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ORIGINAL PAPER

Production of dissolved organic carbon and low-molecular weight organic acids in soil solution driven by recent tree photosynthate

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Abstract Dissolved organic carbon (DOC) is an important component in the terrestrial carbon cycle. Yet, the relative importance of different inputs of DOC to the soil solution remains uncertain. Here, we used a large-scale forest girdling experiment to examine how the supply of recent photosynthate to tree roots and their mycorrhizal fungi affects DOC, in particular low-molecular weight organic acids (LMWOA). We also studied effects of tree girdling on non-structural carbohydrates in microorganism, and examined the effects of freezing of soil and the presence of roots in the soil samples on soil solution DOC and LMWOA in this experiment. The concentration of DOC was reduced by 40%, while citrate was reduced by up to 90% in the soil solution by the girdling treatment. Other LMWOA such as oxalate, succinate, formate and propionate were unaffected by the girdling. We also found that girdling reduced the concentrations of trehalose (by 50%), a typical fungal sugar, and of monosaccharides (by 40%) in microorganisms in root-free soil. The effect of freezing on DOC concentrations was marked in samples from control plots, but insignificant in samples from girdled plots. Release of DOC from cell lysis after freezing was attributed equally to roots and to microorganisms. Our observations suggest a direct link from tree photosynthesis through roots and their mycorrhizal fungi to soil solution chemistry. This direct link should impact solute transport and speciation, mineral weathering and C dynamics in the soil compartment. Importantly, our finding of a substantial photosynthate driven production of DOC challenges the paradigm that DOC is mainly the result of decomposition of organic matter.

Keywords Citrate · DOC · Low-molecular weight organic acid · Carbohydrates · Forest soil · Microorganisms · Mycorrhizae · Tree photosynthesis · Trehalose · Soil solution

Abbreviations

DOC	dissolved organic carbon
LMWOA	low-molecular weight organic acids

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EG early girdling
ECM ectomycorrhizal

Introduction

Production, decomposition and fluxes of dissolved organic carbon (DOC) are important processes in the carbon (C) cycle in terrestrial ecosystems. However, the relative importance of different inputs of DOC to forest soils remains uncertain. Recent litter and humus have been suggested to be the two major sources of DOC (see review by Kalbitz et al. 2000) and the decomposition of litter (either above- or below-ground), humus or microbial necromass are considered as the major processes directly contributing to DOC in soil solution (Kaiser et al. 2001; Park et al. 2002). Root exudation is as another source (Jones 1998; Jones et al. 2004; van Hees et al. 2005) but is, so far, not represented quantitatively in models of DOC fluxes in forest ecosystems (Neff and Asner 2001; Currie and Aber 1997). However, recently we found a 45% reduction in extractable organic C after girdling of a tree-stand (Högberg and Högberg 2002), a treatment which stops the flux of photosynthate from the tree canopy to roots and their associated microorganisms.

The release of low-molecular weight organic acids (LMWOA), highly labile components of soil DOC, is considered to be an important plant and microbial strategy to enhance the availability of nutrients, especially phosphorus (P) and iron (Fe) (see reviews by Jones 1998 and Hinsinger 2001). In forest ecosystems, the highest concentrations of LMWOA are generally found in the humus layer of soil profiles (Strobel et al. 1999; van Hees et al. 2000) especially in association with ectomycorrhizal (ECM) fungal mats (Cromack et al. 1979; Griffith et al. 1994). However, LMWOA generally contribute to less than 5% of the DOC. These small organic molecules are, nevertheless, important C sources for many soil micro-organisms; their low concentrations in the soil solution may, in fact, reflect rapid consumption rather than low production (van Hees et al. 2005).

Recent studies using tree girdling in boreal and temperate forest ecosystems have shown that the direct flow of photosynthate to tree roots supports up to 40–65% of total soil respiration (Högberg et al. 2001; Bhupinderpal-Singh et al. 2003; Subke et al. 2004; Andersen et al. 2005; Olsson et al. 2005; Scott-Denton et al. 2006). Total soil respiration includes respiration by roots, their mycorrhizal fungal symbionts and other microorganisms in the rhizosphere, and by heterotrophic organisms decomposing above- and below-ground litters. Tree girdling terminates the transfer of photosynthate to roots and their associated microbiota, especially mycorrhizal fungi, leaving decomposition by heterotrophic microorganisms as the major source of soil respiratory activity and DOC. It has also been found that the rate of the C flux from canopy photosynthesis to soil respiration is fast with a time lag between the former and the latter of one or a few days in forests with 20- to 35-m-tall trees (Ekblad and Högberg 2001; Steinmann et al. 2004; Ekblad et al. 2005). Högberg et al. (2002) estimated that c. 50% of the total annual C uptake (net photosynthetic production = photosynthesis – foliar respiration) is allocated to root respiration, which also includes the flux of C to mycorrhizal fungi and other root-associated microorganisms, in a boreal pine forest.

