Biol Fertil Soils (2002) 35:18–26
DOI 10.1007/s00374-001-0434-4

ORIGINAL PAPER

Rota Wagai · Phillip Sollins

Biodegradation and regeneration of water-soluble carbon in a forest soil: leaching column study

Received: 9 January 2001 / Published online: 31 January 2002

Abstract We hypothesized that water-soluble C is a major substrate for microbial activity and studied the susceptibility of water-soluble C both to leaching and to microbial degradation. Soil columns, consisting of A-horizon top soil with and without tree seedlings, were leached every 2 weeks for 20 weeks. Water-soluble material was extracted from the soils before and after the 20-week study. Biodegradability of dissolved organic C (DOC) was assessed by solution incubation. DOC in leachates was constant over the 20 weeks and the extractable C pool declined by 31–40% between the start and end of the experiment. The amount and biodegradability of both leachates and extracts were lower in the presence of seedlings. Water extracts contained 8–17 times more DOC than leachates. Percentage biodegradable DOC was 13–16% in leachates and 18–27% in extracts. A soil C destabilization model was proposed based on the measured pools (particulate, water-extractable, and leachable C) and estimates of soil respiration and microbial biomass from the same soil. Leaching loss accounted for 8–14% of the total C destabilized. Due to its low concentration and biodegradability, we concluded that leachable C was not a significant substrate for heterotrophic soil respiration in the studied system. The role of water-extractable C as a major substrate was less clear, as the regeneration rate of the extractable C in soil is still unknown.

Keywords Dissolved organic carbon · Water-extractable carbon · Biodegradation · Ammonium · Nitrate · Leaching · Below-ground carbon dynamics

R. Wagai (✉) · P. Sollins
Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA
e-mail: rota.wagai@umit.maine.edu
Tel.: +1-207-5633146, Fax: +1-207-5633119

Present address:
R. Wagai, Darling Marine Center, University of Maine, Walpole, ME 04573, USA

Introduction

A mechanistic understanding of soil C degradation, which accounts for roughly half of the CO_2 evolution from vegetated soil surfaces, is critical to predict the response of the soil C pool to the increase in atmospheric CO_2 as well as other natural or human-caused environmental changes (McGill et al. 1986; Sollins et al. 1996; Gregorich et al. 2000). Availability of soil C to heterotrophic microbes influences the degradation of soil organic matter (SOM) and, consequently, nutrient availability for plants and microbes. Thus, identification and operational separation of labile C from bulk SOM is of great interest. Despite the vast amount of organic C present in soil, microbial activity is often energy-limited, presumably because most of the soil C is chemically recalcitrant and/or physically inaccessible to microbes. What C substrates in soil do heterotrophic microbes utilize? We hypothesized that microbes gain a significant proportion of their energy from the water-soluble C pool because of its small molecular size and easy accessibility to microbes.

Two lines of findings in the literature are consistent with this hypothesis. First, solution incubation studies (see below) have shown rapid biodegradation of dissolved organic C (DOC) in soil. Despite limitations, the incubation technique used in these studies has provided a way of gauging the availability of DOC to the native microbial community. The amount and biodegradability of DOC are known to be affected by the depth in soil profile (Zsolnay and Steindl 1991; Qualls and Haines 1992; Nelson et al. 1994; Boyer and Groffman 1996), season (Qualls and Haines 1992; Nelson et al. 1994; Boyer and Groffman 1996; Yano et al. 2000), soil type (Boissier and Fontvieille 1993), land-use (Boyer and Groffman 1996), N fertilization level (Yano et al. 2000), liming (Andersson and Nilsson 2001), nature of extractant (Nelson et al. 1994), and type of chemical fractions (Jandl and Sollins 1997). The degradation rate of DOC is not constant over time and the labile portion of DOC can be consumed very rapidly. Roughly 15% of the hydro-

philic neutral fraction of water-extract was degraded in 3 days using the same soil as in the current study (Jandl and Sollins 1997). Roughly 22% (deciduous forest) and 29% (cornfield) of the C extracted from top soil was degraded within 2 weeks (Boyer and Groffman 1996). For C leached from the A-horizon of a mixed forest, up to 28% was lost in 48 h and 50% after 4 days (Boissier and Fontvieille 1993). Dahm (1981) reported that roughly 85% of the freshly leached C from alder foliage was degraded in 48 h. Yano et al. (2000) reported 10–45% degradation (in 5 h) of leached C collected beneath an O-horizon using a flow-through bioreactor with a column colonized by native microbes.

Second, laboratory soil incubation studies have shown a strong positive correlation between CO_2 respired and the amount of extractable C among a wide range of soils from temperate regions (Burford and Bremner 1975; Powlson and Jenkinson 1976; Seto and Yanagiya 1983; Davidson et al. 1987; Zak et al. 1990; Cook and Allan 1992a, b). Furthermore Brooks et al. (1999) found a close link in the field between microbial activity and water-soluble C production: over-winter soil respiration and DOC leaching from the O-horizon during snowmelt correlated strongly across sites in the Rocky Mountains, Colorado. To link the two sets of findings, information about whether soils can regenerate labile DOC at a rate sufficient to meet long-term microbial energy requirement is needed.

To answer this question, we constructed columns with A-horizon soil (where soil C is most concentrated), leached them every 2 weeks for 20 weeks, and quantified the amount and biodegradability of the leachate. Dissolved inorganic N species were also measured. Columns were replicated with and without Douglas-fir tree seedlings to assess the influence of a C-fixing plant on the below-ground dynamics of water-soluble C. A conceptual model was developed with two operationally defined water-soluble C pools (leachable and extractable C). Thus, we viewed water-soluble C as a key pool resulting from soil C destabilization, with microbial respiration and leaching as the two major fates of destabilized C in mineral soil.

