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Dynamics, chemical properties and bioavailability of DOC in an early successional catchment

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Abstract

The dynamics of dissolved organic carbon (DOC) have been intensively studied in mature ecosystems, but little is known about DOC dynamics and the significance of DOC as a substrate for microbial activity in early-successional catchments. We determined the concentration, chemical composition, source, radiocarbon age, and bioavailability of DOC along the hydrological flow path from soil solution to a downstream pond in a recently constructed catchment (Chicken Creek Catchment, Germany). Soil solution, upwelling ground water, subsurface water in an alluvial fan, and pond water all had high DOC concentrations (averages of 6.0–11.6 mg DOC L^{-1}), despite small carbon stocks in either vegetation or soil of the early-successional catchment. The mean ^{14}C age of DOC in upwelling ground water was 2600 to 2800 yr. Solid-state CPMAS ^{13}C NMR revealed a higher proportion of aromatic compounds (32 %) and a lower proportion of carbohydrates (33 %) in upwelling ground water than in pond water (18 % and 45 %, respectively). The ^{14}C age and ^{13}C NMR spectra suggest that DOC was partly mobilized from charred organic matter of the Quaternary substrate. In an experimental 70-days incubation experiment, 20 % of the total DOC was found to be bioavailable, irrespective of the water type. Origin of microbial communities (enriched from soil, stream sediment or pond water) had only marginal effects on overall DOC utilization. Overall, these data suggest that the old DOC can support microbial activity during early ecosystem succession to some extent, although the largest fraction is recalcitrant DOC that is exported from the catchment once it has been mobilized.

1 Introduction

Dissolved organic carbon (DOC) sustains an important fraction of microbial biomass production and respiration in terrestrial and aquatic ecosystems (e.g. Kalbitz et al., 2000; Battin et al., 2008). The potential of DOC to sustain these microbial activities, i.e. DOC bioavailability, is controlled by the supply rate and composition of DOC

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(e.g. Findlay et al., 2003; Kalbitz et al., 2003; Ågren et al., 2008), in addition to a range of environmental factors and the nature of the microbial communities, which vary across the landscape from upland soils to streams and lakes (Judd et al., 2006). In mature ecosystems, young and labile DOC derived from primary producers and allochthonous terrestrial sources are the primary drivers of microbial activity in the upper soil horizon, streams and lakes (Wetzel, 1992; Kalbitz et al., 2003; Tranvik et al., 2009). Between 10 and 44 % of the DOC is readily available for microbial metabolism in soils (see Kalbitz et al., 2000), and even up to 70 % in streams and rivers (Wiegner et al., 2006).

Microbial degradation of labile DOC fractions and conversion of a portion into more refractory DOC components (Tranvik, 1993; Berggren et al., 2009) reduces the bioavailability of the DOC pool during downstream transport from upland soils to receiving waters (Sobczak and Findlay, 2002; Romaní et al., 2006). However, supplies from local sources can increase the average DOC bioavailability along this hydrological flow path. Selective sorption at mineral surfaces further contributes to changes in chemical properties and bioavailability of DOC (McDowell, 1985; Fiebig and Marxsen, 1992; McKnight et al., 1992). For example, low-molecular weight DOC is preferentially adsorbed to carbon compounds (Kilduff et al., 1996), whereas high-molecular weight DOC such as humic and fulvic acids shows greater affinity to metal oxides and clays (e.g. McKnight et al., 1992; Meier et al., 1999; Specht et al., 2000; Kaiser et al., 2002). Thus, there is considerable potential for DOC to be modified during transport from upland soils to streams and lakes. Microbial communities may vary along this hydrological flow path and could be adapted to the composition of the local DOC pools.

Unlike mature ecosystems, recently formed ecosystems such as deglaciated soils or sand dunes are characterized by sparse vegetation cover and low soil organic matter content (Bardgett and Walker, 2004; Bernasconi et al., 2011; Schaaf et al., 2011). These characteristics affect the sources, quantity and chemical properties of DOC in such catchments. For example, glacier-fed streams in the recently formed landscape of a partly deglaciated coastal catchment along the Gulf of Alaska and in the Austrian

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Alps, the ^{14}C age of stream water DOC was estimated at several millennia (Hood et al., 2009; Singer et al., 2012). This notwithstanding, the DOC originating from the melting glaciers was found to be readily bioavailable to microbial communities, possibly due to a high protein and low aromatic carbon content (Hood et al., 2009; Singer et al., 2012).