Such a large flow of C from the tree canopy to the soil compartment may affect DOC concentrations in the soil as, indeed, suggested by the decrease in extractable organic C found upon tree girdling (Högberg and Högberg 2002). In a tree-girdling experiment in sub-alpine pine forest Scott-Denton et al. (2006) also found lower extractable organic C in girdled plots, and also a winter-time pulse of sucrose, a plant sugar, in the soil of control plots, but not in girdled plots. They attributed this pattern to release of sucrose from tree roots damaged by freezing. Freezing may also cause lysis of microbial cells in the soil (O'Leary 1989; DeLuca et al. 1992, Schimel and Clein 1996).

We, therefore, hypothesized that soil solution DOC and LMWOA as well as extractable non-structural carbohydrates in the microorganisms are critically dependent upon the inflow of recent tree photosynthate to the below-ground compartment.

In order to test these hypotheses we studied in detail soil solution DOC and LMWOA in the organic horizon of the boreal pine forest girdled in 2000 (Högberg et al. 2001). We also report on the effect of tree girdling on non-structural carbohydrates in the microorganisms, and on the effects of freezing and the presence of active roots in the soil samples on DOC and LMWOA. The effect of freezing is attributed to root and microbial cell lysis (Christ and David 1994) and the experimental setup with plots with girdled and non-girdled trees should enable us to distinguish effects on ectomycorrhizal fungi and roots from effects on other microorganisms.

Materials and methods

Field site and experimental design

The study area is located at Åheden within the Vindeln Forest Research Park (64°14́N, 19°46́E, 175 m a. s. l.), 55 km northwest of Umeå, in northern Sweden. The mean annual temperature is 1.1°C and the average annual precipitation is 593 mm, of which about 40% falls as snow. The growing season (daily mean temperature >5°C) starts at the end of May and lasts until the end of September. There is usually snow cover for six months between late October and early May.

The stand studied was a naturally regenerated 45–50-year-old Scots pine forest (*Pinus sylvestris* L.) with a sparse field layer of the dwarf shrubs *Calluna vulgaris* L. and *Vaccinium vitis-idaea* L. The bottom layer consisted of mainly *Cladonia* spp. lichens and *Pleurozium schreberi* mosses. The soil was classified as an Entic Haplocryod (Soil Survey Staff, 1998) developed on sandy silt sediment. The mor layer (Oe horizon) was about 2 cm thick with an underlying eluvial horizon (E) with an average thickness of 1 cm and an illuvial (B) horizon about 30 cm thick. The mor layer had the following characteristics (means and standard deviations, $n = 9$): pH(H_2O) 4.0, bulk density 0.16 (0.05) g cm^{-3}, C: N ratio 40 (0.1) and organic matter content 76% (Högberg and Högberg 2002).

The experiment comprised nine quadratic plots of 900 m^2 each with about 120 trees (Högberg et al. 2001). The experiment was divided into three blocks. The girdling of the pine trees was performed in three plots in early June 2000 (early girdling, EG) and in three other plots in mid-August 2000 (late girdling); leaving three untreated plots as controls (C). In this study, we analyzed the EG and C plots. The effects of girdling on soil respiration and microbial biomass proved to be lasting in this particular experiment with the reductions of soil respiration by c. 50%, and of soil microbial biomass by c. 30%, still observed 4 years after treatment (P. Högberg, unpubl. and M.N. Högberg (2006), respectively). Moreover, sporocarp production by ECM fungi, which is critically dependent on the supply of photosynthate, has been extremely sparse after girdling and has likely been supported by non-girdled trees outside these plots. The girdled pine trees retained their foliage for around 3 years after girdling. At the time when the last sampling for this study was made, August 2003, an increase in needle litter-fall could be observed in the EG plots. However, in this forest needles become deposited onto the bottom-layer of mosses and lichens, which is removed before the sampling of the underlying mor layer. Thus, we suppose that there could have been an elevated leaching of DOC from needle litter in girdled plots, but we observed the reverse, lower concentrations of these compounds in EG plots, as reported below.

Determination of sugars in soil microorganims

On 12 September 2000, i.e. 3 months after the girdling treatment, humus soil from the mor-layer was sampled, by use of a 0.1-m diameter corer, for determination of sugars in soil microbial biomass. Sampling was performed along the border of the central 100 m^2 of each plot. Five composite samples made from 10 cores each was taken from each plot and analyzed separately. Non-structural carbohydrates were analyzed in the same extracts as those used for quantification of microbial C (Högberg and Högberg 2002). These compounds were made extractable by applying the chloroform fumigation-extraction method (modified from Vance et al. 1987). The difference in non-structural carbohydrate content between fumigated soil and non-fumigated soil extracts

was assumed to reflect their content in the microorganisms. Before extraction roots were carefully sorted out by hand. Ten grams of root-free soil was fumigated for 19 h using $CHCl_3$ in a desiccator at room temperature. At the same time as the fumigation process was started, the non-fumigated soil was shaken (150 rev min^{-1}) for 30 min with 50 ml 0.5 M K_2SO_4, thereafter the slurry was filtered (Munktell 00H filters, which are equivalent to Whatman 42 filters).

