Biol Fertil Soils (2006) 43: 199–207
DOI 10.1007/s00374-006-0093-6

ORIGINAL PAPER

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Effects of the addition of forest floor extracts on soil carbon dioxide efflux

Received: 16 May 2005 / Revised: 27 December 2005 / Accepted: 29 December 2005 / Published online: 5 April 2006

Abstract Composition and effects of additions of fibric (Oi) and hemic/sapric (Oe + Oa) layer extracts collected from a 20-year-old stand of radiata pine (*Pinusradiata*) on soil carbon dioxide (CO_2) evolution were investigated in a 94-day aerobic incubation. The ^{13}C nuclear magnetic resonance spectroscopy indicated that Oi layer extract contained greater concentrations of alkyl C while Oe + Oa layer extract was rich in carboxyl C. Extracts from Oi and Oe + Oa layers were added to a forest soil at two different polyphenol concentrations (43 and 85 μg g^{-1} soil) along with tannic acid (TA) and glucose solutions to evaluate effects on soil CO_2 efflux. CO_2 evolution was greater in amended soils than control (deionized water) indicating that water-soluble organic carbon (WSOC) was readily available to microbial degradation. However, addition of WSOC extracted from both Oi and Oe + Oa layers containing 85 μg polyphenols g^{-1} soil severely inhibited microbial activity. Soils amended with extracts containing lower concentrations of polyphenols (43 μg polyphenols g^{-1} soil), TA solutions, and glucose solutions released 2 to 22 times more CO_2-C than added WSOC, indicating a strong positive priming effect. The differences in CO_2 evolution rates were attributed to chemical composition of the forest floor extracts.

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Keywords Radiata pine (*Pinus radiata*) · Oi and Oe + Oa extracts · Dissolved organic carbon · Water-soluble organic carbon · Polyphenols · ^{13}C nuclear magnetic resonance spectroscopy

Introduction

Decomposition of litter is of primary importance for sustainability of forest ecosystems because of its role in maintaining soil fertility and organic matter (Kalbitz et al. 2000). Nutrient turnover in forest soils is influenced by a supply of readily available carbon (C), which includes water-soluble organic carbon (WSOC) (Harris and Safford 1996). The WSOC enters the soil profile as a leachate from live and decaying above-the-ground phytomass (Cook and Allan 1992), soil humus, root exudates (Kalbitz et al. 2000), and microbial biomass and metabolites (Christ and David 1996). The chemical composition of WSOC has a major influence on soil carbon dioxide (CO_2) efflux (Borken et al. 2004). It was reported that the presence of polyphenols in water-soluble extracts of forest floor materials is particularly important (Heng and Goh 1984). The readily biodegradable fraction of WSOC ranges from 12 to 44% (Gron et al. 1992; Yano et al. 1998). The fraction of the WSOC that is not decomposed is thought to be bound to the mineral soil and may play an important role in soil C dynamics (Vance and David 1992; Guggenberger and Kaiser 2003; Kaiser and Guggenberger 2003).

Soil microbial response to differences in WSOC composition can be variable and from a general ecological and evolutionary understanding, one would expect the indigenous microbial population to be best adapted to the optimal utilization of the organic compounds present in a certain soil (Block et al. 1992). In contrast, Kalbitz et al. (2003) reported that in a study involving degradation of dissolved organic matter (DOM) collected from four sites (beech forest, spruce forest, peat, and agricultural soil), the highest DOM degradation was not observed with the native inoculum. Although the reasons for the different inoculum efficiencies are not known, these results clearly show that

DOM biodegradability depends on the type and origin of the microorganisms.

Carbohydrates and amino acids are highly decomposable in soils and are utilized preferentially by microorganisms during degradation of different compounds in DOM solutions (Amon et al. 2001; Kalbitz et al. 2003), whereas tannins and lignins are resistant to microbial degradation and generally accumulate during decomposition (Kögel-Knabner et al. 1992; Ganjegunte et al. 2005a). Boissier and Fontvieille (1993) found that polyphenols were closely related to the amount of nondegradable DOM in incubation experiments. In forests, polyphenolic compounds in canopy leachate, leaf litter, coarse woody debris, root exudates, and microbial metabolites tend to accumulate and influence C cycling in forest floor and in soil (Hernes et al. 2001; Kraus et al. 2003).

