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Instream release of dissolved organic matter from coarse and fine particulate organic matter of different origins

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Abstract Dissolved organic matter (DOM), produced through leaching from particulate organic matter (POM), is an essential component of the carbon cycle in streams. The present study investigated the instream DOM release from POM, varying in size and chemical quality. We produced large and medium sized fine particulate organic matter (L-FPOM, 250–500 μm; M-FPOM, 100–250 μm) of defined quality by feeding five types of coarse particulate organic matter (CPOM) to shredding amphipods (*Gammarus* spp.). Microscopic observations showed that L-FPOM and M-FPOM mainly consisted of the fecal pellets of amphipods, and incompletely eaten plant fragments, respectively. DOM release experiments were conducted by exposing CPOM and M- and L-FPOM fractions in natural stream water over a two week period. For CPOM, the release of dissolved organic carbon (DOC) by leaching was highest during the first 6 h (3.64–23.9 mg C g C^{-1} h^{-1}) and decreased rapidly afterwards. For M- and L-FPOM, the DOC release remained low during the entire study period (range: 0.008–0.15 mg C g C^{-1} h^{-1}). Two-way ANOVA revealed that the DOC release rate significantly differed with POM source and size fraction, both at day 1 and after a week of exposure. Multiple regression analyses revealed a significant correlation of elemental contents and lignin content to DOC release rate after a week of exposure. Overall, the results indicated that DOC release rate of FPOM, on a carbon basis, is comparable to that of CPOM after leaching, while size and source of POM significantly affect DOC release rate.

Keywords CPOM · Decomposition · DOM · FPOM · Leaching · Microbial respiration

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Introduction

In rivers and streams, dissolved organic matter (DOM) accounts for a large proportion of the organic carbon pool (Fisher and Likens 1972; Burney 1994).

The DOM supplies energy and carbon to heterotrophic microorganisms, and thus fuels stream metabolism and the instream carbon cycle (Findlay et al. 1986; Weigner et al. 2005). In streams, DOM covers a wide range of molecular sizes including polysaccharides, amino, fulvic, and humic acids (Volk et al. 1997). The DOM composition is primarily controlled by riparian conditions, such as riparian vegetation and land use, as well as by instream processes such as microbial transformation. Thus, the bioavailability of DOM depends on its source, pathway, and diagenetic state (Battin et al. 1999; Findlay and Sinsabaugh 1999; Weigner and Seizinger 2001).

Refractory DOM, due to its low reactivity, mainly contributes to organic carbon transport rather than the local carbon cycle (Thurman 1985). Fulvic and humic acids are generally refractory and are often referred to as the final products of microbially mediated degradation of plant material (Ertel and Hedges 1985; Aitkenhead-Peterson et al. 2003). Hence, refractory DOM can be used as a tracer to elucidate the carbon cycle at the continental and at the global scale (Hedges 1992; Amon and Meon 2004). Chemical characterization has revealed such DOM to be important for the speciation and reactivity of nutrients (Carlsson et al. 1993; Bushaw-Newton and Moran 1999) and metals (Sholkovitz and Copland 1981) in both freshwater and marine systems.

Coarse particulate organic matter (CPOM; >1 mm in diameter), such as leaves and woody debris, serves as a major energy source in forested headwater streams (Fisher and Likens 1973; Meyer et al. 1998; Strauss and Lamberti 2002). The leachates from allochthonous CPOM are mainly labile (Dahm 1981; Strauss and Lamberti 2002) and consist of polysaccharides, amino acids, and fatty acids (Volk et al. 1997; Fischer et al. 2002; Benner 2003). For example, McDowell and Fisher (1976) found that in a forested second-order stream, leaf leachates contributed 42% to the total DOM pool in autumn.

Labile DOM, produced through leaching, is essential for the local carbon cycle (Kirchman 2003; Weigner et al. 2005), and it is important to understand the various transformation pathways of POM to DOM. Especially, fresh leaves are known to produce labile DOM (Baldwin 1999; Strauss and Lamberti 2002). However, limited information is available on the instream release of DOM from POM, and no information is available on the biochemical transformation of fine particulate organic matter (FPOM; <1 mm in diameter). The FPOM is predominant in streams and has a large relative surface area compared to CPOM although it is primarily considered as refractory (Jackson et al. 1995; Alvarez and Guerrero 2000).

In the present study, we investigated the transformation to DOM of 16 types of POM, each of which was produced from a defined single source. They are classified by size into three groups: CPOM (>1 mm, 6 types), L-FPOM (100–250 μm, 5 types), and M-FPOM (250–500 μm, 5 types). Among each size group, POM differed in chemical property, which was determined by elemental composition and fiber contents. We conducted DOM release experiments to test two hypotheses: (1) DOM release rate from CPOM and FPOM reflects their source and generation processes, and (2) FPOM, having a relatively large surface area, plays a significant role in organic matter dynamics in streams, despite being refractory. DOM release rate was determined by POM incubation in flasks for 14 days, and we discuss the results in relation to POM nutrient composition and microbial activity measured by oxygen consumption.

Materials and methods

CPOM preparation

Five CPOM types that differed in texture and chemical quality were collected in the riparian forest along a mid-order section of the Tagliamento River (island-braided reach at river-km 85), a morphologically intact 7th-order river in northeastern Italy (Tockner et al. 2003). Leaves of ash (*Fraxinus excelsior* L.), alder (*Alnus incana* L.), and oak (*Quercus robur* L.) were collected during senescence in October 2002, and twigs of black poplar (*Populus nigra*) (approximately 1 cm in diameter), and filamentous green algae were sampled along the margin of the main river channel. The sixth type of CPOM consisted of macroinvertebrate bodies (*Gammarus* spp.), which were collected as described below and air-dried for 10 min on paper tissue.

The leaves and twigs were air-dried and stored in the dark. The algal samples were transported in cooling boxes to the laboratory, lyophilized and stored at −20°C. A few days before the start of DOM-release experiments, leaves were cut into 14 mm diameter

disks, and twigs were cut into 1 cm long pieces. The ratio of oven- (60°C, 2 days) to air-dry mass of each CPOM was determined to estimate the oven-dry mass of CPOM used for the experiments.

FPOM preparation

Fine particulate organic matter was produced by biological breakdown of the collected CPOM (except *Gammarus* bodies). We produced two size fractions of FPOM (100–250 and 250–500 µm) by feeding amphipods with five types of CPOM that differed in chemical quality, following the protocol of Yoshimura et al. (2008). In total 29 aquaria (25 × 40 × 25 cm) were set up in a greenhouse at 15°C. A polyester screen (500-µm mesh) installed 5.5 cm above the bottom partitioned each aquarium into a bottom and top chamber. Each aquarium was filled with 10 l of sieved (100-µm mesh) river water and constantly aerated by aquarium pumps.