The two types of salt extracts, from non-fumigated and fumigated samples, respectively, were diluted 1:4 with distilled carbon-free water, and injected onto an ion chromatography system with pulsed-amperiometric detection (Peterbauer et al. 1998). Soluble sugars were separated on an anion-exchange column (Dionex Carbopac PA 10, 250 × 2 mm) with a linear gradient of 20–250 mM NaOH in 25 min at 0.25 mL min^{-1}. After each sample the column was re-conditioned with 500 mM NaOH for 10 min. Individual sugars were identified by comparison of the retention times with authentic standards; the identity of carbohydrates was additionally confirmed by GC-MS analysis. We report detailed data on trehalose and monosaccharides, the sugars for which there were significant differences between girdled and control plots.

Soil solution extraction and determinations of DOC and LMWOA

On 16 August, 13 September and 16 November 2000 and 11 August 2003, humus soil from the mor-layer was sampled for soil solution extractions using a 0.04-m diameter auger. All initial sample handling was performed in the field. One composite sample was taken from each plot, with each composite sample made up from 10 cores. The cores were taken along the border of the central 100 m^2 of each plot. Samples taken in 2000 were immediately frozen using dry ice in a cooler box to minimize the time between sampling and freezing. The samples where thus exposed to a treatment, which is likely to cause cell lysis and release of DOC into the soil upon thawing. In 2003, each composite soil sample was split in four equal sub-samples after gentle mixing in a plastic bag. Roots were removed from two of the sub-samples. Two sub-samples, one with and one without roots, were immediately thereafter frozen using dry ice as above. The two remaining two sub-samples, one with, and the other without roots, were kept in a cooler at 5°C until further analysis in the laboratory. The two non-frozen sub-samples were centrifuged within 4 h after sampling to extract the soil solution using a centrifuge drainage technique (Giesler and Lundström 1993). Sub-samples of about 11 g (fresh weight) of humus soil were weighed into 10 mL syringes containing glass microfibre filters (Whatman GF/C) in the bottom. The syringes were placed in a 50 mL acid-washed centrifuge tube and centrifuged for 30 min at 20,000 rpm using a Beckman J2–21 M/E centrifuge with a JA 20 rotor to extract the soil solution. The extracted soil solutions were filtered (Millex-HV 0.45 μm sterile filter) and then immediately frozen. The frozen soil samples taken in 2000 were thawed, roots were removed, and the samples centrifuged and filtered as described above.

DOC was analyzed on a total organic C analyzer (Shimadzu TOC-5000). LMWOA were analyzed using capillary zone electrophoresis (Westergaard et al. 1998). Di- and tricarboxylate ions were quantified with TD electrolyte in a 50/57 cm capillary at 30°C with hydrodynamic (3.45 kPa) sample introduction for 10 s. Cation interference was prevented by adding 10% v/v of Na4-EDTA solution to the samples. Monocarboxylates were quantified with TTT electrolyte in a 70/77 cm capillary at 20°C with 20 s sample introductions.

Statistical analysis

The effect of girdling on non-structural carbohydrates in micro-organisms and soil solution concentrations of DOC and LMWOA were tested using Student's *t*-test. Each plot was represented by the average value to avoid pseudo-replication giving a $N = 3$ for both the girdled (EG) and control (C) plots. The treatment effects of freezing and root presence in relation to girdling were tested using a three-way analysis of variance (ANOVA). Multiple comparisons among treatments were done with Tukey's test. Significant differences refer to the $P < 0.05$ level.

Results

Non-structural carbohydrates in soil microorganisms

We found reductions in trehalose of 49% ($P < 0.05$) and in total monosaccharides of 38% ($P < 0.05$) in soil microorganisms 3 months after tree-girdling (Fig. 1). Glycerol and disaccharides were also analyzed, but showed no differences between treatments and contributed only one tenth relative to trehalose and total monosaccharides. There were significant positive correlations among monosaccharides, trehalose and chloroform-labile C (Fig. 2, data for organic C released by fumigation are from the same samples as reported by Högberg and Högberg 2002). In the non-fumigated samples there were no significant differences in non-structural carbohydrates between the girdled and control plots.

DOC and LMWOA

Comparison of soil solutions from girdled and control plots using non-frozen samples

The DOC concentrations in the non-frozen samples were about 40% lower in the EG compared to the C plots ($P = 0.013$, Fig. 3); the average concentrations were 57 and 33 mmol dm^{-3}, respectively. Among the LMWOA, citrate was about 90% lower in the EG plots (Fig. 4, Table 1). Acetate and malate also tended to be lower in the EG plots (Table 1 and 2), Table 2. 2a. whereas no effects were observed for the other organic acids. The C in LMWOA comprised approximately 5% of DOC, and especially the di/tri-carboxylic acids citrate, succinate and malate correlated strongly to DOC (Table 3, Fig. 5), whereas no significant correlations were found among DOC and the monocarboxylic acids, acetate, formate and propionate (Table 3). The monocarboxylic acids acetate and formate exhibited the highest average soil solution concentrations.