Short-rotation radiata pine (*Pinusradiata*) plantation forestry is a major land use in New Zealand and there is little information on the composition of WSOC from radiata pine forest floor and its influence on soil organic matter (SOM) decomposition rates. It was reported that radiata pine fine litter and coarse woody debris litter contain relatively high concentrations of recalcitrant compounds (Ganjegunte et al. 2004, 2005a). Although effects of the addition of plant residues on soil CO_2 efflux were extensively studied (Vanlauwe et al. 1994; Bell et al. 2003), only a few studies investigated soil CO_2 efflux induced by water soluble organic substrates (Shen and Bartha 1997; Falchini et al. 2003), mostly in arable topsoils. Information on effects of the addition of water-soluble organic substrates on CO_2 efflux from forest soils is very limited. Addition of WSOC from forest floor of monoculture radiata pine can have significant impacts on SOM decomposition and consequently the nutrient availability in these forest soils. The main objectives of this study were to: (1) evaluate the composition of fibric (Oi) and hemic/sapric (Oe + Oa) forest floor extracts and (2) evaluate impacts of the addition of forest floor WSOC, polyphenols in particular, on the CO_2 efflux from soils under fast growing radiata pine.

Materials and methods

Preparation and analysis of forest floor extracts

Forest floor materials were collected from 20-year-old radiata pine stands growing in Bottle Lake Forest near Christchurch (43°30′S, 172°40′E). The forest was planted on windblown greywacke sand dunes (Waikuku sand) and there was a significant build up of forest floor with clearly distinguishable Oi and Oe + Oa layers. Forest floor samples were collected in July 2000 when the site was dry. Forest floor samples were collected from three subplots of 2×2-m area within two selected stands. Approximately 20 kg of intact Oi and Oe + Oa layer material was sampled from each subplot (4 m^2). Depths of Oi and Oe + Oa layers were 1.0 and 3.5 cm, respectively. In the laboratory, Oi and Oe + Oa layers were separated and air-dried. Moisture content in air-dried samples was determined by oven drying subsamples from each plot.

Aqueous extracts of Oi and Oe + Oa layer materials from three subplots from two stands of Bottle Lake Forest plot were prepared separately by shaking 200 g (oven-dry equivalent) of material with 2 l of deionized water for 8 h at 20°C, followed by sequential filtration through Whatman 44, 5 μm and 0.45 μm (Millipore nylon) membrane. Duplicate subsamples of the six filtrates were analyzed for soluble carbohydrates, polyphenols, and total organic carbon (TOC). Soluble carbohydrates were determined using Anthrone reagent method (modified Doutre et al. 1978). Polyphenols were determined using Folin–Denis assay as described by Allen et al. (1974). Total organic C was determined using a Shimadzu TOC-5000A Analyzer.

After the extracts were analyzed they were bulked and concentrated using freeze-drying. The freeze-dried Oi and Oe + Oa extracts were analyzed by nuclear magnetic resonance (NMR) spectroscopy. Solid-state cross-polarization magic angle spinning, total suppression of side-bands (CPMAS-TOSS) ^{13}C NMR spectra was obtained using a Bruker MSL 300 operating at 75.5 MHz. The samples were spun at magic angle spinning rates of 4.8 kHz in a 7-mm OD rotor. Spectra were acquired with 1 ms contact time, 1 s relaxation delay, and 6,000 scans. The spectra were processed using 50 Hz line-broadening and baseline correction. Spectra were plotted with a standard height assigned to the tallest peak. Peak areas were measured electronically using standard Bruker software for the following chemical shift regions (Randall et al. 1995): (1) alkyl C, 0–45 ppm; (2) *N*-alkyl C, 45–65 ppm; (3) *O*-alkyl C, 65–95 ppm; (4) acetal C, 95–108 ppm; (5) aromatic C, 108–140 ppm; (6) phenolic C, 140–160 ppm; and (7) carboxyl C, 160–220 ppm.

Incubation experiment

The annual input of polyphenols to soil from organic matter in radiata pine forest floor in New Zealand is approximately 85 μg g^{-1} soil (Heng and Goh 1984). In this study, the impacts of the addition of Oi and Oe + Oa layer extracts at two concentrations of polyphenols (100 and 50% of the annual soil input of polyphenols) on soil CO_2 evolution rates were evaluated. To obtain the desired concentration of 85 μg polyphenol g^{-1} soil, the aqueous extracts were bulked and concentrated using freeze-drying. Three liters of Oi layer and 1.7 l of Oe + Oa layer extracts (Table 1) were concentrated to 100 ml. The concentrations of polyphenols, carbohydrates, and TOC in the final (concentrated) solutions of Oi and Oe + Oa extracts are shown in Table 2.