Gammarus pulex [L.] and *Gammarus fossarum* Koch were collected from a local stream (Chriesbach at Dübendorf, Switzerland) from November 2002 to January 2003. Both species are widespread and abundant in European headwater streams, and efficiently shred leaves (Kelly et al. 2002, Dangles et al. 2004). Approximately 200 individuals of *Gammarus* were transferred to the top chambers of each aquarium within 1 h after collection.

Twigs and three species of uncut leaves were conditioned for 10 days in bags (mesh size: 1 mm) in the Chriesbach stream (10°C) before being offered to the *Gammarus*, filamentous algae were not conditioned. Twigs offered here were small woody debris collected on the stream shore in the mid-order section of the Tagliamento River. CPOM was added to each aquarium (a single CPOM type for every aquarium), and the *Gammarus* were allowed to feed ad libitum. After the first 2 days of feeding, the aquaria were carefully washed to remove all particles, including *Gammarus* feces, which may have originated from the Chriesbach stream.

Twenty-four hours after washing, the FPOM produced by the *Gammarus* was collected by passing the particles settled in the lower aquarium chambers over 250 and 100-µm mesh screens to obtain two size fractions: L-FPOM (250–500 µm) and M-FPOM (100–250 µm). Thus, we obtained ten types of FPOM (two size fractions from each of five CPOM types). Microscopy revealed that *Gammarus* transformed CPOM into two main FPOM fractions: (i) L-FPOM: 250–500 µm, comprising cylindrical, coarse, cohesive feces (Fig. 1a, b), and (ii) M-FPOM: 100–250 µm,

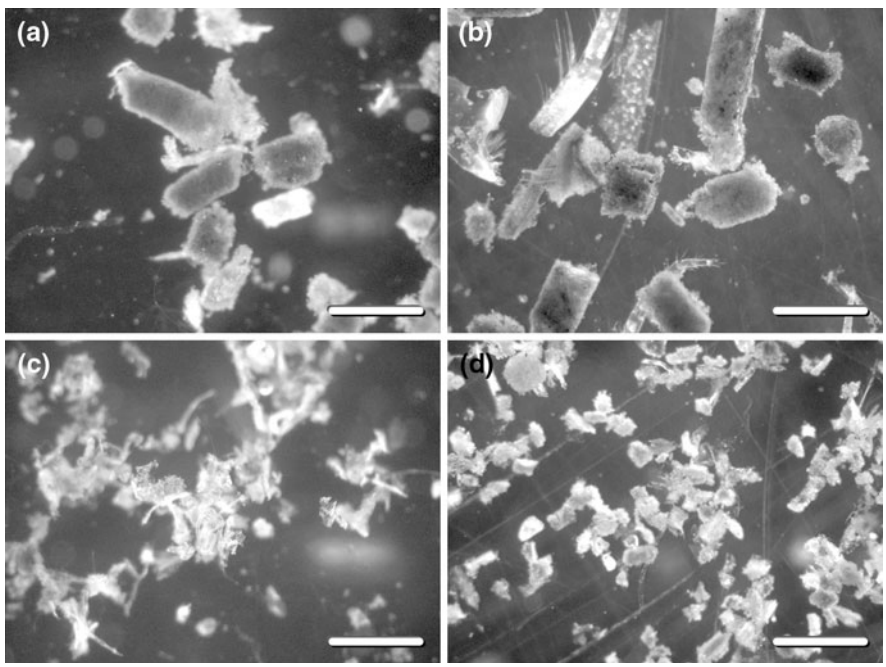

Fig. 1 Photomicrographs of (**a**, **b**) L-FPOM, which were cylindrical, course, cohesive feces, and (**c**, **d**) M-FPOM, which were fragments left over from feeding; both size fractions of FPOM were produced by *Gammarus* spp. POM source: ash (**a**, **c**) and oak (**b**, **d**) leaves. Scale: 500 µm

finer fragments, apparently produced during feeding, and not digested (Fig. 1c, d). Within 3 h of FPOM collection, the DOM release experiments started.

DOM release experiment

Each of the 16 POM types of differing size and source (6, 5, and 5 types of CPOM, L-FPOM, and M-FPOM, respectively), were separately added to 100 ml filtered stream water (GF/F filter, pore size 0.7 μm) in Erlenmeyer flasks and stirred at 100 rpm at 12°C for 14 days. The amount of CPOM and FPOM added was 90.8–142.8 and 6.5–88.0 mg, respectively as oven-dry mass. The stream water was collected from an unpolluted 4th-order stream (Orüti) southeast of Zurich, Switzerland. The mean pH was 8.5, dissolved organic carbon (DOC) was 1.7 mg l^{-1}, total dissolved inorganic nitrogen was 0.65 mg l^{-1}, and soluble reactive phosphorus (SRP) was below the detection limit (<5 μg l^{-1}). Flasks were covered with perforated aluminum foil to allow air exchange. The experiment was run in triplicate.

On days 1, 3, 7, and 14, the entire contents of each flask were filtered through a polyester sieve (mesh size: 11 μm), and subsequently through a GF/F filter; for the CPOM types, samples were also taken after 6 h to determine initial DOM release (leaching). The POM fractions retained by the 11-μm mesh were returned to the flasks, 100 ml filtered stream water was added, and the experiment continued. The mass loss of the 0.7–11 μm fraction was 0.3–7.6% of initial mass for 14 days of the experiment. Note that DOC release rate was expressed as an average between two sampling points, for example the release rate determined by the measurement on day 14 showed an average rate in the second week.

The DOC concentration of the filtered water was analyzed on each sampling occasion (see below). The carbon content of POM was determined at the beginning and at the end of the experiment (days 1 and 14). The DOC release rate was standardized to particulate organic carbon (POC of POM > 11 μm) at each time interval, which was interpolated by fitting a first-order rate model to POC on days 1 and 14.