Materials and methods

Soil sample source

The study site, at the Andrews Experimental Forest in the central Cascade Mountains of Oregon, lies on a gentle north-facing slope at approximately 630 m elevation and 44°13′ N latitude. Precipitation, mostly falling in winter, has averaged 2,200 mm year^{-1} over the past 41 years at the main meteorological station about 6 km SW of the study site. Annual temperature has averaged 9.0°C and 9.5°C at the two nearest reference stands. The soil is a clay loam Inceptisol developed in alluvial and colluvial andesitic parent material. The soil profile was described by Jandl and Sollins (1997). The stand is dense second-growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) averaging 25–30 cm diameter. After old-growth was clear-cut and burned in 1952, Douglas-fir seedlings were planted and some established naturally. Douglas-fir was replanted in the fall of 1956 and in 1957.

Surface mineral soil (0–8 cm in depth) was collected after removal of the litter layer in October 1996 for pilot experiments and in June 1997 for a replicated experiment. The soil was brought back to the laboratory and stored at 5°C for several days. Identifiable litter, woody debris, and roots were removed by hand and the soil sieved (5 mm) and homogenized. A 5 mm sieve was used (instead of 2 mm sieve) to minimize soil handling effects on aggregates, hasten the sieving process, and avoid soil drying (Hart et al. 1994). The sieved soil was stored at 5°C until it was used for the soil water-extraction experiment.

Pilot experiments

In the first pilot experiment, soil was extracted with deionized water (soil:water = 1:3 by weight) on an orbital shaker (approx. 40 rpm) for 30 min. Immediately after shaking, the extracts were centrifuged at 5°C for 30 min (maximum g = 9,715). The centrifuged samples were filter-sterilized through a 0.2 μm polycarbonate membrane (Poretics). The membrane was soaked in 0.5 N HCl, rinsed, and the filtration unit rinsed with deionized water, prior to sample filtration. A vacuum suction of <69 kPa was applied for the filtration to avoid lysing microbial cells. The membrane size of 0.2 μm was used instead of more commonly used 0.45 μm to exclude microbes and submicron organomineral particles (Jandl and Sollins 1997).

The filtered solutions were incubated for 3 months at room temperature (24–27°C) to determine the biodegradability of DOC. Filtrate was separated into three 200-ml aliquots and NH_4Cl was added to achieve final N concentrations of 0, 1, and 5 mg l^{-1} to check for N limitation on DOC degradation. Flasks were covered with aluminum foil, placed on an orbital shaker (roughly 40 rpm), and kept in the dark to prevent CO_2 fixation by algae but allow oxygen diffusion. Dissolved oxygen content was measured after a 3-month incubation with a dissolved oxygen meter with a two-electrode probe (Orion 840). Oxygen concentrations ranged from 83% to 86% saturation (6.6–7.0 mg l^{-1}) suggesting that enough oxygen was available to the heterotrophic microbes for DOC degradation.

Inoculum was prepared by mixing 10 g of the sieved moist soil with 50 ml of deionized water. The mixture was shaken for 30 min and left at room temperature overnight in a gentle shaker. Soil solution was filtered through a 5-μm membrane that kept bacteria and fungal spores/fragments in suspension but removed major grazers (protozoa and nematodes; Frey et al. 1985). Filtrate was added to each incubation flask (inoculum:sample = 1:100, volume basis). One 12-ml aliquot for DOC analysis and two 15-ml aliquots for dissolved N analysis were removed before inoculation. Another 12 ml was removed immediately after inoculation, then 6 and 12 h and 2, 4, 7, 21, 42, and 90 days later for DOC analysis. For dissolved N analysis, another two 15-ml aliquots were collected immediately after inoculation, then 7 and 90 days later. All samples were stored frozen in glass scintillation vials until analysis for DOC and dissolved N.

In the second pilot experiment, a single soil leaching column was leached every 2 weeks for 20 weeks (ten leachings) and the biodegradability of leachates was measured. The soil column was constructed with a tissue culture filter unit (12 cm tall, 1 liter, Nalgene). Surface mineral soil (0–8 cm depth) was collected from the same location in Andrews Forest (January 1997) and sieved (5 mm mesh) as described above. After acid-washing the column, a 1 mm mesh nylon sheet was placed at the bottom and acid-washed silica sand (200±5 g, 16 mesh) was placed on the sheet to minimize filter clogging and soil particle loss during the repeated leachings. Then the field-moist soil (670 g) was gently packed into the column above the sand and nylon mesh layer.

We prepared a synthetic throughfall solution containing 0.24 mg Na^+ l^{-1}, 0.040 mg K^+ l^{-1}, 0.050 mg Mg^{2+} l^{-1}, 0.15 mg Ca^{2+} l^{-1}, 0.16 mg SO_4^{2-}-S l^{-1}, and 1.55 mg Cl^- l^{-1}, similar to the field throughfall solution measured at a nearby site (Sollins et al. 1980). The solution pH was adjusted to 5.29 (5.28–5.31) by adding HCl, to match the pH of the throughfall. Organic N and total P

concentrations in the field throughfall were very low (0.047 and 0.006 mg l^{-1}), therefore these were not added to the synthetic throughfall solution. Inorganic N was also omitted so that we could study dissolved N species in leachates and extracts. The column was leached with the throughfall solution (470 ml) every 2 weeks for 20 weeks. The column was kept covered at room temperature in the laboratory; moisture loss between leachings was roughly 2.5% of the total soil weight. Leachates were centrifuged, filtered through 0.2 μm membrane filters, and incubated up to 2–3 months. Aliquots were taken for DOC and dissolved N analysis during the incubation as described for the incubation of extracts above.