Soil used in the incubation was the same as the ones used in microcosms described by Girisha et al. (2003). Soil samples were air-dried and sieved to pass through a 2-mm sieve. The organic C content of soil (SOC) was 9.42% and because the amount of C in amendments (forest floor extracts) added were very small in comparison to SOC

Table 1 The concentrations [mean±SD (mg l^{-1})] of soluble carbohydrates, polyphenols, and TOC in aqueous extracts of Oi and Oe + Oa layers from Bottle Lake Forest (n=6)

Forest floor	Carbohydrates	Polyphenols	TOC
Oi Layer	136.3±0.5	44.1±0.1	372.8±7.4
Oe + Oa Layer	124.5±0.1	33.3±0.4	299.8±13.2
LSD (0.05)	0.51	0.34	21.76

content, soil was mixed with acid-washed fine sand (1:1, weight) to reduce background soil respiration so that the effect of the extracts on soil respiration could be accurately determined. Selected chemical properties of bulked soil–sand mixture are shown in Table 3. The organic C concentration of soil–sand mixture was 3.2% and C:N ratio was 23. Soil–sand mixture was preincubated at 25°C for 10 days to stabilize the respiration rate.

After preincubation, about 30 g of air-dried soil mixture was placed in small, round plastic containers (45 mm in diameter and 27-mm-deep) and moisture content was adjusted to 80% of field capacity by adding either 5 ml of deionized water (in the case of the control) or 5 ml of extracts or standard [glucose or tannic acid (TA)] treatment solutions. Ten treatments and a control (soil treated with deionized water) were evaluated (replicated four times) in the present experiment (Table 4). About 2.07 ml of concentrated Oi extract was diluted to 5 ml and added to 30 g of soil–sand mixture to achieve desired concentration of 85 μg g^{-1} soil for Oi 85 treatment. Similarly, 4.88 ml of concentrated Oe + Oa extract was diluted to 5 ml and added to soils to attain the required concentration of 85 μg polyphenols g^{-1} soil in Oe + Oa 85 treatment. Half of the volumes of concentrated solutions were used for the Oi 43 and Oe + Oa 43 treatments (43 μg polyphenols g^{-1} soil). Because the study was designed to examine the effects of water-soluble polyphenols on soil CO_2 evolution rates, hydrolysable tannin standards (TA) at equivalent concentrations (85 and 43 μg g^{-1} soil) were included. TA solution of 510 mg l^{-1} was prepared and 5 ml of this was used for TA 85 treatment and 2.5 ml of this solution diluted to 5 ml was added to TA 43 treatment soils. We included four different glucose treatments (G) (G 259, G 130, G 210, and G 105 μg g^{-1} soil) to represent the soluble carbohydrate concentrations in the four forest floor extract treatments (Oi 85, Oi 43, Oe + Oa 85, and Oe + Oa 43), respectively. A glucose solution of 5,000 mg l^{-1} concentration was added in different volumes to achieve the required concentrations of soluble carbohydrates.

Plastic containers containing soils amended with extracts/deionized water were placed in 1 l glass jars and incubated at 25°C for 94 days. Soil moisture content was maintained at 80% field capacity by adding deionized water based on weight loss measured on a weekly basis.

Table 2 The concentrations (mg l^{-1}) of soluble carbohydrates, polyphenols, and TOC in concentrated aqueous extracts of Oi and Oe + Oa layers from Bottle Lake Forest

Sample	Concentrated[a]		
	Soluble carbohydrates	Polyphenols	TOC
Oi layer	3,750	1,229	10,518
Oe + Oa layer	1,360	524	4,461

[a]3 l of Oi layer extract was concentrated to 100 ml by freeze-drying; 1.7 l of Oe + Oa layer extract was concentrated to 100 ml by freeze-drying

Table 3 Selected properties of the soil–sand mixture (1:1) used for incubation

Properties	Value
pH (1:2.5)	5.5
Olsen P (mg kg^{-1})	6
Total P (mg kg^{-1})	304
Extractable K (cmol $_+$ kg^{-1})	0.47
Extractable Ca (cmol $_+$ kg^{-1})	2
Extractable Mg (cmol $_+$ kg^{-1})	1.15
Extractable Na (cmol $_+$ kg^{-1})	0.14
Cation exchange capacity (cmol $_+$ kg^{-1})	12.1
Base saturation (%)	32
Density (Mg m^{-3})	0.97
Field capacity (%)	17
Total C (%)	3.2
Total N (%)	0.14
C:N ratio	23

CO_2 evolution from soil

The CO_2 evolved during the incubation experiment was determined using alkali traps according to the method outlined by Alef and Nannipieri (1995). Briefly, this method involves trapping of CO_2 in a NaOH solution and quantifying by titrating with HCl. During the first week of incubation, CO_2 evolution was measured after 1.0, 1.5, and 5.0 days. Thereafter, CO_2 evolution was determined at weekly intervals.

Statistical analysis

The significance of treatment effects on CO_2 production for each incubation period was determined by using one-way ANOVA (Genstat 4.1). Least significant differences (LSD) procedures were used for treatment mean separation. All statistical evaluations are based on $p<0.05$, unless otherwise mentioned.