The recent construction of an experimental catchment on Quaternary substrates in an opencast mining area (Gerwin et al., 2009) provides an opportunity to study the behavior of DOC along hydrological flow paths during the early phases of ecosystem succession, independent of any legacy effects that occur in deglaciated landscapes. Less than 2.2 mg soil organic matter g^{-1} of the Quaternary substrate (Gerwin et al., 2009) and low rates of soil and sediment respiration (Gerull et al., 2011) reflect the early stage of ecosystem succession in this catchment. This contrasts, however, with remarkably high DOC concentrations (5.1 to 18.3 mg DOC L^{-1}) measured in soil solution, ground, stream, and pond water (Elmer et al., 2011; Gerull et al., 2011), suggesting that DOC is an important driver of microbial activity in the catchment.

In the present study conducted along a hydrological flow path from upland soils to a downstream pond in the early-successional catchment we aimed (i) to identify the sources of DOC; (ii) to determine changes in DOC quantity and chemical properties; (iii) to ascertain whether DOC drives microbial activity; and (iv) to assess whether microbial communities are adapted to utilizing DOC of varying quality. We hypothesized that DOC in the catchment originates primarily from ancient organic matter of the Quaternary substrate, that DOC of ancient origin is the primary substrate for microbial activity, and that chemical properties and bioavailability of the DOC shift along the hydrological flow path from upland soils to the downstream pond.

2 Study site

The study was carried out in the Chicken Creek Catchment constructed in 2004 and 2005 in an opencast pit-mining area (Welzow-Süd) of Lower Lusatia, Germany (51° 37′ N, 14° 18′ E). A detailed description of the site is given in Gerwin et al. (2009).

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The catchment covers a total area of 6 ha (400 m × 150 m). The south-facing slope has a mean gradient of 3.5 %. The substrata used for constructing the catchment were sands and loamy sands deposited as a terminal moraine during the Saale-glacial period. The substrate was removed from the upper 20 m of the glacial deposits, resulting in a dominance of C-horizon material with low organic carbon content (Gerwin et al., 2011).

A clay layer underlying the sand at a depth of 1 to 3.5 m facilitated rapid development of a local aquifer. Within two years after construction of the catchment, surface runoff had carved a network of rills, gullies and three main stream channels from the initially smooth catchment surface. Upwelling ground water delivered permanent flow over short stretches of the stream channels (20 to 43 m length) in which discharge ranged from 0.01 to 0.1 $L s^{-1}$ (Gerull et al., 2011). Downstream, the water seeped into the subsurface of an alluvial fan that had formed from substrate eroded during peak flows of up to 0.4 $L s^{-1}$. Subsurface water in the alluvial fan was a mixture of ground water and stream water. All surface and ground water draining from the catchment discharged into a small pond just downstream of the alluvial fan. The mean water residence time was estimated at < 1 day in the streams (calculated based on daily mean discharge) and at < 300 days in the pond. Annual precipitation in the catchment averaged 569 mm between September 2007 and November 2010 (Elmer et al., 2011), similar to the long-term mean of 559 mm recorded at a nearby meteorological station of the German Weather Service (station Cottbus) between 1971 and 2000.

During the first years after catchment construction, the soil surface was covered by soil crusts that increased soil water holding capacity and surface runoff (Elmer et al., 2011). By 2010, patches of forbs and grasses had established on the initially bare substrate. Common reed, *Phragmites australis*, spread on the alluvial fan, along parts of the pond margin, and in the downstream portions of the stream channels. Submerged macrophytes colonized the pond, particularly *Potamogeton*, *Myriophyllum* and *Chara* species. Annual chlorophyll *a* concentration in the pond water ranged from 5 to 7 $\mu g L^{-1}$

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between September 2007 and December 2009, whereas up to 25 µg L^{-1} were recorded in 2010 (Elmer et al., 2011).

3 Materials and methods

3.1 Sample collection

Dynamics of DOC concentrations in soil solution, ground water, stream water, and pond water were determined between September 2007 and November 2010. Soil solution was collected biweekly in soil pits at 30 and 80 cm depth using porous borosilicate glass suction plates (10 cm diameter) with a permanent pressure of −10 kPa. A weir and an H-flume equipped with ultrasonic sensors and a V2A tipping counter (2 FT-H-flume for flow rates up to 315 L s^{-1}; UGT GmbH, Müncheberg, Germany) were installed to monitor the flow of ground water and stream water in one of three main stream channels, respectively. Continuous records were also taken at the pond outlet using a V-notch weir combined with a tipping counter. Both weirs and the H-flume were equipped with automated water samplers (ISCO 6712 or ISCO 3700; Teledyne Isco, Inc., Lincoln, NE, USA) to monitor concentrations of DOC and other chemical parameters. Water samples were taken daily, pooled in the laboratory to obtain two-week composite samples and stored at 4 °C.