The water content in the EG plots was not significantly higher than in the C plots ($P = 0.24$, *t*-test), 187 and 164 % water (dry weight; dw) respectively. Neither did the concentration of DOC expressed as mg C g^{-1} soil (dw), in combination with the assumption that the concentration measured in the centrifuge soil solution is representative for the total water content, change the relationship between the EG and C plots (0.093 and 0.063 mg C g^{-1} dw, respectively).

Effects of freezing and the presence of roots on DOC and LMWOA

Freezing of soil samples gave a general increase in DOC, oxalate, succinate, malate and citrate independent of whether roots were present or not, and the effect was most pronounced in the C plots (Figs. 3 and 4). For instance, freezing of soil from EG plots gave only slightly, but not significantly, higher DOC concentration (Fig. 3). In the C plots, freezing resulted in a two-fold increase in DOC when comparing samples without roots and a three-fold increase when comparing samples with roots. The difference in DOC concentration between the samples with and without roots was not significant in the non-frozen samples, but significant in the frozen samples ($P = 0.017$, Tukey's multiple comparison). This was also the case for malate ($P = 0.021$, Tukey's multiple comparison), but was not observed for the other

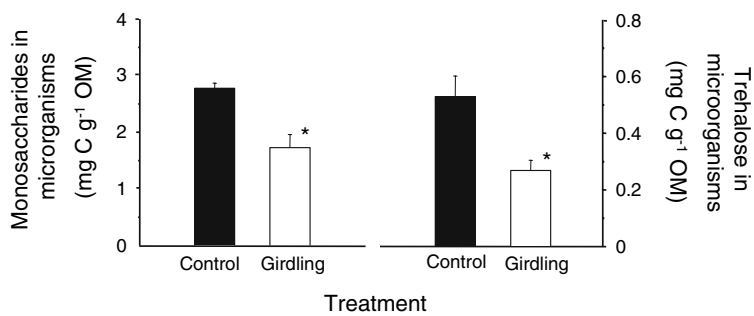

Fig. 1 Average concentrations (and 95% confidence interval) of monosaccharides and trehalose in soil microorganisms in control and tree-girdled plots in a boreal pine forest (sieved humus samples)

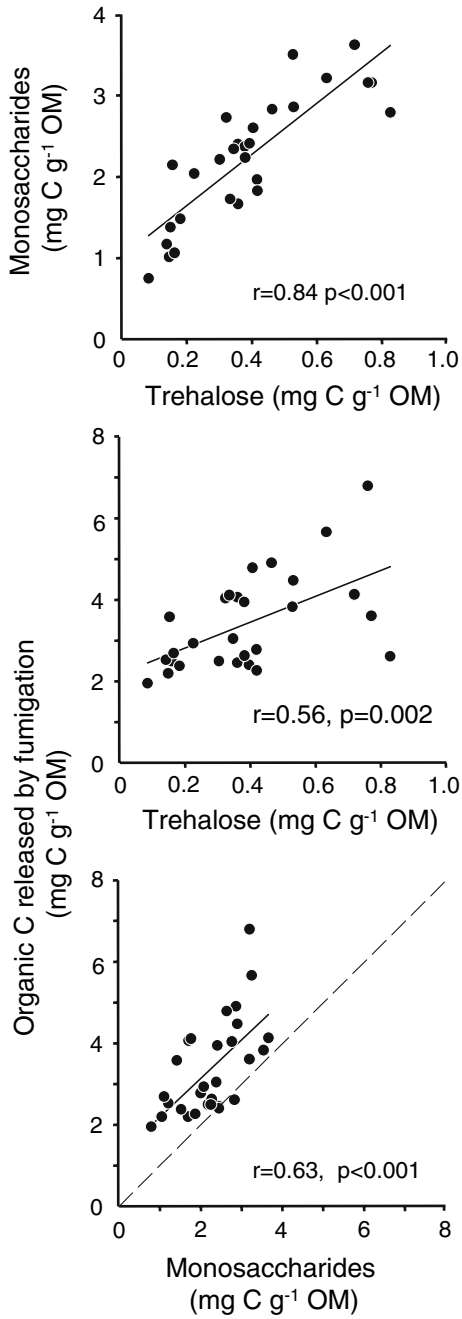

Fig. 2 Correlations among microbial monosaccarides, trehalose and organic C released by fumigation extraction of soils from a boreal pine forest. Data are from tree-girdled and untreated control plots