Results

Qualitative analysis of Oi and Oe + Oa extracts

Concentrations of polyphenols, soluble carbohydrates and TOC in Oi layer extract were significantly greater than those in the Oe + Oa layer extracts (Table 1). The NMR spectra indicated a relatively greater proportion of alkyl-C

Table 4 Amounts of TOC, soluble carbohydrates, and polyphenols added to soil in forest floor extracts and standards

Treatment	Description	Amendment	Milliliter added	TOC in amendment (mg kg^{-1} soil)	Soluble carbohydrates (mg kg^{-1} soil)	Polyphenols (mg kg^{-1} soil)
Control	Control (deionized water)	Deionized water	5	–	–	–
Oi 85	Oi layer extract with polyphenol concentration of 85 μg g^{-1} soil	Oi extract concentrated	2.07 diluted to 5	728	259	85
Oi 43	Oi layer extract with polyphenol concentration of 43 μg g^{-1} soil	Oi extract concentrated	1.04 diluted to 5	364	130	43
Oe + Oa 85	Oe + Oa layer extract with polyphenol concentration of 85 μg g^{-1} soil	Oe + Oa extract concentrated	4.88 diluted to 5	723	211	85
Oe + Oa 43	Oe + Oa layer extract polyphenol concentration of 43 μg g^{-1} soil	Oe + Oa extract concentrated	2.44 diluted to 5	362	105	43
TA 85	Tannic acid ($C_{76}H_{52}O_{46}$) standard for Oi and Oe + Oa extracts with polyphenol concentration of 43 μg g^{-1} soil	Tannic acid (510 mg l^{-1})	5	46	–	85
TA 43	Tannic acid ($C_{76}H_{52}O_{46}$) standard for Oi and Oe + Oa extracts with polyphenol concentration of 43 μg g^{-1} soil	Tannic acid (510 mg l^{-1})	2.5 diluted to 5	23	–	43
G 259	Glucose standard for Oi layer extract with polyphenol concentration 85 μg g^{-1} soil	D-glucose (5,000 mg l^{-1})	1.55 diluted to 5	104	259	–
G 130	Glucose standard for Oi layer extract with polyphenol concentration 43 μg g^{-1} soil	D-glucose (5,000 mg l^{-1})	0.78 diluted to 5	52	130	–
G 210	Glucose standard for Oe + Oa layer extract with polyphenol concentration 85 μg g^{-1} soil	D-glucose (5,000 mg l^{-1})	1.26 diluted to 5	84	211	–
G 105	Glucose standard for Oe + Oa layer extract with polyphenol concentration 43 μg g^{-1} soil	D-glucose (5,000 mg l^{-1})	0.63 diluted to 5	42	105	–

(23%) in the Oi layer extract than the Oe + Oa layer (14%) (Fig. 1 and Table 5). However, the signal intensities for the *O*-alkyl C, *N*-alkyl, and aromatic and phenolic regions were similar for the Oi and Oe + Oa layer extracts. The Oe + Oa layer extract contained relatively greater amount of carboxyl-C (17%) compared to the Oi extract (13%). No statistical analysis of spectra was possible because NMR spectroscopy was carried out on a composite sample extracted from six forest floor samples.

CO_2 evolution

The rate of evolution of CO_2 was greater during the initial stages of the incubation experiment and it decreased with time (Figs. 2 and 3). Data indicated that significant differences in CO_2 evolution were observed between Oi 85 and Oe + Oa 85 treatments up to 46 days, after which, there were no significant differences (Fig. 2). However, no significant differences in rates of CO_2 evolution were observed between Oi 43 and Oe + Oa 43 treatments throughout the incubation (Fig. 2). The differences between Oi 85 and Oi 43 were not significant until

Table 5 Relative signal intensities (%) at different regions of NMR spectra of Oi and Oe + Oa extracts

Shift region	Oi	Oe + Oa
Alkyl (0–45 ppm)	23	14
N-alkyl (45–65 ppm)	12	12
O-alkyl (65–95 ppm)	29	28
Acetals (95–108 ppm)	8	12
Aromatics (108–140 ppm)	10	11
Phenolics (140–160 ppm)	4	6
Carboxyls (160–220 ppm)	13	17