Characterization of organic matter

The DOC concentration was measured with a total carbon analyzer (TOC-5000, Shimadzu, Kyoto, Japan). The initial and the remaining POM fractions as well as conditioned CPOM were ground, homogenized, and characterized as follows. Their dry mass (60°C, 2 days) and organic carbon, nitrogen, and phosphorus contents were measured. POC content was calculated by subtracting the inorganic from the total carbon content. Total carbon and nitrogen contents were determined with a CHNS-Analyzer (EuroEA 3000, EuroVector S.p.A., Milan, Italy). Inorganic carbon was measured with a CO_2 coulometer (UIC Inc., Joliet, IL) linked to a total inorganic carbon autosampler (CM5240, ORBIS BV, Dronten, The Netherlands). Phosphorus was determined as SRP after digestion of POM with $K_2S_2O_8$ in an autoclave at 121°C (Ebina et al. 1983). Fiber content of the initial POM was measured gravimetrically as acid detergent cellulose and acid detergent lignin (Gessner 2005), and expressed as proximate cellulose and lignin contents, i.e. as the percentage of the carbon in POC, assuming 44% carbon in cellulose and 50% in lignin. The relative analytical errors for C, N, and P were <3, 1, and 0.1%, respectively, and the analytical error for cellulose and lignin was <4%.

The microbial respiration was measured as oxygen consumption for initial and remaining POM. The measured portion of the POM sample was transferred to 300-ml Winkler bottles filled with filtered (0.45-μm-pore size) stream water from the study stream (Orüti). The bottles were then sealed while avoiding air bubble formation, and incubated at 12°C for 3 days in the dark. Dissolved oxygen concentration was measured before and after the incubation with a portable probe (Model 26131, Orbisphere Laboratories, Switzerland). After subtracting the oxygen consumption in the control bottle containing only filtered stream water, the respiration rates were standardized to the POC added to each bottle.

Statistical analyses

Two-way ANOVA analyses, with subsequent Tukey's tests, were used to test for the effects of POM size and source on DOC release rate. Multiple regression analyses were applied to determine the relationship of the chemical quality of POM to DOC release rate and microbial respiration, except for *Gammarus* bodies (forward selection with $F > 2.0$). Dependent variables were log-transformed if necessary to ensure normality. Dependent and independent

variables included organic matter, total nitrogen, total phosphorus, cellulose, and lignin contents, and C:N, N:P, and lignin:N ratios. Statistics were conducted using Statistica (Version 7.1, StatSoft Inc., Tulsa, Oklahoma).

Results

POM transformation

The chemical quality of the POM differed greatly with source and size fraction (Table 1). Plant-derived CPOM of different sources (leaves and twig) showed similar organic carbon content (45.5–53.3%). Twig and filamentous algae exhibited the highest (108.7) and the lowest (11.8) C:N ratio, respectively (excluding *Gammarus* bodies). Twig was also characterized by the highest cellulose content (45.4% C) while the highest proximate lignin content was shown by oak leaf (26.8%), followed by alder leaf (24.3%) and twig (23.9%). Among all CPOM, the *Gammarus* bodies had the highest average nutrient content (N: 8.3%; P: 0.99%) and the lowest C:N ratio (4.8).

After the instream conditioning, all leaves increased in nitrogen content (3.29–4.63%) and decreased in phosphorus content (0.08–0.19%) although their carbon content remained constant at about 50%. The nitrogen content of twig also increased to 0.64%. As a result, the instream conditioning raised the C:N ratios and reduced the N:P ratios of leaves and twig. The fiber contents of all leaves also increased. Their lignin content increased by 20% (oak leaf) to 33% (ash leaf).

After the transformation from conditioned CPOM to FPOM by *Gammarus*, the organic carbon content of all leaves and twig decreased. Among them the lowest content was found for oak leaf-derived L-FPOM (26.2%), followed by twig (28.8%). The nitrogen content of leaf-derived FPOM also declined, whereas twig increased in nitrogen content compared to the conditioned state. As a result, leaf-derived FPOM showed higher C:N and lower N:P ratios than conditioned leaves, except for L-FPOM derived from oak leaf. C:N and N:P ratios of twig decreased. The cellulose and lignin contents of conditioned leaves and twig decreased or remained the same. Compared to fresh CPOM, plant-derived FPOM remained high in nitrogen and lignin contents. Regarding green algae, the C:N ratio stayed in the same range, but the N:P ratio declined from 26.2 to 15.0 (L-FPOM) and 19.9 (M-FPOM).

Overall, C:N ratios and cellulose content varied over a wider range in CPOM (C/N: 11.8–108.7; cellulose 12.9–45.4% C; excluding *Gammarus* bodies) than in L-FPOM (C/N: 9.1–22.1; cellulose: 14.7–38.0%) and M-FPOM (C/N: 12.3–38.1; cellulose: 16.5–37.0%). The range of lignin content was 20.8–36.5% for L-FPOM and 26.2–45.7% for M-FPOM, whereas its content in CPOM was 5.7–26.8% excluding *Gammarus* bodies. Comparing the elemental ratios between the two sizes of FPOM derived from the same source, both C:N and N:P ratios were higher for M-FPOM than L-FPOM. In addition, M-FPOM showed a higher lignin content than the L-FPOM derived from the same source.

If plant and algae-derived POM were pooled, the mean C:N ratios were 41.4, 15.1, and 21.4 for CPOM, L-FPOM, and M-FPOM, respectively, and the mean lignin contents were 18.1, 28.5, and 35.0% C, respectively. Mostly, CPOM exhibited the highest C:N ratio and the lowest lignin content among the three size fractions derived from same sources. L-FPOM showed the lowest C:N ratio and M-FPOM showed the highest lignin content for each source.

DOM release from POM

The release of DOC from CPOM was up to two orders-of-magnitude higher at the beginning of the experiment (>3.64 mg C g C^{-1} h^{-1}; day 1) than at the end (<0.26 mg C g C^{-1} h^{-1}; determined as an average in week 2) (Fig. 2). During the first 6 h, release was particularly high from leaves (alder leaves: 23.9 mg C g C^{-1} h^{-1}) compared to green algae (3.64 mg C g C^{-1} h^{-1}). During week 2, the release rate was highest for *Gammarus* bodies (0.23 mg C g C^{-1} h^{-1}) and lowest for oak leaves (0.01 mg C g C^{-1} h^{-1}).