Bioavailability and chemical properties of DOC were determined in April 2010 during a period of constant stream flow. Three spatially independent samples each were taken of four water types to account for spatial heterogeneity in the catchment and all other sources of variability. Soil solution was collected at 30 cm depth in three soil pits over a period of 3.5 weeks. Upwelling ground water was collected in each of the three main stream channels at the sites where ground water surfaced. For this purpose, perforated tubes covered by a 220-µm mesh screen were inserted into the stream sediment to a depth of 5 cm. The inflowing water was collected with a syringe. Subsurface water in the alluvial fan (hereafter called subsurface water) was sampled in three previously

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installed standpipes by slowly pumping water from 60 to 70 cm depth to the surface. All tubes and wells for sampling upwelling ground water and subsurface water were emptied three times before taking samples. In the pond, three mixed water samples taken at 0.5, 1.0 and 1.5 m depth were collected with a Ruttner water sampler. All water samples were filtered through pre-combusted GF/F filters and 0.2 µm cellulose-acetate membrane filters (pre-washed three times with ultrapure water and autoclaved) prior to analyses or experimental incubations. Temperature, oxygen concentration, pH and conductivity were measured at each site. Oxygen depth profiles in the upper 20 cm of stream sediment were measured with an oxygen microsensor (0.9 mm diameter, Microx TX 3, PreSense Pecision Sensing GmbH, Regensburg, Germany; Gerull et al., 2011) at sites characterized by upwelling ground water, perched-flow and downwelling stream water in the three main stream channels both in submerged and parafluvial sections.

3.2 Experimental set-up to assess DOC bioavailability

Three distinct microbial communities sampled from soil, stream sediment taken in the upwelling section, and pond water were used as inocula to assess DOC bioavailability in a 70-day incubation experiment. To prepare the inocula, soil and sediment cores (3 cm diameter) were taken to a depth of 5 cm. The upper 1 cm was discarded to avoid phototrophs. Three cores were taken at each of the two sites and pooled. In the laboratory, 40 g wet mass of the soil and sediment cores were suspended in 250 mL of Volvic mineral water and vigorously shaken before diluting 10-mL aliquots 25 fold. Volvic mineral water has a relatively low DOC concentration of 0.6 mg l^{-1} and has been successfully used as a culture medium for microbial-organisms (Le Dû-Delepierre et al., 1996). Volumes of 250 mL of diluted soil and sediment suspensions as well as of the mixed pond water sample were subsequently incubated in Erlenmeyer flasks (acid washed and autoclaved) in the dark at 20 ± 1 °C with gentle shaking (orbital shaker at 100 rpm). After 14 days, the resulting suspensions were passed through a 10-µm mesh screen to remove any metazoan grazers. Bacterial abundance in the soil inoculum was

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1.2×10^6 cells mL^{-1}, in the stream-sediment inoculum 0.3×10^6 cells mL^{-1}, and in pond water 3.4×10^6 cells mL^{-1} (see below for analytical procedures).

A total of 33 microcosms (1-L glass flasks) were filled with 700 mL of filtered water (see above) collected in the field (triplicates of each water and inoculum type) or deionized water as a control (one replicate per inoculum type), 5 glass slides (35 × 17 mm) to provide surface area for biofilm development, and a magnetic stirrer. All glassware and other materials were acid-washed and autoclaved. Upwelling ground water, subsurface water, and pond water were inoculated with 1 mL each of the three enriched microbial communities. Soil solution was inoculated exclusively with the enriched soil microbial community, because the available water volume was too small for additional treatments. This resulted in a total of 10 treatments. To prevent nutrient limitation during the experiment, microcosms received a buffered mineral salt solution containing $NaNO_3$ and Na_2HPO_4 at final concentration of 100 mg L^{-1} and 10 mg L^{-1}, respectively. The average total dissolved nitrogen concentration in filtered (pre-washed 0.45-µm cellulose acetate filters; Sartorius, Göttingen, Germany) water samples was 21.0 ± 1.1 mg L^{-1}, determined with a TOC/TN analyzer (TOC-VCPH TNM-1, Shimadzu, Tokyo, Japan) as described below. Addition of the nutrient solution increased conductivity by 20–30 % above the initial value. Microcosms were incubated in a climate chamber at 20 ± 1 °C in the dark for 70 days. Every day, microcosms were placed on a magnetic stirrer for 5 min to carefully mix the water column and to ensure oxygen saturation during the experiment.