Replicated leaching experiment

Soil columns, 33.6 cm long with a bottom chamber for leachate collection, were constructed by attaching a PVC pipe to a tissue culture filter unit (12 cm tall, 1 liter, Nalgene). An acid-washed nylon sheet and silica sand (500±1 g, 16 mesh) were placed at the bottom of each column as described for the pilot leaching experiment. After mixing the soil thoroughly, 2.1–2.3 kg of field-moist soil was placed gently in each soil column, resulting in a soil depth of 25–28 cm. Six columns were planted with tree seedlings. Three seedlings of Douglas-fir (seeds from southwest Oregon, grown for a year in nursery) were planted in each column after gently washing the peat moss off the roots with water. Three soil columns contained soil but no seedlings as controls. Two columns contained only nylon mesh and the sand layer to control for background contamination.

All eleven columns were kept in a greenhouse from July to October, allowing time to recover from the soil disturbance, stabilize soil structure, and establish a root system. Air temperature in the greenhouse fluctuated between 18°C and 34°C. Soil moisture was brought back to 80% of field capacity every 3–14 days, depending on season, with the synthetic throughfall solution. Leaching losses from the columns were minimal during this period (total of 2.2–15.2 ml per column).

Roughly 470 ml of the synthetic throughfall solution was applied to each column every 2 weeks from 10 November 1997 to 14 March 1998. The total volume of throughfall solution applied during the repeated leaching study was approximately 20% of the annual precipitation for the study area. The throughfall solution was applied gently to minimize soil compaction. Leachate was collected from the bottom chamber of each column by applying a gentle vacuum (<14.2 kPa). Soils were in contact with the throughfall solution for 6–25 min (15 min on average). The leachates were centrifuged and filtered as described above. Soil particles were collected after the centrifugation, air-dried, and the weight of leached particles was measured.

Inoculum was prepared in the same way as in the pilot study after each leaching from the soil from one extra soil+tree column (prepared for this purpose). Unlike the pilot study, all samples were filtered (0.2 μm) each time to remove particulate organic C from the incubated solution. All the filtered samples were stored frozen in glass scintillation vials until analysis for DOC and dissolved N.

The soil remaining after column preparation was stored at 5°C until March 1998 and extracted with water (soil:water = 1:5, 350 rpm) for 30 min (initial extraction). The extract was centrifuged and filtered as described above. Immediately after the extraction of refrigerated pre-leaching soil (initial extraction), the extract was separated into three aliquots. The first and second aliquots received the inoculum extracted from the refrigerated soil in the same way as for leachate inoculation. Before inoculation, K_2HPO_4 was added to the second aliquot (final phosphate concentration of 0.25 μmol l^{-1}) to avoid phosphorus limitation of DOC degradation. No P limitation was observed. Inoculum for the third aliquot was prepared by rinsing the 0.2-μm membrane with the filtrate to collect the material caught by the membrane. This was done to check the effectiveness of this inoculum preparation compared with 5.0-μm filtrate (see Incubation of leachates). No significant difference in DOC degradation was found between the two inoculum preparations. At the end of the repeated-leaching experiment, soils were extracted with deionized water (final extraction). Soils from all columns were sieved (2 mm) to remove any fine roots. An extensive root system was present and the tap root from each seedling was 14–28 cm long, often coiling at the bottom of the column. A 2 mm sieve was used instead of 5 mm to minimize addition of fine roots into soil. The field-moist soil from each column was extracted, centrifuged, and filter-sterilized as in the initial extraction.

Soil and solution analyses

DOC was calculated as the difference between total C and inorganic C in solution based on platinum-catalyzed combustion/nondispersive infrared gas analysis (TOC-5000A; Shimadzu). Samples in scintillation vials were thawed and the biodegradable DOC (BDOC) was determined as the decrease in DOC between the preinoculation sample and the sample after a 3-month incubation. Percentage BDOC (%BDOC) was calculated as $BDOC/DOC_{pre\text{-}inoculation} \times 100$.

A few flasks from the incubation showed small increases in DOC over time: theoretically DOC should not increase over the incubation time due to no C input. The higher values may have been caused by contamination during the 3-month incubation or by heterogeneity of the incubated solution: the solution was mixed gently before sampling to reduce damage to the microbial population. Analytical errors are also possible for extracts incubated longer, which had DOC concentrations close to the detection limit of the Shimadzu TOC analyzer.

Ammonium and NO^{3-} samples were thawed and analyzed with a Lachat autoanalyzer (QuikChem 4200; Lachat Instruments). Ammonium and NO^{3-} standards were prepared using both deionized water and the artificial throughfall solution. The solution matrix had no effect on Lachat results, thus deionized water was used to make standards.

Total C and N in each soil column and seedlings were determined with a Carlo-Erba CNS analyzer after dismantling of the leaching columns. Soil was air-dried, manually ground, and sieved (60 mesh) before analysis. All three seedlings from each column were air-dried and ground. The soil used to make the columns in the beginning of study was also analyzed for total C and N. Soil pH from each column was determined with a pH/ISE meter (Orion) after mixing air-dried sieved soil with deionized water (soil:water = 1:10, weight basis) for 60 min.