The release of DOC from FPOM was lower than from CPOM for the first two days. During week 1, L-FPOM from oak leaves and green algae, and M- and L-FPOM from twig had negative DOC release rates (−0.24 to −0.03 mg C g C^{-1} h^{-1}; Fig. 3), indicating DOC uptake. Two-way ANOVA also revealed significant effects of size and source on the DOC release rate, both on day 1 and in week 2 (Figs. 4, 6). The results from ANOVA for day 1 were

Table 1 The chemical properties of fresh CPOM, conditioned CPOM, L-FPOM, and M-FPOM before the DOM release experiments (mean, $n = 3$). The properties of CPOM, L-FPOM, and M-FPOM after DOC release experiments (day 14) are additionally shown after the slash ($n = 3$ for OC, TN and C/N)

Fraction	Source	OC (%)	TN (%)	TP (%)	C/N (molar ratio)	N/P (molar ratio)	Cellulose (% C)	Lignin (% C)	Lignin/N (mass ratio)
Fresh CPOM	Ash leaf	45.4/47.3	1.50/3.31	0.31	35.2/16.7	10.1	15.7	5.7	3.4
	Alder leaf	53.3/51.4	3.21/4.27	0.12	19.4/14.0	55.3	12.9	24.3	8.1
	Oak leaf	49.9/45.3	1.83/1.87	0.19	31.8/28.3	20.2	17.8	26.8	14.6
	Twig	49.6/48.8	0.53/0.93	0.01	108.7/65.2	91.0	45.4	23.9	44.8
	Green algae[†]	15.8/32.9	1.56/1.77	0.13	11.8/21.6	26.2	17.6	10.0	2.0
	Gammarus	34.3/15.1	8.31/2.97	0.99	4.8/5.9	17.7	13.9	2.9	0.2
Conditioned CPOM	Ash leaf	52.5	4.63	0.19	12.8	54.2	19.5	38.9	8.8
	Alder leaf	55.0	4.27	0.08	14.6	112.8	18.4	52.2	13.4
	Oak leaf	50.5	3.29	0.11	17.9	66.0	19.3	46.4	14.3
	Twig	48.6	0.64	0.01	86.1	101.6	42.5	29.2	44.6
L-FPOM	Ash leaf	47.0/48.9	3.86/4.70	0.18	14.2/12.1	44.2	15.9	31.5	7.7
	Alder leaf	43.6/46.5	3.27/3.84	0.17	15.6/14.1	40.4	14.7	36.5	9.7
	Oak leaf	26.2/39.0	2.13/2.60	0.38	14.3/17.5	12.0	17.7	28.6	7.0
	Twig	28.8/35.3	1.59/1.55	0.26	22.1/26.7	12.7	32.2	25.0	9.0
	Green algae[†]	9.7/25.9	1.31/2.93	0.19	9.1/10.3	15.0	38.0	20.8	3.1
M-FPOM	Ash leaf	51.1/47.6	3.15/3.90	0.14	18.9/14.3	47.5	16.5	35.5	11.5
	Alder leaf	51.2/50.0	3.70/3.86	0.07	16.2/15.1	106.8	18.5	45.7	12.6
	Oak leaf	44.7/45.5	2.42/2.69	0.10	21.6/19.7	47.9	17.2	39.0	14.4
	Twig	38.6/41.4	1.18/1.17	0.07	38.1/41.2	35.2	33.1	28.8	18.8
	Green algae[†]	6.1/25.1	0.64/2.79	0.07	12.3/10.5	19.9	37.0	26.2	5.0

Cellulose and lignin contents are expressed as a percentage of their carbon to total organic carbon. [†] May contain inorganic particles

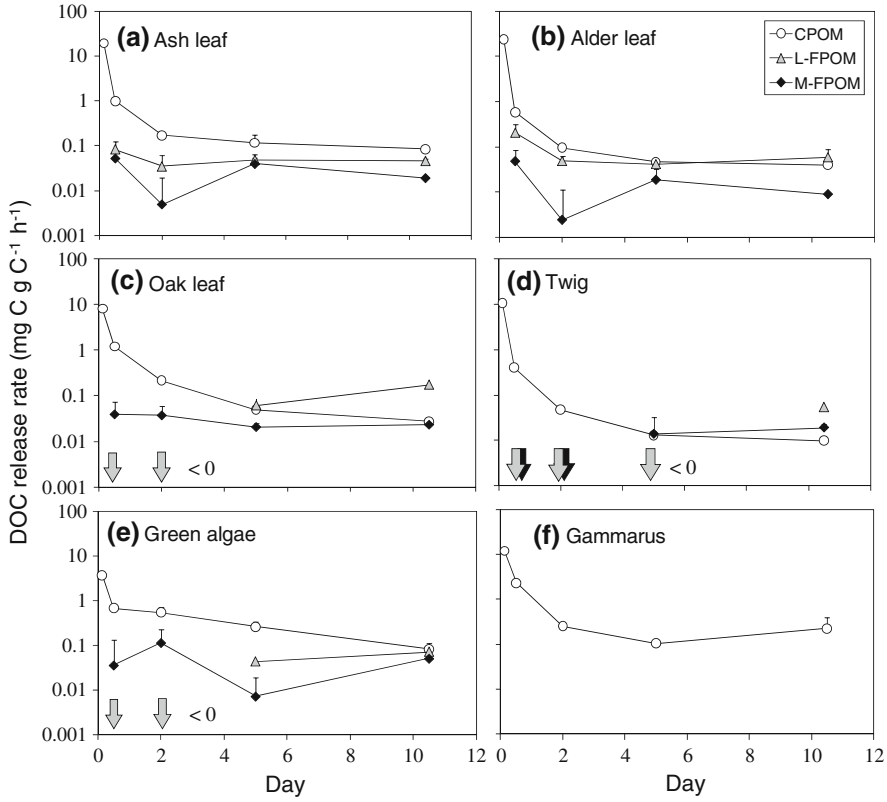

Fig. 2 DOC release rates (mean ± SD, $n = 3$), standardized to POC, from CPOM, L-FPOM and M-FPOM. Error bars (SD) are shown on the upper side of the mean (some bars are not visible since they are too small.) *Arrows* indicate negative values (*gray* for L-FPOM, *black* for M-FPOM), corresponding to DOC uptake (see Fig. 3). Note the logarithmic scale for *y*-axis. Note that DOC release rates determined by the measurement on day 14 were plotted at day 11.5 since the rates express the average in week 2

$F_{45,2} = 2{,}370$ ($p < 0.001$) for size, $F_{45,4} = 155$ ($p < 0.001$) for source, and $F_{45,8} = 126$ ($p < 0.001$) for the interaction (size × source). The DOC release rate from CPOM was significantly higher than from FPOM (Fig. 4a). Regarding POM source, alder leaf and green algae exhibited the highest and lowest release rates, respectively, and the differences here to other sources were significant (Fig. 5a).