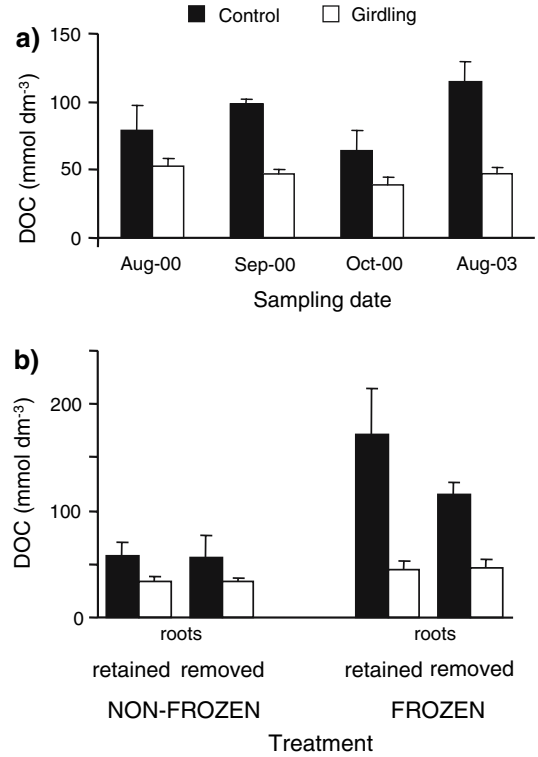

Fig. 3 Average concentrations (and 95% confidence interval) of DOC in soil from tree-girdled and control plots in a boreal pine forest. (**a**) frozen root-free humus soil samples from 2000 and 2003; (**b**) non-frozen or frozen soil samples, with roots retained or removed; these samples were taken in 2003

LMWOA. The freezing effect in the EG plots was minor compared to the C plots and only significant in the case of citrate (Fig. 4).

The three LMWOA acetate, formate and propionate did not show any regular patterns with regard to the different treatments (Fig. 6). Acetate was higher in samples with roots; however, the effect was not related to girdling nor to freezing (Fig. 6).

A comparison of the DOC concentrations in frozen samples without roots sampled in August–November 2000 (Fig. 3) showed that there was a significant treatment effect ($P < 0.001$; two-way ANOVA with treatment and sampling date as main factors) already three months after the girdling.

Discussion

Previous work in this girdling experiment has demonstrated that the termination of the

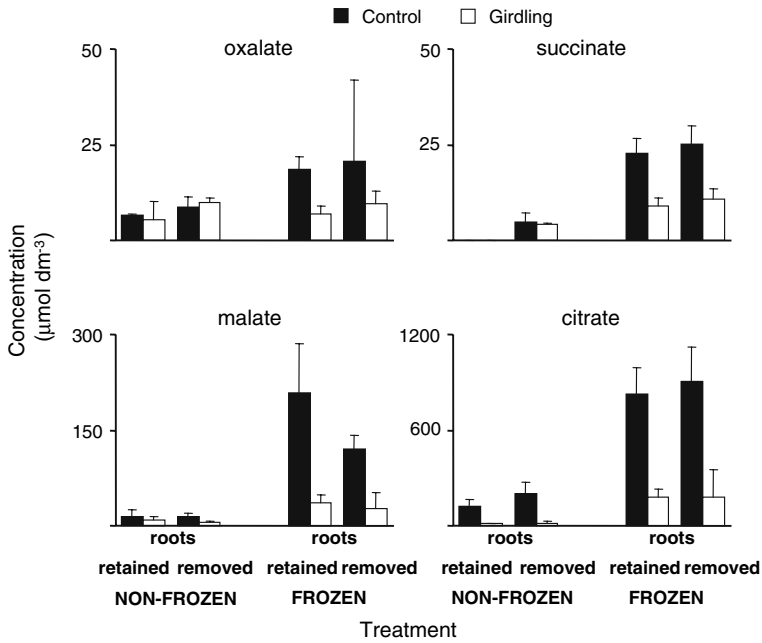

Fig. 4 Average concentrations (and 95% confidence interval) of four low-molecular weight organic acids in soil from tree-girdled and control plots in a boreal pine forest, and in which the soils were frozen or not frozen after sampling and in which roots were removed or retained. The acids succinate, malate and citrate are all part of the citric acid cycle

Table 1 Probability values for students *t*-test comparing soil solution concentrations of DOC and some low-molecular weight organic acids

DOC	Citrate	Oxalate	Malate	Succinate	Acetate	Formate	Propionate
$P = 0.013$	$P < 0.001$	$P = 0.965$	$P = 0.119$	$P = 0.579$	$P = 0.059$	$P = 0.237$	$P = 0.319$

Table 2 Probability values for main and interaction effects for three-way ANOVA

Treatment effects	DOC	Citrate	Oxalate	Malate	Succinate
main effects					
Storage	$P < 0.001$	$P < 0.001$	$P = 0.043$	$P < 0.001$	$P < 0.001$
Roots	$P = 0.065$	$P = 0.369$	$P = 0.320$	$P = 0.040$	$P = 0.004$
interactions					
Girdling × storage	$P < 0.001$	$P < 0.001$	$P = 0.054$	$P < 0.001$	$P < 0.001$
girdling × roots	$P = 0.052$	$P = 0.402$	$P = 0.708$	$P = 0.095$	$P = 0.675$
storage × roots	$P = 0.074$	$P = 0.959$	$P = 0.840$	$P = 0.057$	$P = 0.218$
girdling × storage × roots	$P = 0.054$	$P = 0.965$	$P = 0.885$	$P = 0.090$	$P = 0.933$