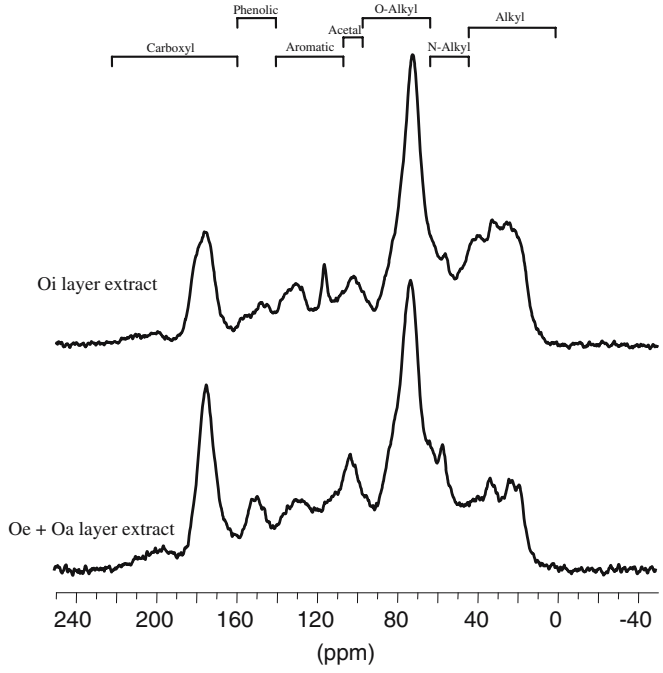

Fig. 1 ^{13}C CPMAS NMR spectra of freeze-dried aqueous extracts of Oi and Oe + Oa layers

68 days; thereafter, CO_2 evolution from Oi 43 was significantly greater than from the Oi 85 treatment. The Oe + Oa 43 treatment recorded significantly greater rates of CO_2 evolution than Oe + Oa 85 throughout the incubation period (Fig. 2).

CO_2 evolution from Oi 85 was significantly greater than its TA equivalent (TA 85) during the first 48 days of incubation, after which, there was no significant difference. The differences in CO_2 evolution between Oi 43 and TA 43 were significant up to 90 days and thereafter, no significant differences were observed (Fig. 3a). The Oe + Oa 85 and TA 85 did not differ significantly in CO_2 evolution throughout the incubation period. In contrast, Oe + Oa 43 produced significantly greater CO_2 than its polyphenol standard TA 43 throughout the incubation period (Fig. 3b). The CO_2 evolution from glucose standard (G 259) for Oi 85 treatment was significantly greater than Oi 85 only after 90 days of incubation.

Significant differences between Oi 43 and its glucose standard G 130 were observed only during the first 14 days of incubation (Fig. 3c). The CO_2 evolution from Oe + Oa 85 was significantly lower than its glucose standard G 210 after 38 days of incubation and the differences remained significant throughout the end of incubation. Significant differences existed between Oe + Oa 43 and its equivalent glucose standard (G 105) up to 46 days (Fig. 3d).

Discussion

Chemistry of Oi and Oe + Oa extracts

The Oi layer mainly consisted of freshly fallen needles and contained greater amounts of water-soluble carbohydrates and polyphenols compared to the Oe + Oa layer. This difference between the two readily identifiable layers is in agreement with other studies (Gamble et al. 1996; Bhat et al. 1998; Kraus et al. 2004). The differences were primarily attributed to the greater degree of leaching and microbial degradation of Oe + Oa layer (Gamble et al. 1996; Bhat et al. 1998; Kraus et al. 2004). The greater degree of decomposition not only influenced the total amount of TOC in the Oe + Oa layer but also the nature of C as more readily available substrates are utilized early in the decomposition process (Hopkins and Chudek 1997).

The NMR spectra of freeze-dried aqueous extracts indicated that while the relative signal intensities of alkyl C region of Oi extract spectra was comparatively greater than Oe + Oa extract, the carboxyl signal intensity for Oe + Oa extract was greater than the Oi layer extract (Fig. 1 and Table 5). Similar results were reported by Fröberg et al. (2003) for a 35-year-old Norway spruce forest floor layer. The alkyl signals are mainly from compounds that are resistant to decomposition such as surface waxes, cutins, suberins, and resins (Baldock and Preston 1995). This was expected because Oi layer material is mostly composed of

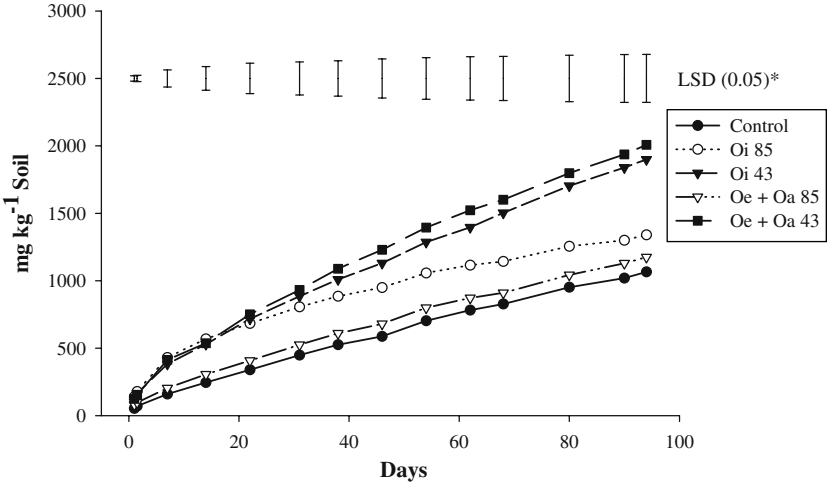

Fig. 2 Cumulative CO_2-C evolution over a period of 94 days from litter extract amendments at 100 and 50% annual input polyphenol concentrations rate (n=44, i.e., four replications × 11 treatments including control). *LSD *bars* are the mean LSDs for all five *curves*