During week 2, the DOC release rate from FPOM increased, that is L-FPOM increased from 0.046 to 0.175 mg C g C^{-1} h^{-1}, and M-FPOM from 0.009 to 0.050 mg C g C^{-1} h^{-1} on average, and was comparable to the release rate from CPOM (0.01–0.23 mg C g C^{-1} h^{-1}, Fig. 2). The results from ANOVA for week 2 were $F_{45,2} = 48.1$ ($p < 0.001$) for size, $F_{45,4} = 18.1$ ($p < 0.001$) for source, and $F_{45,8} = 17.5$ ($p < 0.001$) for the interaction. In general, the release rate from L-FPOM was significantly higher than from M-FPOM (except for green algae). In week 2, the highest release rate was from *Gammarus* bodies (0.23 mg C g C^{-1} h^{-1}), and among the POM from vegetation, the L-FPOM from oak leaf showed the highest release rate (0.18 mg C g C^{-1} h^{-1}), while the M-FPOM from alder leaf showed the lowest release rate (0.009 mg C g C^{-1} h^{-1}). Two-way ANOVA also showed that the mean release rate from L-FPOM was significantly higher than from CPOM and M-FPOM (Fig. 4b). In terms of source, oak leaf and green algae showed the highest release rates while alder leaf and twig showed the lowest release rates, where the difference was significant (Fig. 5b). During the 14 days, the smallest fraction, M-FPOM, had a narrower range of release rate, regardless of its source, compared to CPOM and L-FPOM.

On a carbon basis, organic carbon flux from POM to DOM for 14 days ranged from 29 to 89 mg C g C^{-1} for CPOM, −1.1 to 8.8 mg C g C^{-1} for L-FPOM, and 1.5 to 4.2 mg C g C^{-1} for M-FPOM (Fig. 6). As mentioned above, initial leaching of soluble compounds on the first day largely contributed to the high flux of CPOM, especially for plant-derived materials and *Gammarus* bodies. Regarding plant-derived materials, more than 82.5% of the total DOC released from CPOM in 14 days was released on the first day. In contrast, this figure was <24.5% for L-FPOM and M-FPOM. For plant- and algal-

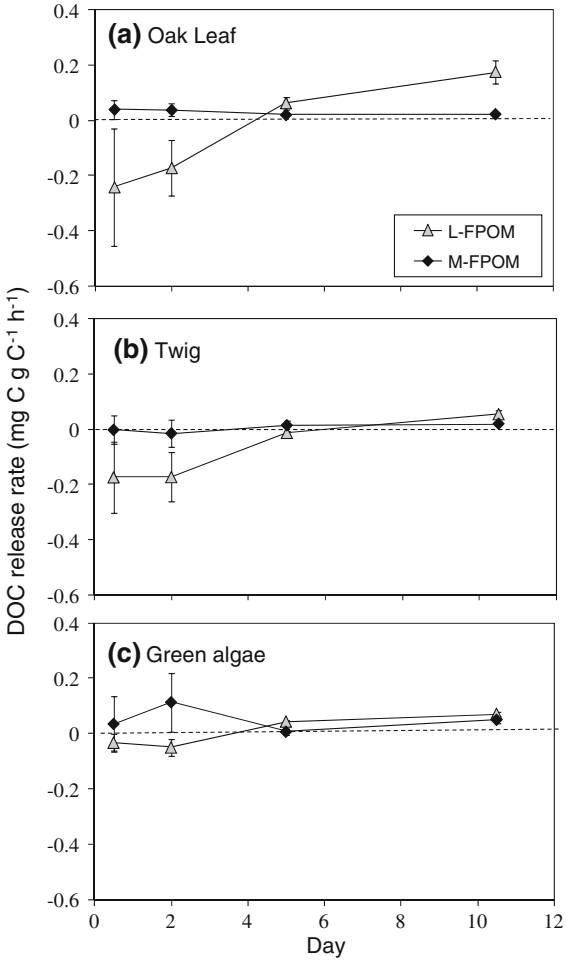

Fig. 3 DOC release rates (mean ± SD, $n = 3$), standardized to POC, from L-FPOM and M-FPOM derived from oak leaves (**a**), black poplar twigs (**b**), and green algae (**c**). Negative values correspond to DOC uptake. Note that the *y*-axis scale is linear and that DOC release rates determined by the measurement on day 14 were plotted at day 11.5 since the rates express the average in week 2

derived POM, DOM release for 14 days led to a significant increase in %N (except for oak leaf as CPOM), and their C:N ratios decreased accordingly (Table 1). FPOM derived from twig and green algae exhibited significantly higher OC content than at the start of the experiment while the C:N ratios were unchanged. For *Gammarus* bodies, the nitrogen content decreased from 8.3 to 3.0%, and the C:N ratio increased slightly from 4.8 to 5.9 during the 14 day experiment.

The microbial respiration rate of CPOM ranged from 0.22 (twig) to 3.9 mg O_2 g C^{-1} h^{-1} (*Gammarus* bodies) at the start of the DOM release experiment, and from 0.12 (twig) to 4.5 mg O_2 g C^{-1} h^{-1} (*Gammarus* bodies) at the end of the experiment (Fig. 7). Among the vegetation sources, green algae showed the highest respiration rates for CPOM (2.53 mg O_2 g C^{-1} h^{-1}) and M-FPOM (2.57 mg O_2 g C^{-1} h^{-1}) at the start of the experiment, while oak leaf showed the highest for L-FPOM (4.06 mg O_2 g C^{-1} h^{-1}). Among the three size fractions for each source, L-FPOM generally showed the highest respiration rate (except for ash-derived POM). At the end of the experiment, this trait was also observed for oak leaves, twig, and green algae. Amphipod bodies and M-FPOM derived from oak leaves and twigs showed a slight increase in microbial respiration rate on average although their temporal differences were not significant.

Multiple regression analyses revealed significant correlations of the chemical composition of POM to DOC release rate and respiration rate (week 2; Table 2). The overall correlation coefficient was 0.96 ($p < 0.001$) for DOC release rate in week 2 and 0.93 ($p < 0.001$) for respiration rate on POM on day 14. Significant variables ($p < 0.01$) for DOC release rate were found to be organic carbon, total nitrogen, and lignin contents, and C:N and N:P ratios. The DOC release rate was negatively correlated with OC content, N:P ratio, and fiber content; and positively correlated with TN and the C:N ratio. The OC content had the highest partial correlation coefficient (−0.65, Table 2). The microbial respiration rate of POM was negatively correlated to lignin and to N ratio and positively correlated to TP and cellulose contents, while its correlation coefficient with OC content was negative but not significant. Furthermore, there was a significant positive correlation between the DOC release rate and the respiration rate ($R = 0.58$, $p < 0.001$; Fig. 8).