3.3 Analyses of bacterial abundance, respiration, and DOC

Samples of suspended bacteria were taken from the inocula and at days 0, 7 and 70 and fixed with formaldehyde (final concentration 3.7 %). Bacteria from 70-day old biofilms were scraped off the glass slides with a scalpel, suspended in 25 mL autoclaved, particle-free water, and preserved with formaldehyde as above. Aliquots of 0.5 to 2 mL were concentrated on black polycarbonate membranes (0.2 µm; Sartorius,

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Göttingen, Germany), stained with DAPI (4′,6-diamidino-2-phenylindol, final concentration 1 µg mL^{-1}) as described in Nixdorf et al. (2003), and counted by epifluorescence microscopy.

Microbial respiration and DOC concentrations were determined in the four filtered water types, after 1 (microbial respiration only), 7, 14, 42 and 70 days of incubation. Microbial respiration of suspended and biofilm communities was determined with oxygen optodes (PreSens-Precision Sensing GmbH, Regensburg, Germany) by recording the oxygen decline in closed 50-mL glass flasks (Schlief and Mutz, 2011). Glass slides (11.9 cm^2) were submerged in 47 mL of the corresponding water. The flasks were closed, ensuring that they were air-bubble free, and incubated for 14 to 21 h at 19 °C. Oxygen declines in sterile-filtered (0.2-µm cellulose acetate membrane; Sartorius, Göttingen, Germany) controls of each water type were used to correct the measured respiration rates (by 8 to 46 %). Respiration rates (mg O_2 h^{-1}) are presented as sum of the suspended and biofilm communities per respiration chamber (i.e. per 47 mL of water and 11.9 cm^2 of biofilm area).

DOC concentrations were determined in filtered (pre-washed 0.45-µm cellulose acetate filters; Sartorius, Göttingen, Germany) water samples with a TOC/TN analyzer (TOC-VCPH TNM-1, Shimadzu, Tokyo, Japan). DOC added to the microcosms with the inoculum and the nutrient solution was estimated based on the difference in concentrations directly before and after adding the suspensions to the control microcosms. DOC data from day 0 are approximations of the calculated difference and DOC concentration of the sterile filtered water types.

Absorbance spectra, carbohydrate concentrations, and δ^{13}C of DOC were determined in the four filtered water types, after 7 and 70 days of incubation. DOC absorbance (A) between 235 and 375 nm was measured spectrophotometrically (Perkin Elmer, Waltham, MA, USA) in a quartz cuvette at room temperature, and was converted to an absorption coefficient, a (cm^{-1}), according to:

$$a = \frac{2.303 \times A}{S} \quad (1)$$

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where S is the path length of the cuvette (1 cm). Specific UV absorption at 254 nm ($SUVA_{254}$) calculated as a_{254} divided by the DOC concentration was used as proxy of DOC aromaticity (Weishaar et al., 2003), and the ratio of a_{250}/a_{365} served as an indicator of the average size difference of DOC molecules (de Haan and de Boer, 1987).

Total carbohydrates, including mono-, oligo- and polysaccharides as well as their methyl ethers, were analysed by the phenol-sulphuric acid method with absorbance measured at 485 nm (Dubois et al., 1956). Data are reported as mg glucose equivalents L^{-1}. Low-molecular weight organic acids such as formiate, malate, tartrate, oxalate and citrate were measured by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) equipped with an IONPAC® AS19 column and an IONPAC® AG11-HC pre-column.

Isotope-ratio mass spectrometry (IRMS; DELTA V Plus Advantage coupled with a Finnigan LC IsoLink interface, Thermo Scientific, Schwerte, Germany) of acidified samples (pH < 2) was used to determine the $\delta^{13}C$ and $\delta^{12}C$ values of the DOC. The relative abundance of $\delta^{13}C$ (‰) was calculated based on the relation:

$$\delta^{13}C = \left(\frac{Z_{sa}}{Z_{st}} - 1\right) \times 10^3 \quad (2)$$

where Z_{sa} is the $^{13}C/^{12}C$ ratio of the sample and Z_{st} the $^{13}C/^{12}C$ ratio of the international Pee Dee Belemnite (PDB) standard.