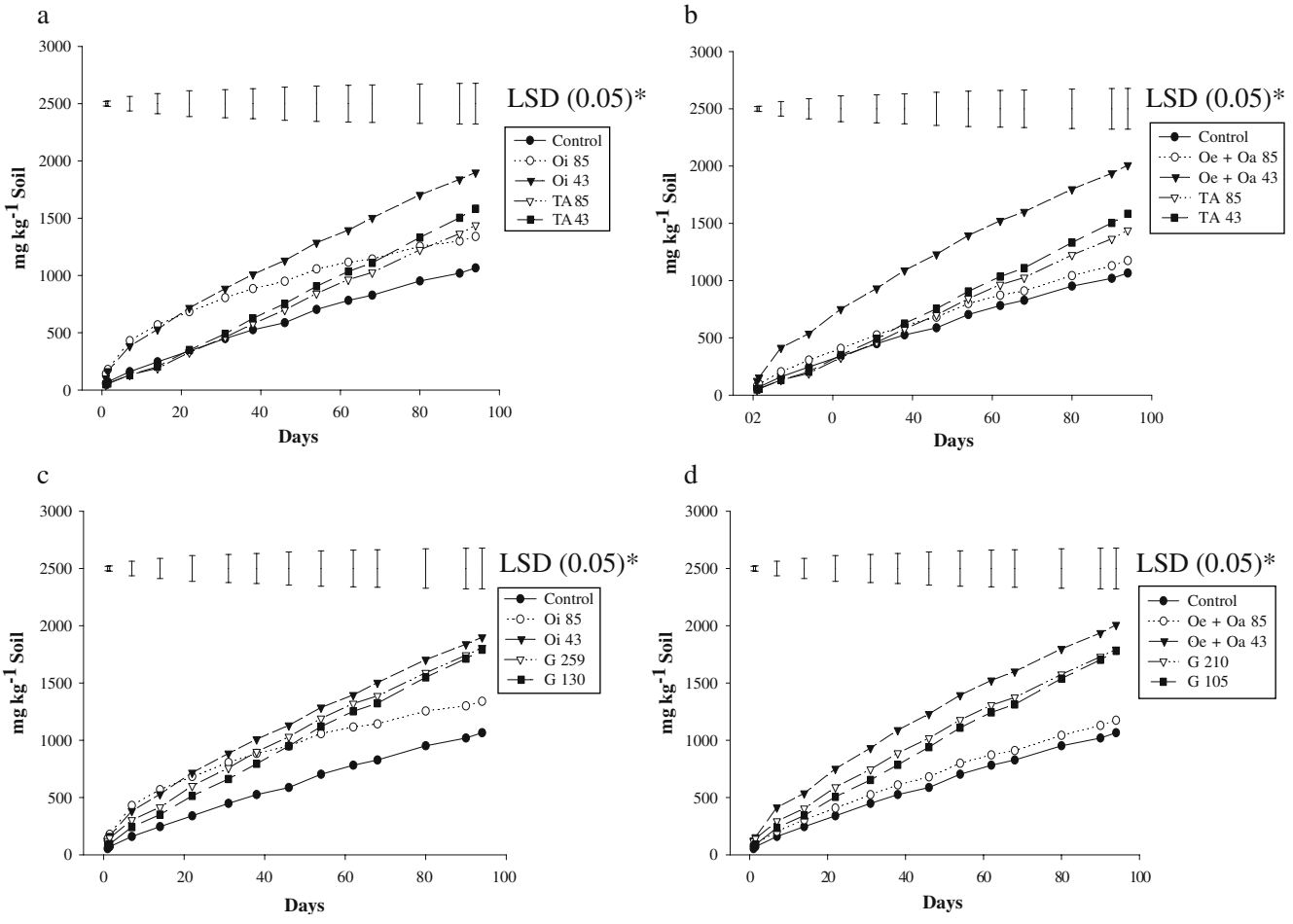

Fig. 3 Cumulative CO_2-C evolution from soils amended with **a** Oi layer extracts and their respective TA standards, **b** Oe + Oa layer extracts and their respective TA standards, **c** Oi layer extracts and their respective glucose standards, and **d** Oe + Oa layer extracts and their respective glucose standards (n=44, i.e., four replications × 11 treatments including control). *LSD *bars* are the mean LSDs for all five *curves*