Statistical analysis

Each column was leached repeatedly and leachates were separately analyzed for DOC and dissolved N. Because the same columns were measured over time, a repeated measures design (univariate and multivariate; SAS Institute 1992) was used to assess the pattern over the nine leachings. Due to the limited replication of soil columns, only three leachings (i.e., 1st, 5th, and 7th leachings) were selected for the repeated measures analysis. The results did not change when other sets (e.g., 2nd, 6th, and 9th leachings) were used. The treatment effect (i.e., addition of tree seedlings to soil column) was examined by *t*-test assuming an unequal variance. The degradation of C during DOC incubation was regressed against incubation time (least-square linear regression; SAS Institute 1992) after log transformation of both DOC and time. There is no biological basis for such a transformation; it simply allowed a comparison of the slope of degradation curves between soil-alone and soil+tree columns.

Unless noted, soil C and N variables were all expressed on a per gram oven-dried soil basis (e.g., μg g^{-1} soil).

Table 1 Dissolved organic carbon (*DOC*), biodegradable DOC (*BDOC*), and the percentage BDOC (*%BDOC*) over the pilot repeated-leaching experiment

[a] Number of days between leachings
[b] After incubating for 70–90 days, leachates were analyzed for DOC without filtration
[c] The first row had regular inoculum and the second row had inoculum made from a fresh litter layer

Leaching number	Leaching interval[a] (days)	Volume (ml)	Incubation period (days)	DOC leached	BDOC[b]	%BDOC (%)
				(μ g g^{-1} soil)		
1[c]	0	443	95	2.36	1.49	63.2
		443	95	–	1.17	49.6
2	14	462	80	6.92	4.84	70.0
3	15	454	66	3.49	0.34	9.7
4	16	450	90	4.57	0.21	4.7
5	16	446	90	5.62	0.44	7.9
6	15	442	92	5.55	0.60	10.8
7	14	465	70	5.12	0.34	6.7
8	14	456	66	4.19	0.27	6.5
9	14	454	42	4.22	0.41	9.7
10	22	454	21	4.22	0.48	11.4

Table 2 Summary of solution incubation experiments (both leachates and water-extracts) from the pilot experiments and replicated leaching study. Mean and (standard error)

Type of sample	Incubation period (days)	n[a]	DOC	BDOC	%BDOC (%)	Remarks
			(μg g^{-1} soil)			
Pilot extracts-N effect[b]	29.2	3	51.84	20.05	38.67	Soil:water=1:3 by wt., n=1 for extraction.
				(1.05)	(2.03)	Incubated samples unfiltered before DOC analysis.
Pilot repeated leachings[c]	21–90	10	4.42	0.96	22.74	Incubated samples unfiltered before DOC analysis.
			(0.42)	(0.41)	(7.55)	
6th leachates-soil only	73.8	2	1.65	0.26	15.93	
			(0.12)	(0.03)	(0.95)	
6th leachates-soil+trees	73.8	2	1.47	0.19	12.96	
			(0.03)	(0.01)	(0.84)	
Initial extract	45.4	3	24.79	6.81	27.36	Solution contained different inoculum and Phosphorus levels.
			(0.58)	(0.80)	(2.62)	
Final extracts- soil only	89.5	3	17.01	4.02	23.13	
			(1.24)	(0.88)	(3.40)	
Final extracts-soil+trees	89.5	5	14.97	2.66	17.69	
			(0.54)	(0.23)	(0.83)	

[a] Sample number for each solution incubation experiment
[b] Water-extract was separated into three aliquots to test any N limitation on DOC degradation
[c] Average value over ten leachings. (see Table 1 for detail)
[d] Only 6th leachate was used for DOC incubation

Results

Pilot experiments

The amount of leached DOC was roughly constant over the ten successive leachings in the pilot study, whereas BDOC as well as %BDOC were greater for the first two leachings than for the remaining eight leachings (Table 1). Water extracts (shaken) contained approximately 12 times more DOC and 20 times more BDOC than did leachates (Table 2).

Leached C and water-extractable C

In the replicated study, the soil continued to lose a relatively constant amount of C over the nine repeated leachings, and water extracts (shaken) contained 10–20 times more C than did leachates (Fig. 1). Similarly the initial extracts had roughly 30 times more labile C than the leachate (Table 2). The sum of leached C in all nine leachings was still less than the amount of extractable C at both extraction times. The initial extracts contained 48–56% more DOC and 81–174% more BDOC than the final extracts. The percent of DOC degraded (%BDOC) was 5–16% higher in extracts than in leachates (Table 2).

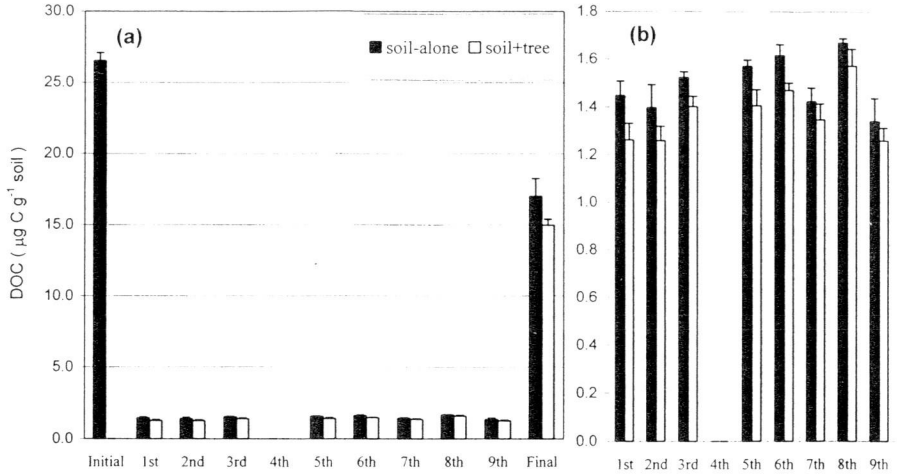

Fig. 1 Changes in dissolved organic C (*DOC*) among the extractions (initial and final extraction times) and repeated leachings (**a**), and among the nine repeated leachings at expanded scale (**b**).
Error bars show standard errors (n=3 for soil-alone column, n=5 for soil+tree column). Two-week intervals between leachings. All the soil columns were leached at fourth leaching but leachates were not analyzed