DOC of acidified (pH < 2) and freeze-dried upwelling ground water and pond water samples (pooled replicates) were analyzed by solid-state ^{13}C NMR spectroscopy (DSX 200 NMR spectrometer, Bruker, Karlsruhe, Germany) using the cross-polarization magic angle spinning (CPMAS) technique (Schaefer and Stejskal, 1976; Peersen et al., 1993). Available volumes of soil solution and subsurface water were insufficient for this kind of analysis. The chemical shifts of ^{13}C are expressed relative to tetramethylsilane (= 0 ppm). The NMR spectra were partitioned into four major chemical-shift regions whose areas were integrated to quantify the relative abundance of four functional

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groups (Knicker and Lüdemann, 1995): 0 to 45 ppm = alkyl C, 45 to 110 ppm = O-/N-alkyl C, 110 to 160 ppm = aromatic C, 160 to 220 ppm = carboxyl C.

Radiocarbon age of the DOC in upwelling ground water and Quaternary substrate was measured from acidified (pH < 2), freeze-dried samples at the Accelerator Mass Spectrometry facility operated by the Paul Scherrer Institute (PSI) and ETH Zurich, Switzerland. Results were corrected for blank values and isotopic fractionation (Stuiver and Polach, 1977). The ^{14}C contents are reported as $\delta^{14}C$ (‰), i.e. relative to the absolute radiocarbon content of the atmosphere in 1950 (Trumbore, 2000). Conventional radiocarbon ages were calculated following Stuiver and Pollach (1977) and calibrated using the OxCal program (Ramsey, 2001).

3.4 DOC fluxes

DOC fluxes were estimated on an annual basis by multiplying DOC concentrations and measured or estimated water fluxes. DOC deposition was quantified by measuring bulk precipitation and its DOC concentrations (Elmer et al., 2011), while annual fluxes of DOC into (input) and out of the pond (i.e. export from the catchment) were determined based on measured discharge and its DOC concentrations. Water inflow into the pond by ground water and stream water (Q_{gs}) was calculated as follows:

$$Q_{gs} = P_{pond} - Q_{pond} - ET_{pond} - \delta V_{pond}, \quad (3)$$

where P_{pond} is the precipitation on the pond surface, Q_{pond} is the outflow from the pond, ET_{pond} is the estimated evapotranspiration from the pond surface, and δV_{pond} is the measured change in water volume of the pond. DOC inputs via ground water, stream water and subsurface water were calculated by multiplying Q_{gs} by the mean annual DOC concentrations measured at the weir and the H-flume. Finally, the DOC removal in the pond was calculated as the difference between DOC inputs by direct precipitation on the pond surface and by ground water and stream water inflow and the DOC export at the pond weir.

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3.5 Statistical analyses

To determine differences in DOC concentration among water types in three successive years (2008 to 2010), mean DOC concentrations were calculated from data collected between February and May each year. Differences were assessed by means of a permutation-based one-factorial analysis of variance (permANOVA). Permutation stopped when the estimated standard error of the estimated p was less than $0.001 \cdot p$ that resulted in 5000 permutations (Anderson, 2001). In case of significant differences among water types, Tukey's post-hoc test was used for pairwise comparisons.

DOC decomposition rates were calculated as:

$$y = R + (1 - R)e^{(-k_L \cdot x)} \quad (4)$$

where R is the recalcitrant DOC pool, $(1 - R)$ corresponds to the labile DOC pool and k_L is the decomposition rate coefficient for the labile carbon. Before the analysis, data were normalized by dividing by the treatment maximum. Data of all water types and microbial communities were pooled.

Differences among and between the four water types in April 2010 were tested with a one-factorial permANOVA and Tukey's post-hoc test. A permANOVA was performed to test for the effects of microbial community, water type and time on microbial respiration, DOC concentration and chemical properties in experimental incubations. Differences in DOC chemical properties between two time points during the incubation were determined by means of a permutation based t-test. Pearson coefficient was used for correlation analyses.

Non-metric multidimensional scaling (NMDS) was used to identify relationships between DOC chemical properties ($SUVA_{254}$, molecular weight, carbohydrate concentration, $\delta^{13}C$) of the four water types and microbial communities at days 7 and 70. NMDS was based on Bray-Curtis similarities. It was restricted to two dimensions. Data were transformed before the analysis by dividing response parameters by their maxima, and treatments were standardized to equal sums. All calculations were performed with the software packages R (version 2.11.1) and Origin Pro 9.

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4 Results

4.1 Environmental parameter in April 2010

Mean monthly precipitation was 20.8 mm. The catchment and pond were covered by snow and ice until late March. At upwelling sites, pore-water oxygen concentrations dropped to hypoxic conditions below 4 cm sediment depth (Fig. 1). Iron oxide deposits indicated reducing conditions in the shallow subsurface at upwelling sites. However, at downwelling sites and sites of perched flow stream water and sediments were well oxygenated (Fig. 1). Pond water sampled at noon was supersaturated with oxygen and had a pH of 8.2 to 8.4 (Table 1).

