Increasing SUVA indicates increasing aromaticity of
596 DOC.

597

598 **Figure 4.** Percentage of dissolved organic carbon ($DOC/(DOC+CO_2)\times100$) leached from
599 columns (n=4, mean ± SE).

600

601 **Figure 5.** Leachate chemistry. Differences in pH measured in leachate (n=4, mean ± SE).
602 Nitrogen as NH_4-N and NO_3-N measured in leachate (n=4, mean ± SE).

603

604 **Figure 6.** Sorption to ferryhydrite shown as a) percentage of C in leachate adsorbed to
605 ferrihydrite and b) ratio between $SUVA_{285}$ before and after sorption. A ratio of 1 means no
606 effect of quality on sorption, while a ratio larger than 1 indicates preferential sorption of
607 hydrophobic compounds and a ratio of less than 1 preferential sorption of hydrophilic
608 compounds. Pooled samples of leachate after 6 (n=6), 9 (n=5) and 19 (n=5) weeks of
609 incubation (mean ± SE).

610

611

Figures

Figure 1a

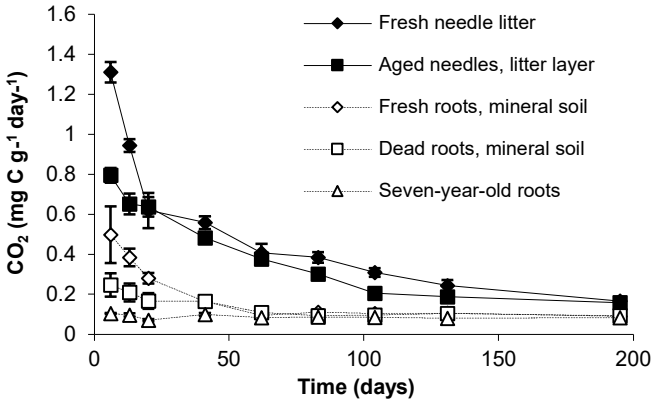
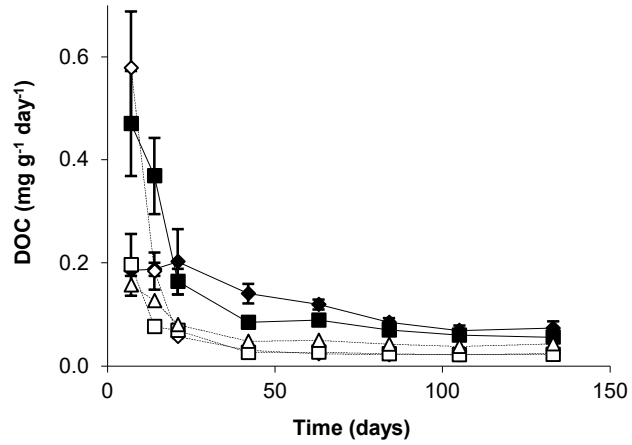


Figure 1b



621 Figure 2

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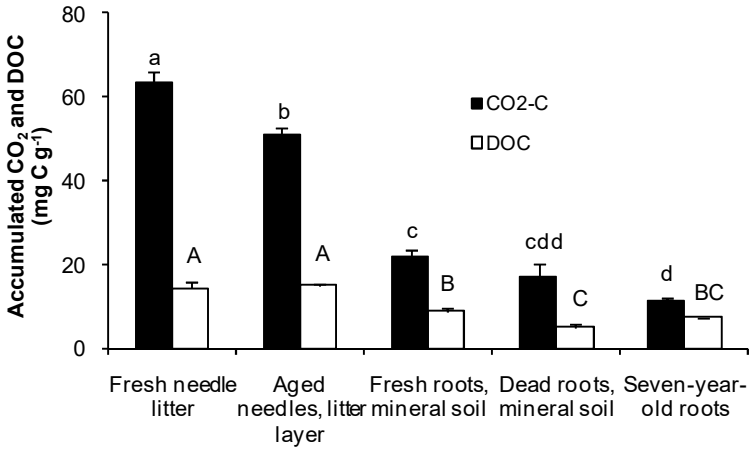

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625 Figure 3

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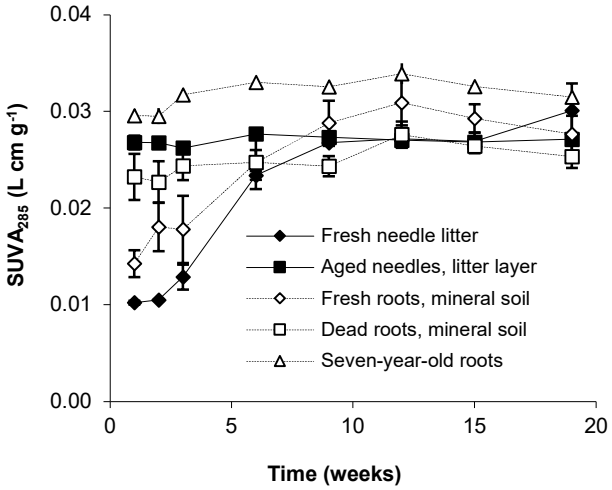

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629 Figure 4

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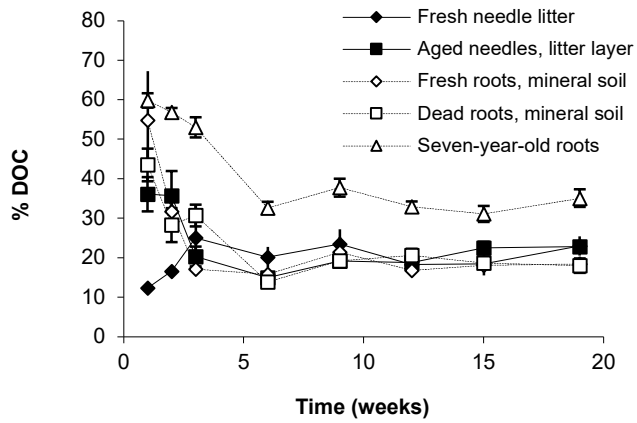