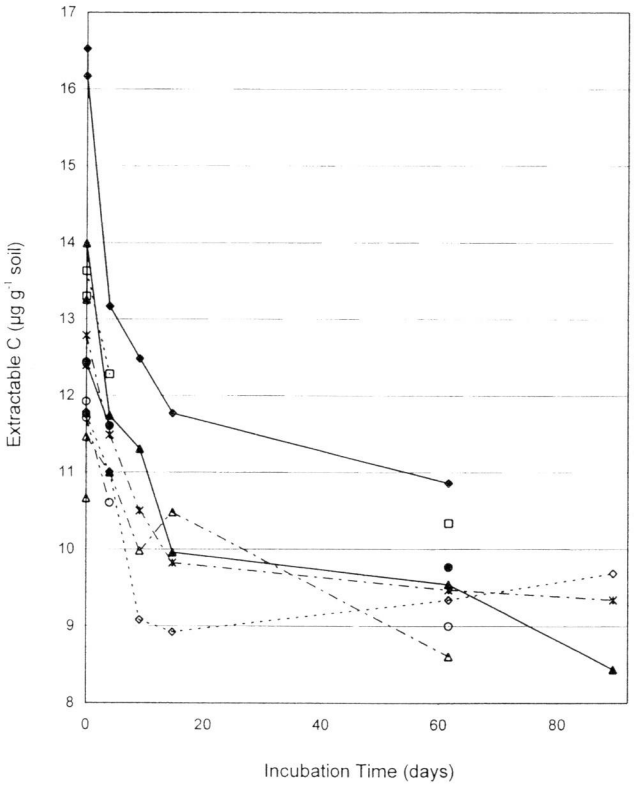

Fig. 2 Degradation of dissolved organic C (*DOC*) from each soil column after the final extraction. *Solid lines with filled symbols* are extracts from the soil-alone columns and *broken lines with open symbols* are from the soil+tree columns. Each *symbol* represents an individual flask

Table 3 Degradation of water-extractable C at the final extraction and the regression analysis of individual degradation curves after log transformation of both DOC and incubation time. Mean and standard error of each column type(n=3 for soil-alone, n=5 for soil+tree column) are shown

	Soil-alone columns		Soil+tree columns	
	Mean	SE	Mean	SE
Total DOC[a]	14.07	(1.25)	12.01	(0.43)
BDOC[b]	4.38	(0.89)	2.62	(0.22)
%BDOC[c]	30.69	(4.65)	21.68	(1.38)
Regression model fit[d]				
r^{2e}	0.85	P value range 0.0002–0.11	0.74	P value range 0.003–0.17
Intercept	2.47	(0.05)	2.37	(0.03)
Slope	–0.030	(0.006)	–0.021	(0.002)

[a] DOC concentration in the extracts prior to inoculation for DOC degradation (μg C g^{-1} soil)
[b] The loss of C during the DOC incubation experiments (μg C g^{-1} soil)
[c] BDOC relative to DOC (%)
[d] Both DOC concentration and incubation time were log-transformed
[e] P value of individual regression fit was 0.0002, 0.0007, and 0.106 for soil-alone columns, and 0.003, 0.093, 0.056, 0.015, and 0.174 for soil+tree columns

The soil-alone columns released more C than soil+tree columns consistently for all leachings (Fig. 1). However, potentially significant differences between the two column types were detected only for the 1st, 3rd, 5th, and 6th leaching (P<0.1). Soil water extracts at the end of the leaching experiment also showed significantly less DOC for the soil+tree columns than for soil-alone columns (P=0.005). Biodegradable DOC in the sixth set of leachates and final extracts was also less in the soil+tree columns than in soil-alone columns but the difference was not significant. Although biodegradability was measured only at the sixth leaching, earlier leachates were expected to contain an equal or greater amount of BDOC than sixth leachates considering the pattern found in the pilot study (Table 1).

Dissolved organic C at both initial and final extraction time showed rapid early degradation followed by a slower stage (Fig. 2). The percentage of DOC biodegradable (%BDOC) appeared to be higher and the slope of the DOC degradation curve appeared more negative (i.e., faster degradation) for the soil-alone columns than for the soil+tree columns (Table 3), but no significant difference was detected. Soil pH did not differ between the two column types at the final extraction time.