4.2 DOC

DOC concentrations significantly differed among water types along the hydrological flow path (MS = mean sum of squares = 18.10, df = 4, $P < 0.001$). Concentrations were highest in stream water (up to 20 mg L^{-1}, 11.6 ± 0.7 mg L^{-1} = mean ±95 % confidence interval) and lowest in soil solution (6.0 ± 0.5 mg L^{-1} at 30 and 80 cm depth). Ground water (7.5 ± 0.5 mg DOC L^{-1}) and pond water (6.6 ± 0.3 mg DOC L^{-1}) had intermediate concentrations (Fig. 2). Mean DOC concentrations of the pond water between February and May significantly increased from 2008 to 2010 (MS = 6.37, df = 1, $P = 0.03$), but similar trends were not observed for soil solution, ground water or stream water.

In April 2010, mean DOC concentrations were similarly high in upwelling ground water, subsurface and pond water, and tended to be slightly lower in soil solution (MS = 8.46, df = 3, $P = 0.21$; Table 2). DOC in soil solution and upwelling ground water showed large variability among the three replicates (coefficient of variation = 42 and 48 %, respectively), which were taken more than 75 m apart from one another. The replicate sampling sites for subsurface and pond water were located more closely together (< 20 m) and coefficients of variation were as low as 8 and 14 %, respectively.

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The $\delta^{14}C$ values of ground water DOC ranged between −280 ‰ and −297 ‰, corresponding to mean ^{14}C ages of 2635 ± 35 and 2830 ± 40 yr. The $\delta^{14}C$ values of the Quaternary substrate were between −317 ‰ and −862 ‰ which corresponds to mean ^{14}C ages of 3065 ± 45 and 15 935 ± 75 yr.

The $\delta^{13}C$ values of DOC decreased from −27.4 ‰ to −29.0 ‰ along the hydrological flow path (Table 2). Intensity distributions of DOC compounds observed by solid-state ^{13}C NMR spectra revealed that upwelling ground water and pond water differed in the composition of aromatic C and O-/N-alkyl C (Fig. 3). DOC from the upwelling ground water showed higher proportions of aromatic C (32 %) than those from the pond water (18 %). Peaks at about 130 ppm in both spectra indicated C- or H-substituted aromatic carbon (Knicker et al., 2005b). In contrast, DOC from the pond water had a higher proportion of O-/N-alkyl C. Resonances at about 72–74 ppm can be attributed to carbohydrate carbon in celluloses and hemicelluloses (Wilson et al., 1983). The proportion of alkyl C was similar at both sites. The signal at 31 ppm in upwelling ground water indicated polymethylene C in long-chain aliphatic structures of varying origin (fatty acids, lipids, cutin acids and other aliphatic biopolymers). In contrast, DOC from the pond water showed a resonance at 23 ppm caused by more short-chain alkyl C structures. These structures can be ascribed to acetyl groups in hemicelluloses (Kögel-Knabner, 1997, 2002). The proportion of carboxyl C was similar at both sites. These resonances at about 172 ppm indicated carboxyl, amide and ester groups (Kögel-Knabner, 1997).

Total carbohydrate concentrations significantly differed among water types (MS = 5.19, df = 3, $P = 0.03$). In agreement with the solid-state ^{13}C NMR spectra, the lowest concentration was recorded in soil solution and the highest concentration in pond water (Table 2). Low molecular weight organic acids were mostly below the detection limit of 0.15 mg L^{-1} in all water types. $SUVA_{254}$ (MS=1.42 × 10^{-4}, df = 3, $P = 0.12$) and the a_{250}/a_{365} ratio (MS = 2.24, df = 3, $P = 0.36$) of all water types were similar along the hydrological flow path (Table 2).

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4.3 Bacterial abundance and DOC bioavailability

During the experimental incubation to determine DOC bioavailability, the abundance and dynamics of suspended bacteria (Fig. 4) was similar among the three microbial communities (3-factorial permANOVA: MS = 8.44×10^9, df = 3, $P = 0.33$) and was not affected by water type (MS = 9.43×10^9, df = 3, $P = 0.27$). Similarly, the density of biofilm bacteria was similar among the three microbial communities (2-factorial permANOVA: MS = 3.30×10^{12}, df = 2, $P = 0.27$) and between water types (MS = 1.42×10^{12}, df = 3, $P = 0.69$). The abundance of suspended bacteria increased 100 to 1000fold by day 7 of the incubation and subsequently leveled off.