Discussion

The release of DOM from CPOM and FPOM is a key transformation process of organic matter in streams. Our results indicate distinct size-effects of POM on DOM release, on both the initial leaching and subsequent microbial decomposition phases. The initially high DOC release rate from CPOM was caused by the leaching of highly soluble components,

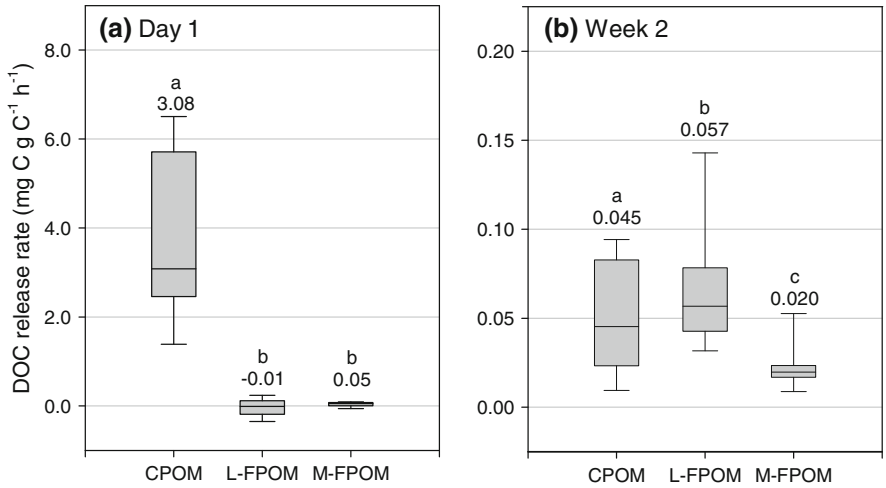

Fig. 4 DOC release rates from CPOM, L-FPOM and M-FPOM at day 1 (**a**) and week 2 (**b**) (all species combined). Boxes show 25th percentile, median, and 75th percentile, and *error bars* indicate 10th and 90th percentiles (a *box* and *whisker plot*). Values above the *boxes* are medians. The different *lower case letters* indicate significant differences between size groups (Tukey's test). Note that DOC release rates in week 2 were determined by the measurement on day 14 as the average in week 2

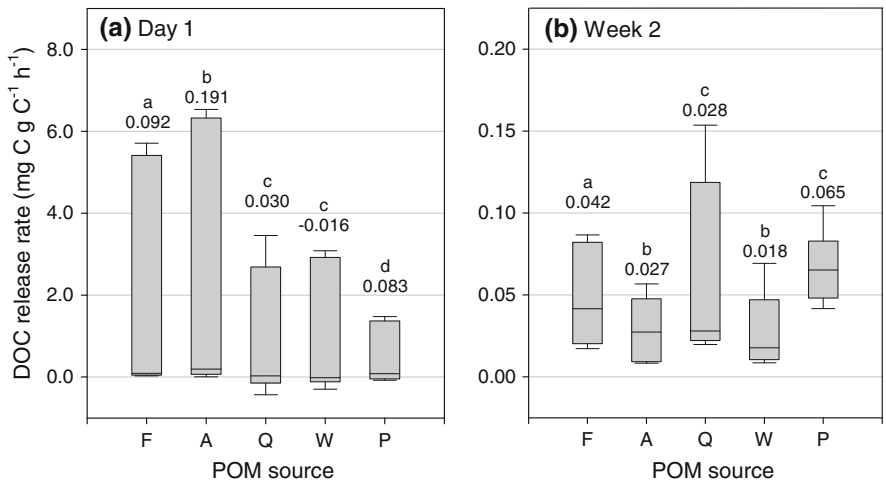

Fig. 5 DOC release rate from five types of POM on day 1 (**a**) and week 2 (**b**) of the leaching experiment (all sizes combined). POM type: leaves of ash (F), alder (A), and oak (Q), black poplar twigs (W), and green algae (P). *Boxes* show 25th percentile, median, and 75th percentile, and *error bars* indicate 10th and 90th percentiles (a *box* and *whisker plot*). Values above the *boxes* are medians. The different *lower case letters* indicate significant differences between source groups (Tukey's test). Note that DOC release rates in week 2 were determined by the measurement on day 14 as the average in week 2

and Gessner et al. (1999) and Wallace et al. (2008) suggested that leaching is the dominant instream process during the first day of CPOM exposure. In contrast, initial DOC release (leaching) from FPOM was almost negligible, even on the day of its formation (day 1), mainly because the sources (CPOM) were already preconditioned for 10 days in a stream before being shredded and digested by the *Gammarus*. In week 2, however, when microbial decomposition dominated, the DOC release rates from CPOM were similar to those from FPOM on a carbon basis as described above. This implies that the DOC release rate is determined by both the labile properties and relative surface area of POM.

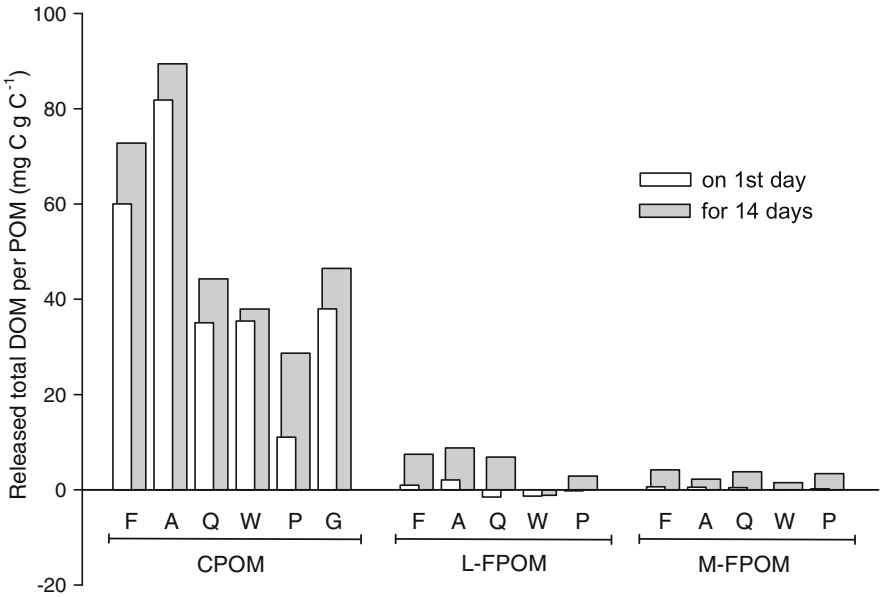

Fig. 6 Released total DOM relative to initial POM on the first day and for 14 days of the experiment (averages of carbon-based values in triplicate). POM type: leaves of ash (F), alder (A), and oak (Q), black poplar twigs (W), and green algae (P)

In our experimental framework, the DOM release experiment from CPOM corresponds to the instream conditioning of CPOM. Although the experiment and the conditioning differed in duration and physical and chemical conditions, nitrogen content and C:N ratio of CPOM increased in common, reflecting microbial colonization. At the same time, fiber contents of leaf litter substantially increased (by a maximum of 33% for ash leaf) while organic carbon content changed by only a few percent and did not show a consistent pattern among all CPOM sources. These findings indicate that the instream conditioning resulted in the release of soluble DOM compounds from CPOM and microbial colonization of CPOM. In other words, soluble organic compounds of POM seem to be replaced with microbial cells, which resulted in the relatively small change in organic carbon content together with the increase in nitrogen content.