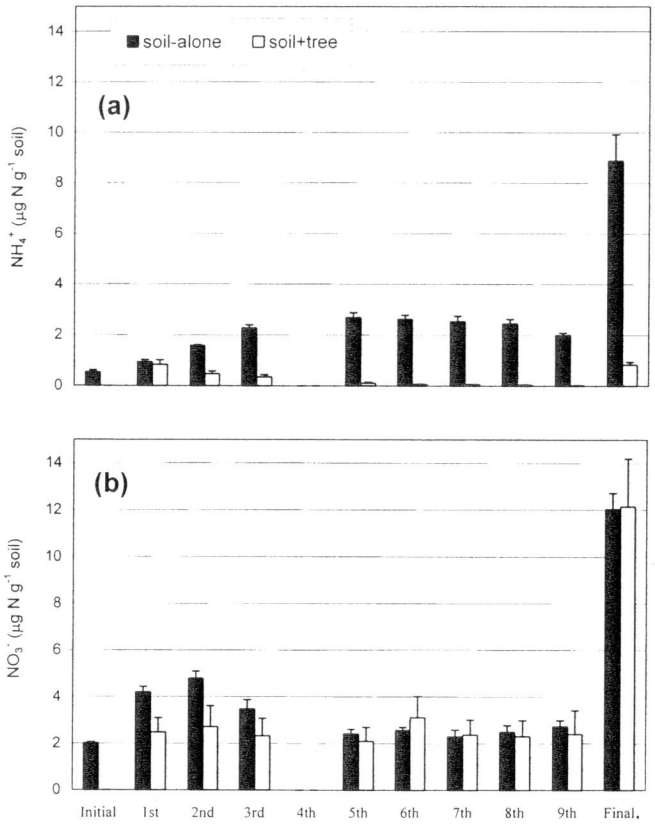

Fig. 3 Changes in NH_4^+ (**a**) and NO_3^- (**b**) between the initial and final extraction time as well as the nine repeated leachings with 2-week intervals. *Error bars* show standard errors (n=3 for soil-alone column, n=5 for soil+tree column)

NH_4^+ and NO_3^-

Ammonium in leachates significantly increased in the soil-alone columns and decreased in the soil+tree columns over the nine successive leachings (P=0.040 and 0.013, respectively, Fig. 3). The treatment effect (of seedlings in soil columns) was significant for all leachings except the first. The final extraction also showed significantly more extractable NH_4^+ in the soil-alone columns than in the soil+tree columns.

Leached NO_3^- decreased significantly across the nine leachings for the soil-alone columns (P=0.009), whereas leached NO_3^- in the soil+tree columns was relatively constant (Fig. 3). The treatment effect was present only at the first and second leachings (P=0.05 and 0.08). In contrast to NH_4^+, the final extraction showed no difference in NO_3^- between the soil-alone and the soil+tree columns.

Discussion

DOC production over repeated leachings

The soil studied here continued to release 4.2 mg C l^{-1} leaching^{-1} of DOC for 18 weeks without inputs of C from plants and O-horizon (Fig. 1). Similarly, Vance and David (1991) reported that 3.6 mg C l^{-1} leaching^{-1} of DOC were continuously leached from Spodosol soil columns (O + B horizon) during weekly repeated leachings over a year.

There are at least two explanations for the constant production of leachable DOC. The first assumes that soil holds a large leachable C pool and a small amount of DOC is leached every time, thus the pool does not diminish during the successive leachings. This view implies a hydrological control on DOC leaching (i.e., the amount of water or the length of leaching time, when water is in contact with soil, determines the amount of C leached). A second explanation is that a constant amount of leachable C is regenerated between leachings by microbial degradation of non-leachable C, even though each leaching removes all or at least a major portion of the leachable C from the soil. A more realistic view may be a combination of the above two explanations: the production of leachable C exceeds the leaching loss, resulting in a constant release of C.

At the ninth leaching, a 470-ml throughfall solution was separated into three portions, leached sequentially, and three leachate samples were collected from one soil-alone column and two soil+tree columns. No consistent pattern of C concentration was found among the three successive leachates, thus the first explanation was not supported. Soils had been incubated for 4 months before the first leaching instead of a 2-week incubation period between leachings. Thus the soils had more time to generate leachable C but released no more DOC at the first leaching than at later leachings. This may support the first explanation: hydrological control of leachable C production. However, this does not rule out the second explanation because it is possible that microbial consumption of regenerated leachable C had kept the pool small.

Christ and David (1996) studied the mechanisms of leaching from Spodosol forest-floor materials by changing the time interval of leachings. Hourly successive leachings of their red spruce litter showed a continuous decline in leached C followed by a steady level, supporting the second explanation: a small but regenerating leachable C pool. When the litter samples were incubated at 10°C for different period up to 14 days after initial intensive leaching, the amount of leached C actually decreased with increasing incubation time. This decline in leachable DOC may be explained by microbial consumption during the incubation period (i.e., the longer the leaching interval, the more leachable C consumed, and thus the less C leached). Between 8% and 34% of the DOC leached from red pine forest floor was degraded in 5 h (Yano et al. 2000). Microbial respiration of the litter materials from the same site used by Christ and David (1996) was 70 μg C g^{-1} soil day^{-1} at 10°C (Gödde et al. 1996). Assuming that the initial fast rate of leachable C production continued for 14 days (i.e., 3,220 μg C g^{-1} soil), the microbial population depended solely on leachable C for their substrate, and the respiration rate was constant, then the microflora degraded 30% of the potentially leachable C during the 14 days. Thus, the de-

cline in the leachable C over time was not likely to be explained solely by the microbial consumption of regenerated C. Christ and David (1996) also chemically fractionated the DOC and found that the ratio of hydrophilic to hydrophobic acids increased over the 8-week incubation period. An increase in this ratio suggests a relative increase in microbial metabolites over plant-derived DOC over time (Guggenberger et al. 1994).

Biodegradability of DOC

Percentage BDOC was only 5–11% for the 3rd to 10th leachates in the pilot study (Table 1), although it was considerably higher in the first two leachings. Only 13–23% of the 6th leachate in the replicated study was degraded (Table 2). These results suggest that the leachable C pool was not highly labile. In contrast, 18–39 % of the extractable C was degradable. Jandl and Sollins (1997) found on average 15% BDOC in water-extracts of a soil from the same location (near the same soil pit). The authors incubated the DOC solution after fractionating into hydrophobic acid (HoA), hydrophilic acid (HiA), and hydrophilic neutral (HiN) fractions. Biodegradable DOC was mostly in the HiN fraction, most of which degraded within 3 days.