DOC in controls with deionized water increased from 0.9 mg L^{-1} to 2.2–3.7 mg L^{-1} following addition of the microbial inoculum and nutrient solution. No further changes were observed during the subsequent incubation of 70 days.

DOC concentrations in all water types decreased until day 14, but remained constant thereafter (Fig. 5, Table 3). A significant effect of water type on DOC was caused by a lower DOC concentration in soil solution. None of the interactions of water type, microbial community and time had a significant effect on DOC concentrations. Application of a simple exponential decay model to the data pooled across water types and microbial communities suggested that 20 % of the DOC was labile and 80 % was recalcitrant. The estimated decay rate coefficient of the labile DOC fraction was 0.19 day^{-1}. No decomposition of the recalcitrant DOC fraction was detected during the 70 days of incubation.

Three-factorial permANOVA indicated that water type did not affect microbial respiration (Table 3). However, a significant interaction between microbial community and time indicated that the dynamics of respiration differed significantly among the three studied microbial communities during the incubation. Peak respiration rates during the first 14 days corresponded to decreasing DOC concentrations (Fig. 5). Microbial communities from soil, stream sediment and pond water showed decreasing respiration rates after either day 42 or day 14, respectively. In contrast to soil and pond water

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communities, respiration of the stream sediment community increased to the initial level on day 70. Microbial respiration was not related to DOC concentration (R = 0.08, P = 0.41).

Water type and microbial community affected the chemical properties of DOC during the incubation. The microbial communities of soil, stream sediment and pond water used 8.6 % (permutation t-test: P = 0.83), 33.1 % (P = 0.05) and 36.8 % (P = 0.10) of the pond water carbohydrates between days 7 and 70, respectively (Fig. 6). The isotopic difference between days 7 and 70 was significantly affected by water type (2-factorial permANOVA: MS = 1.09, df = 3, P = 0.004) but not by the microbial community (MS = 0.03, df = 2, P = 0.87). The estimated isotopic difference ranged from 0.9 to 2.2 ‰ between days 7 and 70. The smallest differences were detected in soil solution and pond water. Larger isotopic differences between days 7 and 70 were observed in upwelling ground water and subsurface water that might be attributable to changes in aromaticity and molecular weight of the DOC over time (Fig. 6).

4.4 DOC fluxes

Our flux estimates (Table 4) indicated DOC input from the catchment into the pond of 84–243 kg yr^{-1} in 2008 to 2010. The highest input was measured in 2010 when precipitation and, hence, surface runoff that was discharged in the streams were higher than in the previous years. In general, the DOC exported from the catchment at the pond outlet was substantially smaller than the input via stream water, ground water and subsurface water, indicating that DOC removal in the pond was approximately 40–70 kg yr^{-1}. This corresponds to an annual DOC removal of 10–17.5 g m^{-2} of pond area. During the three-year observation period from 2008 to 2010, DOC export from the catchment increased by 38 % whereas DOC removal in the pond decreased by 42 %.

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5 Discussion

5.1 DOC in an early-successional catchment

DOC concentrations in the recently constructed Chicken Creek Catchment with sparse vegetation cover and minimal soil development were surprisingly high. The measured concentrations of up to 7 mg L^{-1} in soil solution are similar to those of temperate grassland and forest mineral soils (Fahey et al., 2005; Nambu et al., 2008; Sanderman et al., 2008; Tipping et al., 2012). Likewise, DOC concentrations in ground, subsurface and surface waters with up to 20 mg DOC L^{-1} are similar to, or even higher than, those of mature ecosystems, including streams (Ziegler and Brisco, 2004), lakes (Pace and Cole, 2002; Steinberg, 2003; Berggren et al., 2009), and forest and grassland catchments (e.g. Hagedorn et al., 2000).

Radiocarbon ages of the Quaternary substrate C and upwelling ground water DOC indicate that old inherited carbon, possibly derived partly from the Quaternary substrate, was a minor source of this DOC and that recent, plant-derived C accumulating in the catchment since 2005, made a larger contribution. Stream water DOC supplied by the melting glacier of a partly deglaciated watershed in Alaska with a radiocarbon age of up to 4000 yr (Hood et al., 2009) is older than DOC in the Chicken Creek Catchment. Likewise, in recently ice-free soils of the Austrian Alps, the radiocarbon age of soil-respired CO_2 was 7000 yr (Bardgett et al., 2007). Further evidence from 26 glaciers in the European Alps points to a positive relationship between the ^{14}C age and the bioavailable fraction of the DOC pool (Singer et al., 2012). These data imply that old C can be a significant source of C available in early-successional ecosystems. Nevertheless, results from grassland and forest soils as well as from humic lakes indicate that carbon turnover times increase with increasing carbon age (Gaudinski et al., 2000; Berggren et al., 2009; Budge et al., 2011), suggesting that younger DOC is depleted during soil passage or along hydrological flow paths such that stream water DOC carryies older ^{14}C signals (B. Marschner, personal communication, 2012).