As shown by photomicrographs (Fig. 1), L-FPOM mainly consisted of cylindrical particles, which were obviously the fecal pellets from the *Gammarus*. In contrast, M-FPOM appeared to be mechanically produced by incomplete feeding. This difference in these processes was clearly exhibited also by microbial respiration. If the respiration rates of CPOM after the DOM release experiment were considered as "conditioned states", the changes in microbial respiration enable us to infer the digestion effect of *Gammarus* on POM. All pairwise comparisons showed higher respiration of L-FPOM than conditioned CPOM on average for all individual sources (Student *t* test showed that four among five pairs were statistically significant at $\alpha = 0.05$). In contrast, respiration of M-FPOM was not significantly higher than conditioned CPOM in any of the cases (Fig. 7). *Gammarus* caused a slight decrease in nitrogen contents of leaf-derived POM compared to the corresponding conditioned states, probably due to its selective assimilation of nitrogen, which is related to the stoichiometric unbalance between *Gammarus* and its food source. However, the contents were still higher than those of fresh CPOM (Table 1).

These findings may imply that the digestion process of *Gammarus* activates microbes on POM and enhances POM decomposition, which accounts for the lower fiber contents of L-FPOM than those of M-FPOM. This implication might be further related to DOM immobilization on refractory L-FPOM derived from oak leaves, twigs, and green algae, which was observed during the first week of the experiment (Fig. 3). The negative DOC release rate of L-FPOM represents DOC loss from bulk water, indicating DOM adsorption onto POM or microbial assimilation of DOM (see Fischer 2003) rather than POM decomposition. Our data are insufficient to explain how particle-associated microbes interact with DOM. However, at least, the diet of *Gammarus* was likely to enhance microbial activities and thus

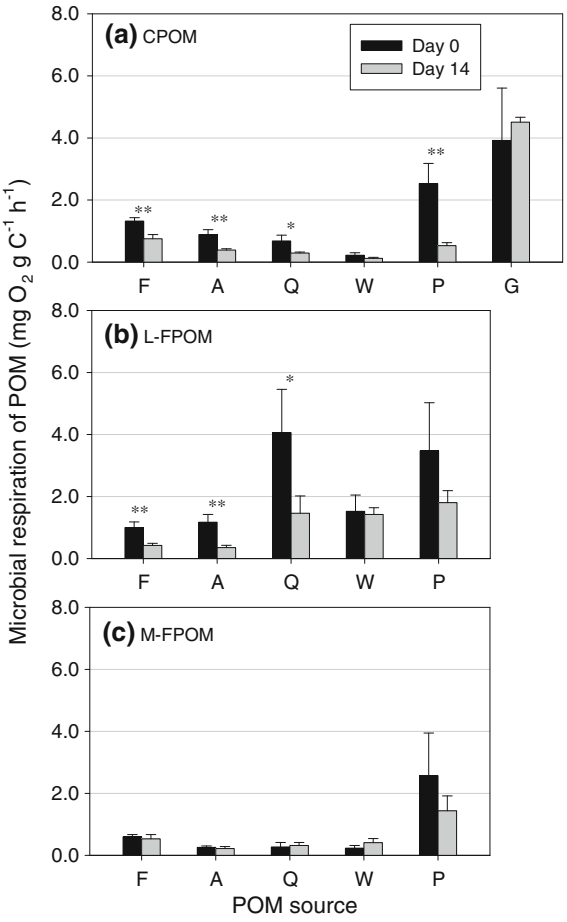

Fig. 7 Microbial respiration (mean ± SD, $n = 3$) on CPOM (**a**), L-FPOM (**b**), and M-FPOM (**c**) on days 0 and 14. Results are standardized to POC. POM type: leaves of ash (F), alder (A), and oak (Q), black poplar twigs (W), green algae (P). *Gammarus* bodies (G). *Asterisks* indicate significant differences between FPOM and CPOM as revealed by *t* tests. ** $p < 0.01$; * $0.01 < p < 0.05$

the activity of degradative enzymes on POM in their intestine, as it has been found for slipper lobster (*Thenus orientalis*, Johnston and Yellowlees 1998).

DOM uptake by L-FPOM continued for the first few days. However, in week 2, L-FPOM released DOC at a higher rate than did M-FPOM and CPOM for alder and oak leaves and black poplar twigs. It is unlikely that physical desorption of organic matter in week 1 contributed to this sharp increase in DOC release rate of L-FPOM because bulk water quality was reset to the initial condition after each sampling. Rather, the increase in DOC release rate might be attributed to the microbial community on POM, which was affected by the environmental change from the amphipod intestine, with low pH and low dissolved oxygen, to oxygenated stream water. The decrease in microbial respiration in week 2 suggested that the surface microbes on L-FPOM lowered its activity, and might adapt to the stream water environment during the two weeks of exposure. In contrast, microbial communities simply developed on the surface of M-FPOM because most of the M-FPOM was produced by incomplete feeding on CPOM. Therefore, the differences of DOC release rates between L- and M-FPOM can be attributed to the processes they experience. These findings highlighted one of the organic matter pathways mediated by macroinvertebrates in streams.

Multiple regression analyses revealed that organic carbon quality is an important predictor of both the DOC release rate and microbial respiration. Carbon quality was determined as C:N ratio, cellulose and lignin contents, and lignin to N ratio. Lignin content and lignin to N ratio were negatively correlated to DOC release rate. Lignin is highly resistant to microbial enzymatic activities; hence, initial lignin content is a pivotal factor determining the decomposition rate of plant-derived material (Webster and Benfield 1986; Gessner and Chauvet 1994). The lignin to N ratio has been used as an indicator of the resistance of organic matter to microbial degradation (Melillo et al. 1982; Taylor et al. 1989). The lignin content and lignin to N ratio, indicating refractory properties, were significant factors for decreasing the DOC release rate in the microbially mediated phase (week 2). Further, carbon quality was reflected also by organic carbon content, which was negatively correlated to DOC release rate. Organic carbon content seems to be a reasonable indicator for the resistance because persistent compounds in POM are generally highly carbonaceous. For example, cellulose and lignin are characterized by a high carbon content whereas relatively labile compounds such as polysaccharides and amino acids contain less carbon in general. At the same time, the positive correlation of nutrient content (TN, TP) with DOC release rate and microbial respiration indicated that high nutrient content stimulates microbially mediated decomposition. Overall, the refractory structure of POM and also the nutrient content are the dominant predictors of the DOC release rate from POM, and the POM decomposition rate.