Because the DOC solution incubation technique provided an artificial aquatic environment for microbes, it is possible that some labile DOC was not utilized as efficiently as in a field situation. This and similar techniques used by other workers, however, have shown degradability values ranging up to 85% (Dahm 1981; Zsolnay and Steindl 1991; Qualls and Haines 1992; Boissier and Fontvieille 1993; Boyer and Groffman 1996; Jandl and Sollins 1997), demonstrating the usefulness of the technique for measurement of labile DOC.

Leached C versus water-extractable C

Amounts of water-extractable C and BDOC were 10 and 30 times that of leached C. In addition, %BDOC was consistently higher in extracts than in leachates for both pilot and replicated experiments (Table 2 and Fig. 1), suggesting that a significant amount of water-soluble C and BDOC was present within aggregates rather than on the surface of aggregates. Similarly the top 25 cm of Mollisols from Iowa corn and soybean fields produced roughly 25 times more C by water-extraction than by leaching on a dry soil basis (McCarty and Bremner 1993), assuming the field-moist soil had 30% moisture. Greater release of DOC by extraction can be explained not only by disruption of aggregate structure but also by longer dissolution time. The soils in our study were shaken for 30 min during the extraction before centrifugation at 5°C. The leaching times among the eight columns varied between 6 and 25 min but no clear relation was found between leaching time and DOC production. Thus disruption of aggregates rather than leaching is likely the dominant factor causing greater release of DOC and BDOC during extraction.

Patterns of NH_4^+ and NO_3^- over successive leachings

The columns with and without seedlings showed a contrasting pattern of leached NH_4^+ (Fig. 3). That NH_4^+ was detectable in leachates implies that the gross mineralization rate exceeded the sum of gross nitrification, immobilization, plant uptake (in the case of the soil+tree column), and abiotic fixation and volatilization. The soil+tree columns released significantly less NH_4^+ than did the soil-alone columns but not NO_3^-, suggesting preferential uptake of NH_4^+ by plant roots. The increase in leached NH_4^+ over time in the soil-alone columns could be due to an increase in N mineralization and/or a decrease in any of four processes (nitrification, immobilization, abiotic fixation, and volatilization) over the nine leachings. Nitrate in the soil-alone columns increased, suggesting an increase in gross nitrification and/or decrease in immobilization and denitrification. These N transformation processes, however, were not examined in this study.

Extraction treatment caused roughly a 3 to 4-fold increase in inorganic N release compared to the leaching treatment (Fig. 3). It is noteworthy that the extraction caused substantially more dissolution of DOC than that of inorganic N. This contrasting pattern may suggest a difference in the mechanism of leaching loss between C and N in the studied soil.

Treatment effect (soil-alone vs soil+tree columns) on DOC

Extracted and leached C were consistently lower in the soil+tree columns than in the soil-alone columns (Fig. 1). In addition, final extracts from the soil+tree columns had a lower %BDOC than initial extracts or final extracts from the soil-alone columns (Table 2). These results argue against the hypothesis that the soil+tree columns had more leachable and extractable C than the soil-alone columns due to the C inputs from roots of the seedlings. Live plant root systems release low-molecular-weight organic C as exudates and labile particulate C as fine roots turn over, provide habitats for microbes, and penetrate soil aggregates during growth. Two hypotheses can be proposed to explain our results. First, the microbial population in the soil+tree columns was supported largely by C from roots, while microbes in the soil-alone columns utilized potentially soluble C from the soil matrix to meet their energy requirements. Second, root growth and root-derived C stabilized soil C, making it less water-soluble, although we are unaware of any processes that might produce such an effect.

If the first hypothesis is true, our results might imply that the root-derived C was not stabilized in soil and was rapidly utilized by soil microbes. Using a ^{14}C pulse-labeling technique, Norton et al. (1990) found that 31% of the photosynthesized ^{14}C in a Ponderosa pine seedling was

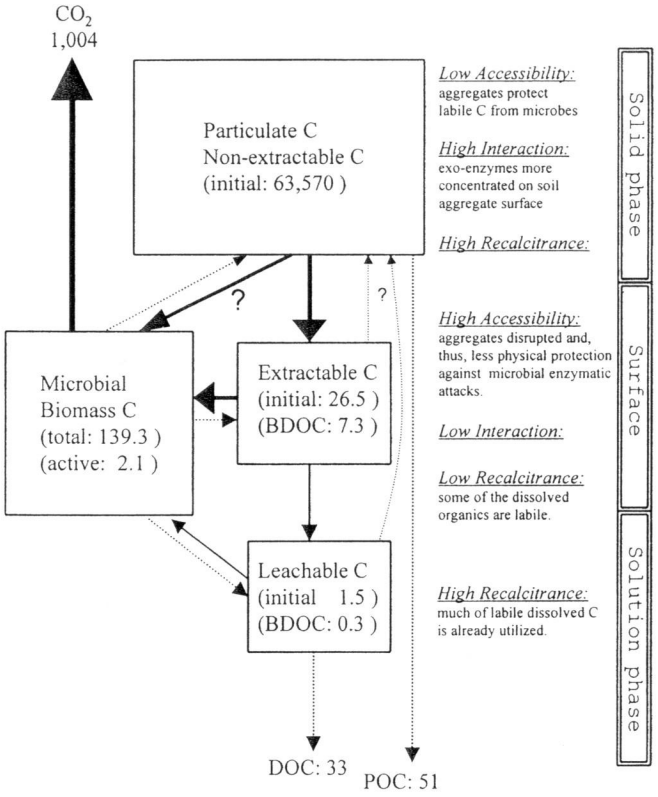

Fig. 4 Soil organic C destabilization model. No C input from roots or litter layer was considered. Values are means from the soil-alone columns (n=3). All the fluxes were expressed in mg C kg^{-1} soil $year^{-1}$ and all the pool sizes were in mg C kg^{-1} soil. The width of fluxes shows relative importance in C mobilization. Microbial biomass and respiration data were from the same study location (Jandl and Sollins 1997)

found in the bulk soil after a 5-day labeling period and over a half of the labeled C in the soil was respired during a 7-day chase period, suggesting rapid release of photosynthates via roots, and their quick utilization by microbes.