Table 2a (continued). Probability values for main and interaction effects for three-way ANOVA

Treatment effects	Acetate	Formate	Propionate
main effects			
Storage	$P = 0.471$	$P = 0.025$	$P = 0.210$
Roots	$P < 0.001$	$P = 0.109$	$P = 0.675$
interactions			
Girdling × storage	$P = 0.020$	$P = 0.319$	$P = 0.589$
girdling × roots	$P = 0.064$	$P = 0.248$	$P = 0.370$
storage × roots	$P = 0.636$	$P = 0.012$	$P = 0.067$
girdling × storage × roots	$P = 0.008$	$P = 0.097$	$P = 0.326$

belowground flux of photosynthate leads to a 40% reduction in soil respiration in 5 days and reductions by up to 50–65% within the first two years (Högberg et al. 2001; Bhupinderpal-Singh et al. 2003), while there was a loss of c. 30% and 45% of total microbial biomass and extractable DOC, respectively (Högberg and Högberg 2002). Previous work also demonstrated a rapid depletion of root starch reserves upon girdling and an almost total elimination of the sporocarp production by ECM fungi (Högberg et al. 2001). These

Table 3 Pearson correlation coefficients matrix for DOC and various low-molecular weight organic acids

	DOC	Oxalate	Succinate	Malate	Citrate	Propionate	Acetate
DOC							
Oxalate	0.625***						
Succinate	0.965***	0.572***					
Malate	0.800***	0.583***	0.844***				
Citrate	0.895***	0.634***	0.911***	0.885***			
Propionate	0.003***	–0.040	–0.277	0.097	0.032		
Acetate	0.114***	0.219	0.252	0.070	0.180	–0.011	
Formate	–0.007***	0.121	0.189	–0.100	–0.075	–0.243	–0.032

*** denotes significance at $P < 0.001$ level

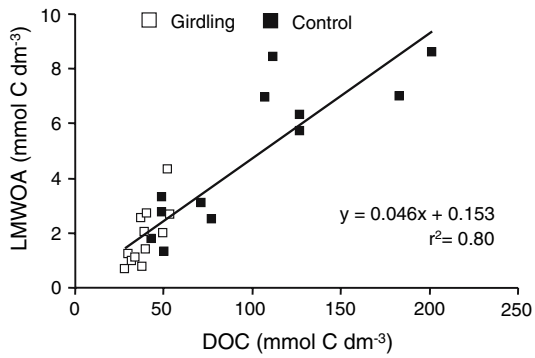

Fig. 5 The relation between DOC and the sum of the low-molecular weight organic acids acetate, citrate, formate, malate, oxalate, propionate and succinate in soil from a boreal pine forest. The samples are from tree-girdled and untreated control plots

effects are noteworthy, since fine roots, mycorrhizal roots, and microorganisms are known to release a variety of C compounds, e.g., sugars, amino acids and carboxyl acids (Hütsch et al. 2002; Jones et al. 2004), several of which were analyzed here. The lower DOC concentrations found in the soil solution from the frozen and non-frozen soil samples from girdled as compared to control plots (Fig. 3a, b) is in agreement with the decline of 45% in extractable DOC in the girdled plots in previously reported for 2000 (Högberg and Högberg 2002), and similar to that found in samples treated in the same way in 2003 (Fig. 3a, b). The effect of girdling on soil respiratory activity, microbial biomass and the production of sporocarps by ECM fungi has been

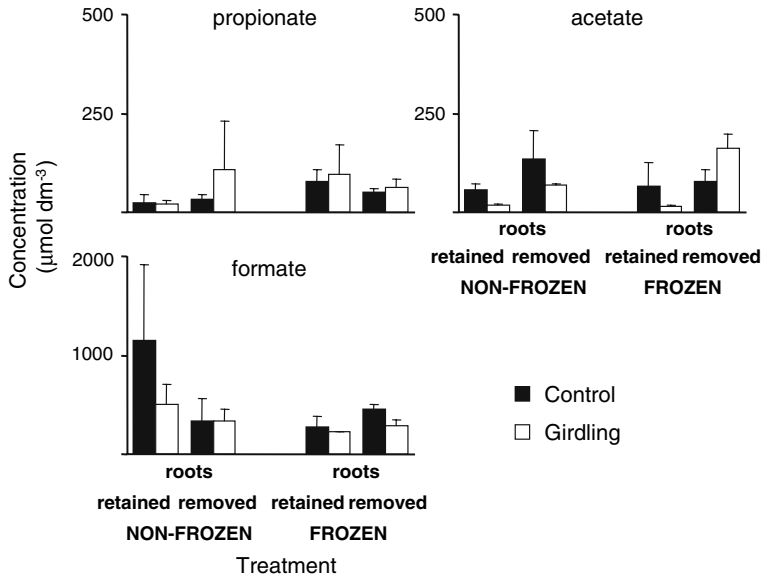

Fig. 6 Average concentrations (and 95% confidence interval) for three low-molecular weight organic acids in soil from tree-girdled and control plots in boreal pine forest, and in which the soils were frozen or not frozen after sampling and in which roots were removed or retained

remarkably stable over the years observed here, and the same seems to be the case with DOC.