recently fallen needles, which contain greater concentrations of surface waxes, cutins, suberins, and resins. The signal intensities in *O*-alkyl and acetal regions for Oi and Oe + Oa extracts were similar, indicating that polysaccharides content in these two extracts were similar (Preston 1996). This observation is in contrast with the results of other studies (Wilson 1983; Baldock and Preston 1995) that have reported decrease in *O*-alkyl and acetal C with decomposition (from Oi to Oa stage). This might be due to loss of soluble sugars and polysaccharides from Oi material either due to leaching or microbial degradation. Zech et al. (1992) investigated the relationship between the *O*-alkyl C signal intensity and loss of polysaccharides during decomposition. They noted that decrease in *O*-alkyl C was not proportional to the decrease in total polysaccharides, indicating accumulation of nonpolysaccharide *O*-alkyl C such as oxygenated C atoms of propane side chain and methoxyl groups of lignin monomers. Lack of differences in *O*-alkyl C could also be due to microbial formation of polysaccharides in the Oe + Oa layer during decomposition. Sollins et al. (1996) stated that many bacteria and fungi release diverse polysaccharides into their immediate environment. Huang et al. (1998) also observed an accumulation of polysaccharides in mineral soil samples with increasing decomposition of organic matter, which could be attributed to microbially synthesized polysaccharides.

The combined signal intensity of *N*-alkyl, aromatic and phenolic regions are attributed to lignin monomers and hydrolysable tannins (Lorenz et al. 2000; Ganjegunte et al. 2005b). Both Oi and Oe + Oa layer extracts recorded similar combined signal intensity of *N*-alkyl, aromatic and phenolic regions. Tannins and lignin are resistant to decomposition and they accumulate during initial stages of decomposition (Kraus et al. 2004; Ganjegunte et al. 2004, 2005a). Lack of differences between the combined signal intensities of *N*-alkyl, aromatic and phenolic C between Oi and Oe + Oa layers suggests that lignin and tannins in Oe + Oa layers may have been decomposed or at least significantly altered. Similar observations were made by other studies (Zech et al. 1992; Martinez 2002). As decomposition progresses (from the Oi to Oe + Oa stage), aromatic (lignin) and aliphatic compounds (polysaccharides) in the Oi layer undergo depolymerization and demethyloxylation to yield carboxyl C (Wershaw et al. 1996) representing organic acids like aldonic and aldaric acids. Thus,

the extract of the Oe + Oa layer registered relatively greater signal intensity in the carboxyl region indicating that it is derived from more decomposed substrates. In addition, accumulation of secondary metabolites and amide C may also contribute to signals in the carboxyl region (Hopkins and Chudek 1997).

Impact of the addition of Oi and Oe + Oa extracts on CO_2 evolution

The amount of C added as WSOC in different treatments was a fraction of the total soil C but in all cases, CO_2 evolution rates were greater than the control over 94 days of incubation period (Figs. 2 and 3). Soluble organic matter is an important substrate for microorganisms (Marschner and Bredow 2002) and is quickly depleted during incubation. Laboratory studies (Boissier and Fontvieille 1993; Boyer and Groffman 1996) have shown that microorganisms can decompose different amounts of the water-soluble organic matter fraction. These studies, which ranged in duration from hours to months, indicated that 10–40% of the water-soluble organic C was decomposable under laboratory conditions. The difference between CO_2-C evolved from soils amended with different WSOC and that of control indicates the fraction of added WSOC that was mineralized. The cumulative CO_2-C evolved from Oi 43, Oe + Oa 43, TA 85, TA 43, G 259, G 130, G 210, and G 105 were 229, 260, 811, 2,252, 713, 1,394, 865, and 1,702% of the added WSOC, respectively. This may be attributed to the "positive priming effects" of added WSOC, a phenomenon that deals with triggering a change (positive or negative) in natural mineralization processes through input of an easily decomposable energy source (Kuzyakov et al. 2000). It was suggested that some microorganisms invest low amounts of energy to maintain the cell in a state of "metabolic alertness," thus being able to react more rapidly to the addition of substrates and it was shown that even trace amounts of easily available substrates trigger microorganisms into activity (De Nobili et al. 2001). Another theory suggests that the addition of easily available substrates only promotes the growth of microorganisms, which are characterized by their ability to respond to the addition of substrate by rapid growth, but are not able to utilize the more complex organic compounds typical for SOM (Fontaine et al. 2003). De Nobili et al. (2001) and Chander and Joergensen (2001) suggested that additional CO_2 evolution from soils after the addition of substrate is only an apparent priming effect, assuming that the additional CO_2 originates from the turnover of native microbial biomass instead of SOM mineralization.