Similarly, NH_4^+ was lower in the soil+tree columns than in soil-alone columns in all leachates (except for the first leaching) as well as at the final extraction time. Both microbial immobilization and plant uptake of NH_4^+ in the soil+tree columns could have caused the lower NH_4^+ in both leachates and extracts.

Significance of water-soluble C with respect to soil C destabilization

Soil C pool sizes and fluxes were estimated from measurements of particulate, water-extractable, and leachable C pools from the soil-alone columns, and from data on respired CO_2 and microbial biomass C (Fig. 4). Inputs from litter layer and plant roots were not considered. All values were extrapolated to an annual basis. We make no claim that these values are accurate estimates of annual fluxes; this budget is rather an attempt to integrate measurable pools and fluxes with mechanisms of soil C destabilization and to assess the importance of the two DOC pools with respect to the total amount of C destabilized. We used a field measurement of soil respiration from a litter-removed plot (226 g C m^{-2} $year^{-1}$) in the same stand where soil had been collected to estimate the CO_2 respired from the columns (Jandl and Sollins 1997). Our microbial biomass C value comes from the same study and was based on a microscopic method (Frey et al. 1985; Ingham et al. 1991).

Microbial respiratory loss of C far exceeded leaching loss (Fig. 4), even after accounting for root respiration. Leaching loss of C (including both DOC and POC) accounted for 8–14% of total C loss (i.e., leached C plus respired C) in our study, depending on the estimated respiration rate.

The contribution of DOC leaching to soil C loss was also low in other forest systems. Cronan (1985) leached intact soil columns from three forest types in New England weekly for 1 year in a greenhouse. The contribution of DOC leaching to total C loss was 30–44, 16–28, and 6–14% for the columns made of O-horizon, O+A, and O+A+B-horizon, respectively. Leached C varied by a factor of two among the forest types, even between two sites with the same soil type (Haplorthod). Vance and David (1991) leached reconstructed columns of Haplorthod soil from a northern hardwood forest weekly for 1 year. DOC leaching accounted for roughly 6, 9, and 30% of total C loss for the O-horizon, O+B, and O+doubled B-horizon columns, respectively. It should be noted that neither study measured particulate C loss and both used less conservative definitions of DOC, 0.45 μm (Cronan 1985) and 0.7 μm (Vance and David 1991), than we did. Also, Vance and David (1991) leached their columns for 2–3 days, during which time leachates were not sterilized and much BDOC might have been lost.

It is very unlikely that microbial degradation of leachable C explains a significant portion of the CO_2 respired from the soil we studied because of the small amount and low biodegradability of the leachable C. But to what extent could extractable C (as opposed to leachable C) fuel microbial respiration? Three lines of evidence suggest its role may be significant.

- The amount of labile extractable C was similar to the amount of active microbial biomass C (Fig. 4), and, depending on its turnover rate, it could account for a significant portion of the microbial respiration.
- The amount of DOC degraded fits well with measured forest-floor respiration. Because natural DOC tends to contain compounds of different recalcitrance, DOC degradation is better modeled by assuming multiple DOC pools with different decay rates (Qualls and Haines 1991). Based on the fitted decay curves for the initial extracts (least-square linear regression), roughly 80% and 10% of BDOC were degraded within 6 and 12 days, or about 3.4 and 0.2 mg CO_2–C kg^{-1} soil day^{-1}. If we can assume that BDOC is kept replenished over time, these respiration rates extrapolate to 98.1 g C m^{-2} $year^{-1}$ from the top 10 cm of the

mineral soil. This estimate of extractable C degradation is about half of the measured forest-floor respiration rate (226 g C m^{-2} $year^{-1}$, Jandl and Sollins 1997), which is reasonable if root respiration constitutes 50% of the total respiration. Using the same approach, Jandl and Sollins (1997) estimated 120 g C m^{-2} $year^{-1}$ for the annual degradation rate of extractable C based on the DOC degradation curve.

- The importance of extractable C as a major energy source for microbes can also be considered from another perspective: the microbes' need for labile substrate. Assuming that the active portion of microbial biomass C (2.1 mg C kg^{-1} soil, Fig. 4) is in steady-state and the microbial assimilation efficiency is 0.4, then microbes require 5.3 mg C kg^{-1} soil of labile C to maintain their biomass, a value quite similar to the biodegradable portion of the extractable C (7.3 mg C kg^{-1} soil, Fig. 4).

Further study on the turnover rate of extractable C pool is necessary to determine whether extractable C can support soil microbial biomass and thus explain a significant portion of soil microbial respiration.

Acknowledgements We wish to thank David Myrold, Bruce Caldwell, Yuriko Yano, Robert Griffiths, and Kate Lajtha for their insightful suggestions. We also thank Lisa Ganio for statistical assistance, Stephen M. Griffith (USDA National Forage Seed Research Center) for the use of a dissolved N analyzer, Carol Glassman for total C and N analysis, and Robert Griffiths for use of laboratory facilities. This paper is a contribution from the Andrews Forest Ecosystems Studies Program.

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