Our results clearly demonstrate that termination of the input of photosynthate C to the belowground system reduces the C status of the microorganisms, and both DOC and LMWOA in the soil solution. The latter changes could, in theory, be due to decreased net production or increased net consumption or to changes in the solubility of DOC. However, we suggest that the decreases in DOC and LMWOA are caused by decreased net production, which is supported by the observations of reductions in microbial biomass and its content of sugars, and that other explanations are counterintuitive. For instance, it is hard to explain the reductions in DOC and LMWOA as effects of increased net consumption, since the decrease in concentration occurs only in some LMWOA, while most of them are readily available C sources for the heterotrophs remaining in the soil, and their residence times are generally assumed to be very short (Jones 1998; van Hees et al. 2005). It also seems unlikely that an increase in net consumption would occur in connection with decreases in soil microbial biomass and in soil respiration. Changes in soil-water content, especially dry-wet cycles, may also affect DOC concentrations (Kalbitz et al. 2000). However, the small differences in water-content between treatments do not suggest that this can explain our observations.

The production of LMWOA here attributed to microorganisms may be the result of microbial substrate overflow metabolism, of the type exploited industrially, (Jennings 1995) and can reflect that mycorrhizal fungi experience an N limitation, but a surplus supply of C from their tree hosts. When this occurs, C is channeled away from anabolic to catabolic metabolism, and incompletely oxidized C compounds are exuded or shunted to secondary metabolism. Excess C can also be stored in the case of fungi as trehalose (Jennings 1995). The greater accumulation of this typical fungal compound in the C plots clearly suggest that the fungi in these plots have a higher C status than in the EG plots. The most logical explanation of this difference is the dependence of ECM fungal mycelium of a supply of photosynthate. Our observation of low amounts of trehalose and monosaccharides in the microorganisms are in agreement with the observations of an almost total elimination of sporocarp production by ECM fungi in the girdled plots through the period observed.

Root litter has been suggested to contribute more to DOC relative to root exudates (Qualls et al. 2002). An increase in root senescence should, thus, increase DOC concentrations in the soil. However, Högberg and Högberg (2002) demonstrated that girdling, which should accelerate root senescence, decreased the microbial C and extractable organic C in root-free soil within 1–3 months. Drastically reduced numbers of ECM sporocarps in girdled plots was also observed (Högberg et al. 2001). Dead ECM mycelium (Högberg and Högberg 2002) and fine roots in the girdled plots may stimulate saprotrophic degradation, due to both the extra C input and the relaxation of competition between ECM and saprotrophs (Lindahl et al. 2001), and could hence potentially increase DOC production. This was, however, not the case here, which supports our suggestion of a decreased net production of DOC and LMWOA by roots and associated microorganisms, especially ECM fungi, as the most likely explanation of the pattern observed.

The girdling treatment would also terminate the production of extracelluar enzymes by ECM fungi and thereby their influence on the decomposition of organic matter. While this could have contributed to the decline in DOC, we cannot speculate about the importance of this process, but believe that it cannot have had a major effect. The DOC/DON ratio in the DOC lost after the girdling was 51 (Högberg and Högberg 2002) suggesting a mix of carbohydrates, LMWOA, amino acids and proteins as expected in root exudates (Smith 1979 and references therein). Moreover, Kalbitz et al. (2003) showed that soil solution DOC from a spruce forest Oe-horizons was highly degradable with a half-life of about 3 days for the labile DOC fraction as would be expected from root exudates; this fraction accounted for more than 87% of the total DOC pool.

The specific freezing effect in the C plots is in line with the above observations. Freezing causes cell lysis and release of C into the soil solution.

The effect seems to partly relate to roots and their associated ECM fungi and other root-associated microorganisms, and partly to free-living microorganisms, but likely also including the extramatrical mycelium of ECM fungi, which also occurs in the soil, from which roots had been removed (Fig. 7). Interestingly, there was no effect of the presence of roots in the frozen samples from the girdled plots. Roots, both from the dying pine trees and the vital, but sparse field layer vegetation, are present in the EG plots. The higher concentrations of DOC in soils with, as compared to without roots, from C plots should, however, relate to freeze damage also to tree roots, the associated ECM fungi and other microorganisms associated to the mycorrhizosphere. It is noteworthy that the freezing effect on the release of C due to cell lysis is small as compared to that of chloroform fumigation, i.e. the C released into the soil solution after freezing was only about 6% of the organic C released by fumigation (Högberg and Högberg 2002).