Although forest floor extracts added to soil contained similar concentrations of polyphenols (85 and 43 μg g^{-1} soil) as the TA standards (85 and 43 μg g^{-1} soil), the cumulative CO_2-C evolution from soils amended with TA 85 as a proportion of added WSOC was 54 and 22 times of that from Oi 85 and Oe + Oa 85, respectively. Similarly, the cumulative CO_2-C evolution from soils amended with TA 43 as a proportion of added WSOC was nine and eight times of that from Oi 85 and Oe + Oa 85, respectively. The results of this study clearly suggest that at lower concentrations, polyphenols present in WSOC were readily available to microorganisms. Similar results were reported in many previous studies (Bending and Read 1996; Bhat et al. 1998; Fierer et al. 2001; Kraus et al. 2004). While Fierer et al. (2001) reported that shorter-chained condensed tannins were more labile and less inhibitory to microbes than longer-chained condensed tannins, the opposite of this was reported by Kraus et al. (2004). Bhat et al. (1998) indicated that hydrolysable tannins are more labile than condensed tannins. Thus, the varying results observed in different studies suggest that tannins/polyphenols of different chemical structure are processed in soil in different ways.

However, higher concentrations of polyphenols and WSOC in Oi 85 and Oe + Oa 85 resulted in severe inhibition of microbial activity, indicating significant "negative priming effects." Possible mechanisms of negative priming are toxicity of the substrate to microorganisms and inhibition of enzyme activities or structural change of organic matter by binding (Gianfreda et al. 1993; Fierer et al. 2001). A preferential utilization of the easily available substrate compared to SOM is a further explanation (Kuzyakov et al. 2000). The cumulative CO_2 evolution was significantly lower for extracts containing relatively greater concentrations of polyphenols (Oi 85 and Oe + Oa 85) than extracts with lower concentrations of polyphenols (Oi 43 and Oe + Oa 43), indicating that the effects of greater concentrations of polyphenols become more important with time as readily available sources of C become depleted (Heng and Goh 1984).

Lack of significant differences in cumulative CO_2-C values between Oi 85 and G 259 up to 90 days suggests that freshly fallen needles may have contained adequate supplies of readily available C to sustain soil respiration for this length of time (Kalbitz et al. 2003). Similarly, Oi 43, which had a lower polyphenols concentration, registered significantly greater CO_2 evolution than its glucose standard G 130 up to 2 weeks. Once the readily available C sources were utilized, differences became nonsignificant. The same argument can be extended to C mineralization rate differences between Oe + Oa 43 and its glucose standard G 105. Significantly lower CO_2 evolution from Oe + Oa 85 than its glucose standard G 210 after 38 days of incubation could be due to the combined effects of greater polyphenols concentration and presence of recalcitrant compounds such as lignin in the Oe + Oa extract. However, the cumulative CO_2-C evolved from soils amended with G 259 and G 130 were 7 and 14 times added WSOC compared to 0.4 and 2.3 times of added WSOC in soils amended with Oi 85 and Oi 43 extracts. Similarly, CO_2-C evolved from soils amended with G 210 and G 105 were 9 and 17 times of added WSOC compared to 0.15 and 2.60 times of added WSOC from soils amended with Oe + Oa 85 and Oe + Oa 43 extracts, respectively. This again confirms the positive priming effects of the addition of glucose solutions.

Conclusions

The study demonstrated positive priming effects of forest floor layer extracts on soil CO_2 efflux. While recalcitrant compounds such as suberins, cutins, and wax controlled CO_2 release in Oi layer extract, carboxyl C representing organic acids like aldonic and aldaric acids may have influenced C release from Oe + Oa layer extract. WSOC compounds were readily decomposed by soil microbes and significant positive priming effects were observed in soils amended with WSOC except Oi 85 and Oe + Oa 85 extracts. In case of Oi 85 and Oe + Oa 85 extracts, which contained greater polyphenol concentrations, the CO_2 efflux was significantly lower indicating severe inhibition of microbially mediated degradation of added WSOC. In forest floor extracts, the presence of readily available source C compounds might have been responsible for greater C release compared to that from their respective TA standards. Further research is needed to examine the influence of different rates, specific types of tannins/polyphenols, and microbial communities on C and N cycling in forest soils.

Acknowledgements The New Zealand Vice Chancellors' Committee through its Commonwealth Scholarship program supported this research. Access to Eyrewell Forest was kindly facilitated by Carter Holt Harvey Forests. We thank Prof. George Vance for helpful comments to improve the manuscript.

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