Table 2 Results of multiple regression analyses on the relationship of initial biochemical properties to DOC release rate in the second week and respiration rate on POM on day 14 (all size fractions pooled)

Dependent variable	Selected independent variables	Standard partial correlation coefficient	T	p
DOC release rate in week 2 ($n = 45$, $R = 0.96$, $F = 50.0$, $p < 0.001$)	OC (%)	−0.65	−5.5	**
	TN (%)	0.58	3.5	**
	N/P (−)	−0.36	−3.0	**
	C/N (−)	0.59	2.9	**
	Lignin (%)	−0.31	−2.9	**
	Cellulose (%)	−0.31	−2.6	*
	TP (%)	0.17	1.9	ns
	Lignin/N (−)	−0.23	−1.4	ns
	Intercept		−2.2	*
Respiration rate on POM on day 14 ($n = 45$, $R = 0.93$, $F = 64.0$, $p < 0.001$)	Lignin/N (−)	−0.65	−6.1	**
	TP (%)	0.37	4.6	**
	Cellulose (%)	0.49	4.1	**
	OC (%)	−0.17	−1.5	ns
	Intercept		−1.6	ns

Gammarus bodies were excluded from this analysis. R multiple regression coefficient. ** $p < 0.01$; * $0.01 < p < 0.05$. *ns* not significant. Note that DOC release rates determined by the measurement on day 14 were averages in week 2

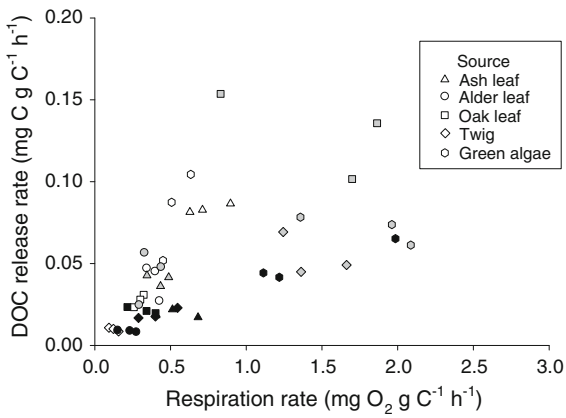

Fig. 8 The relationship between the microbial respiration rate at day 14 and the DOC release rate in week 2. *White*, *gray*, and *black symbols* indicate CPOM, L-FPOM, and M-FPOM, respectively. Note that DOC release rates in week 2 were determined by the measurement on day 14 as the average in week 2

Nutrient availability of the substrate and of the surrounding water influences POM decomposition and thus DOM release in streams (Sinsabaugh et al. 2002; Stelzer et al. 2003) and in soils (Taylor et al. 1989; Kalbitz et al. 2000), although the response of microbes to increased dissolved nutrients was influenced also by POM quality as suggested by Stelzer et al. (2003). In the present study, the significant correlation between TP and respiration rate implied that the associated phosphorous enhanced microbial activity and the transformation of POM to DOM. Dissolved nutrients also increase microbial respiration and fungal biomass on POM (Stelzer et al. 2003). However, this does not necessarily mean that such a microbial shift due to increased dissolved nutrients enhances POM decomposition, as shown by Sinsabaugh et al. (2002). Consequently, it is suggested that when dissolved nutrient concentrations are high, the microbial community on POM depends more on dissolved nutrients than on nutrients associated with POM. Further, the high nutrient content of POM might enhance DOM release and POM decomposition under the condition that dissolved nutrients are limited. In addition, temperature, dissolved oxygen, and diagenetic state of POM are found to be important determinants for DOM release from POM because they control the development of the microbial layer (Kaushik and Hynes 1971; Baldwin 1999; O'Connell et al. 2000).

The DOC release rates measured in this study are the net result of DOC leaching, physical DOM adsorption onto the POM surface, microbially mediated degradation of POM, and DOM assimilation by microbes on POM. At the initial leaching phase,

DOM is mainly released as labile polysaccharides, amino acids, and plant pigments (Kaplan and Newbold 2003), which may serve as an important energy and nutrient source for microbes. Thus, on the first day of the experiment, microbial immobilization of leached DOM might be substantial, and the gross DOC release rate is most likely to be much higher than the net rate determined by the present study. The difference between gross and net rates probably decreases with time because of the concurrent rapid decline in leaching of labile components (Fig. 2). Further, the presented experiments simulated organic matter transformation under conditions common in headwater streams. In real streams, bulk water quality (e.g., pH, dissolved oxygen concentration, and DOC concentration) could influence DOM leaching due to DOM adsorption or desorption on POM as well as different microbial assimilation processes.

Nevertheless, the present study has quantified a key transformation process and pathway of organic matter. DOM released from CPOM forms a major bioavailable DOM pool in streams (Dahm 1981; Meyer et al. 1998; Strauss and Lamberti 2002). A less-recognized pathway, however, is the release of DOM from FPOM. We demonstrated that the DOM release rate from FPOM is comparable to the release from CPOM. Considering the quantitative role of FPOM in streams, this pathway can be substantial throughout year, as shown for fine river sediment (Riggsbee et al. 2008). The release from FPOM most likely occurs during the entire year, thereby serving as an important background source, while DOM is substantially released from CPOM during the short leaf abscission period in autumn. Further, Kaiser et al. (2004) suggested that FPOM in the Tagliamento River is the major source of bioreactive DOM and, in part, of inorganic nutrients, although FPOM collected in the Tagliamento River was found to show lower C:N ratios than those measured in this study. Such bioreactive DOM was known to be the largest pool of DOM in this river (Kaiser and Sulzberger 2004). Thus, its fast microbial turnover implies that DOM release from FPOM is significant for organic carbon transformation throughout the year, which is also supported by our experiments. In addition, FPOM is rich in nitrogen due to precedent conditioning, and is an important resource for macroinvertebrates. Overall, therefore, it is essential to integrate pathways via FPOM for a comprehensive understanding of organic matter processing in streams.

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