The effect of cell lysis on the LMWOA was inconsistent. There was a similar trend for the three LMWOA involved in the citric acid cycle (citrate, succinate and malate) with regard to their quantitative release into the soil solution. They also correlated strongly to DOC, independent of treatment, despite the fact that they only contributed to about 5% of the DOC (Fig. 5). Strong correlations between di/tricarboxylic acids and DOC have also been reported by Strobel et al. (1999) and van Hees et al. (2000). This suggests that processes involved in the production/consumption of DOC and these LMWOA are linked.

The three monocarboxylic acids, acetate, formate and propionate, are typically produced as waste products during anaerobic conditions. The fact that none of them were affected by the presence of roots or freezing (Fig. 3) supports the idea of them being waste products of plant or microbial metabolism. Neither were they correlated to DOC. Previous studies have reported high concentrations of moncarboxylic acids in forest humus soils (see review by Strobel 2001), but also that they do not correlate with DOC (Westergard-Strobel et al. 1999). The high concentrations of especially acetate and formate in the soil solution may indicate anaerobic processes, since exudation of monocarboxylic acids from plant roots is assumed to be small (Jones 1998; Ryan et al. 2001). Forest soils can have anaerobic micro-sites with a high capacity to form LMWOA (Küsel and Drake 1999; Reith et al. 2002), e.g., possibly because of the high oxygen consumption rates in the rhizosphere. Our results may thus reflect heterogeneity of the soil.

How does the annual C flow of current photosynthate compare to other soil C flows and the DOC pool in the humus? The estimated annual C flow of current photosynthate from the tree canopies to roots was approximately 200 g C m^{-2} $year^{-1}$ in our site in the years 2000–2001 measured as the difference in total respiration between control and girdled plots (Bhupinderpal-Singh et al. 2003) and taking into account the partitioning between C used for root growth and for root respiration (Högberg et al. 2002). Leaching losses of DOC from an adjacent and similar pine forest were estimated at approximately 14 g C m^{-2} $year^{-1}$ assuming an average water leaching of 300 mm $year^{-1}$ (Giesler et al. 1996). The soil solution DOC pool in the control plot is approximately 0.3 g C m^{-2} (0.093 mg C g^{-1} (dw) × 160 kg m^{-3} × 0.02 m) out of which 40% (i.e. 0.12 g C m^{-2}) should be directly derived

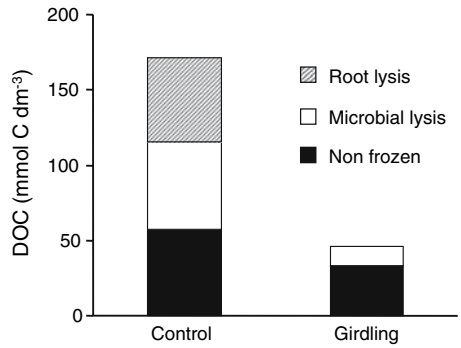

Fig. 7 Summary of plot treatment and soil treatment effects on soil solution DOC plots. We assume that freezing of root-free samples primarily results in cell lyses of microorganisms, but in samples with roots, lyses of both microorganisms and roots. The difference between frozen samples with and without roots is thus the contribution of root lyses. In soil from girdled plots, there was no difference between samples with roots retained or with roots removed, and the increase in DOC concentration due to freezing was thus attributed to primarily microbial lyses

from recent photosynthates according to the differences between C and EG plots observed by us. The small pool of DOC compared to the annual leaching losses indicates a high turnover of DOC in the humus layer. Annual litterfall at a nearby and similar site is roughly 100 g C m^{-2} $year^{-1}$ (Giesler, R., unpublished data), less than 50% of the annual C flow of photosynthate from tree canopies to their roots. Clearly, therefore, photosynthate has the potential to contribute substantially to the soil C flow in this ecosystem and this C input is large compared to the pool of DOC.

Thus, it seems reasonable that changes in the flux of photosynthate C to roots and fungi and other organisms in the rhizosphere could significantly affect the DOC pool in the soil. Such changes could result from changes in the plant C allocation patterns in response to variations in soil nutrient availability. In a recent study of two-year-old spruce saplings, Aitkenhead-Peterson and Kalbitz (2005) showed significant differences in the release of DOC in response to different N treatments, and which appear to be in agreement with plant C allocation theory predicting lower plant C allocation belowground under conditions of higher N supply (Cannell and Dewar 1994). In line with this suggestion, we observed 40% lower respiration by roots, their mycorrhizal fungi and other root-associated microorganisms in fertilized boreal spruce forest, in which stem-wood production was increased by 300% relative to in unfertilized control plots (Olsson et al. 2005).

Conclusions

Our data suggest that the flux of recent photosynthate through roots, their mycorrhizal mycelium and other closely associated microorganisms can support the production of up to 40% of the DOC in the soil solution and accounts for some LMWOA, especially for the production of citrate, in this type of forest. The size of this flux of DOC obviously warrants its inclusion in models of soil C flows. It is likely a very dynamic component responding to seasonality in photosynthesis and plant C allocation patterns, as well as their responses to factors such as drought and nutrient supply.

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