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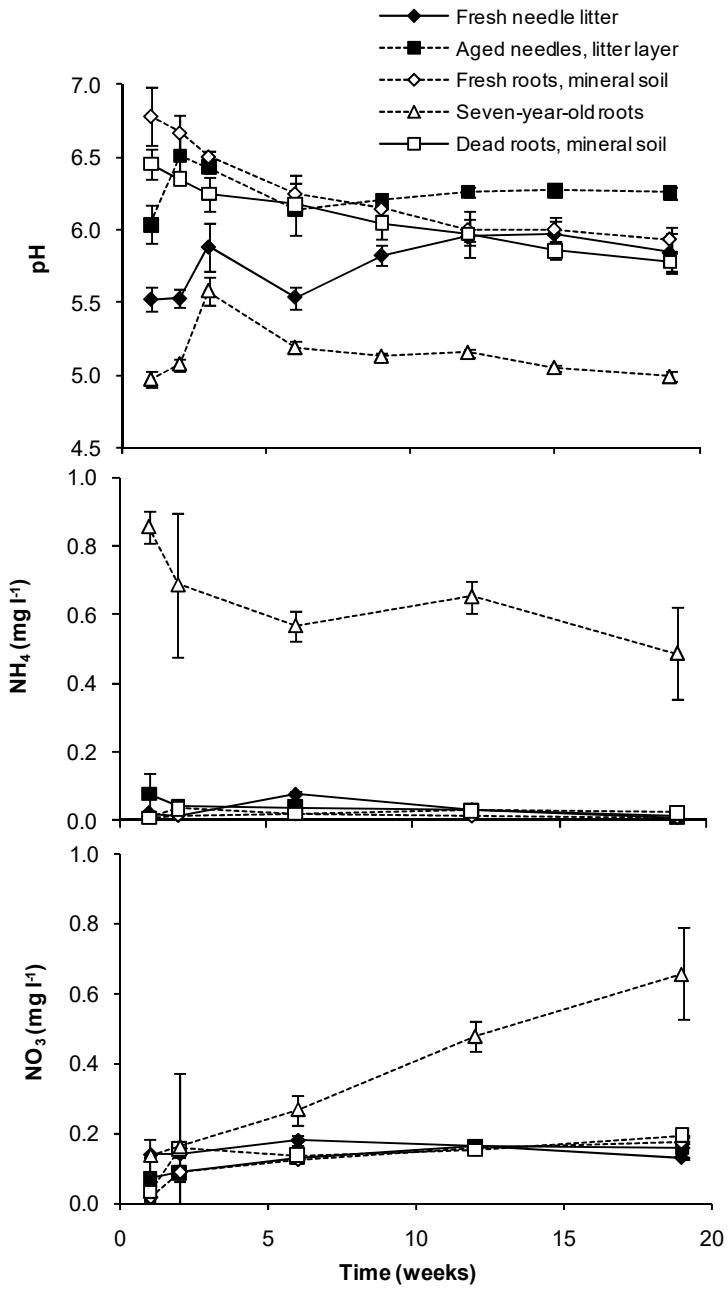
633 Figure 5



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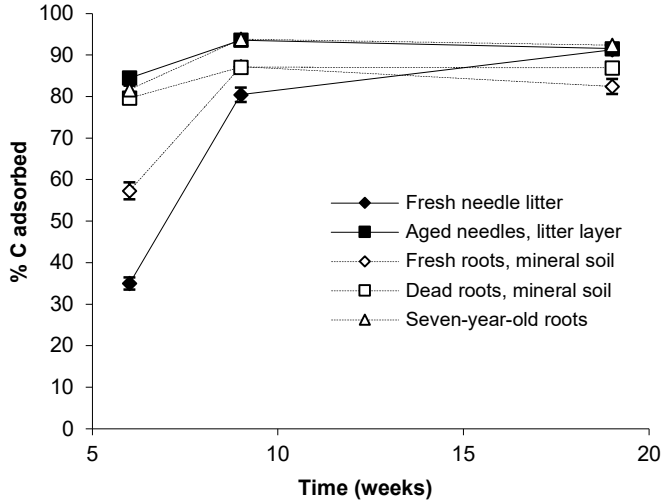
636 Figure 6a



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638 Figure 6b

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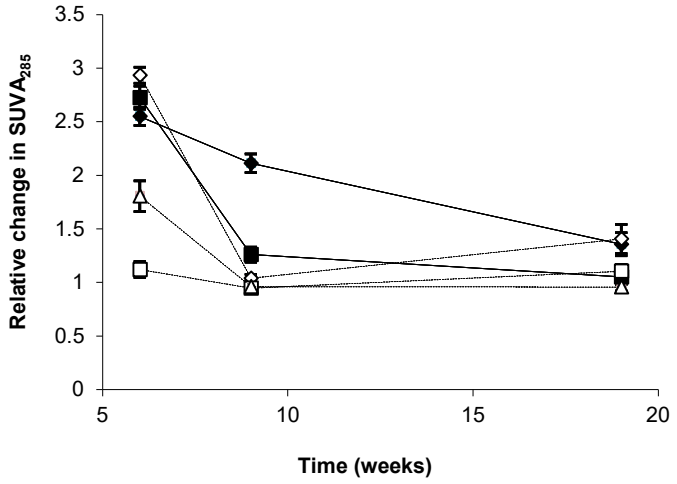

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