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These conclusions are further supported by the large proportion of aromatic carbon recorded in the Chicken Creek Catchment. The observed solid-state ^{13}C NMR peaks at about 130 ppm are commonly assigned to aromatic carbon derived from charred organic material, lignite, soot, or altered plant-derived lignin (Skjemstad et al., 1996; Knicker et al., 2005a). Given that sources of plant-derived lignin were scarce in the early-successional Chicken Creek Catchment (Elmer et al., 2011), it appears that the observed aromatic C was derived from older material. Part of the long-chain aliphatic carbon in the DOC, as represented by alkyl C, could also have originated from old material such as lignite (Rumpel et al., 1998). However, the ^{13}C signature of the DOC (−25 to −30 ‰) indicates that recent organic matter derived from C_3 plants and microbial exudates was a substantial fraction of the DOC pool (see Krull et al., 2005). Thus, although part of the DOC in the Chicken Creek Catchment might have been mobilized from Quaternary substrate, possibly facilitated by the dumping process during catchment construction, a significant portion must be derived from younger material.

Sorption to (and desorption from) mineral phases, leaching from patchily distributed vegetation and leaf litter as well as microbial activities in the subsurface may alter the chemical properties and composition of DOC along the hydrological flow path. This was also observed in the Chicken Creek Catchment, from sites of upwelling ground water to subsurface waters of the alluvial fan, although DOC concentrations varied little. The tendency of total carbohydrate concentrations to decrease points to mineralization in the well-oxygenated hyporheic zone of streams, where carbon is mineralized by microbial communities (Gerull et al., 2011). This is in line with the observed $\delta^{13}C$ depletion of DOC (1.1 to 1.6 ‰) along the hydrological flow path, suggesting preferential microbial degradation or sorption of ^{13}C enriched compounds such as recently formed carbohydrates (Benner et al., 1987; Santruckova et al., 2000).

DOC in pond water was distinctly different from that in all other water types. Differences include a higher pH, a higher carbohydrate concentration, and a markedly higher proportion of O-/N-alkyl C, whereas the proportion of aromatic C was lower. These characteristics indicate a significant organic carbon supply by aquatic primary

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producers. Potential sources include phytoplankton, submerged macrophytes, which densely colonized the pond (Elmer et al., 2011), and litter leachates from *Phragmites australis*, a highly productive emergent macrophyte that rapidly colonized the pond margins. Biological soil crusts and any top-soil material potentially contained in the dumped Quaternary substrate of the catchment may have been additional sources of recent DOC, since the pond received water mostly via surface runoff collected in stream channels during heavy precipitation (Elmer et al., 2011; Hofer et al., 2012).

5.2 DOC bioavailability

Changes in DOC concentrations and microbial respiration during our 70-day experimental incubations of water types from the Chicken Creek Catchment suggest two distinct periods: an initial period characterized by declines in DOC concentrations and relatively high respiration rates between days 0 and 14, and a second period between days 14 and 70 when changes in DOC concentration were minor and respiration rates declined (Fig. 5). The initial sharp decline in DOC is in accordance with data from a wide range of soil solutions (Kalbitz et al., 2003), and suggests that 20 % of the DOC pool was labile. In mature catchments, including forests, agricultural soils, streams, rivers and wetlands, the bioavailable fraction of the DOC pool has been found to range from 3 to 67 %, with a mean of 25 % (e.g. Raymond and Bauer, 2001; Wiegner and Seitzinger, 2001; Marschner and Kalbitz, 2003; Wiegner et al., 2006; Balcarczyk et al., 2009; Hood et al., 2009; Petrone et al., 2009). Thus, despite the sparse vegetation and soil development, the proportion of bioavailable carbon in the DOC pool of the early-successional Chicken Creek Catchment is similar to that in mature ecosystems.

The fraction of bioavailable DOC in the Chicken Creek Catchment was not related to the total DOC concentration, which is in accordance with previous findings (Findlay et al., 2001; Sobczak and Findlay, 2002; Wiegner et al., 2006). However, DOC bioavailability is affected by its quality as defined by chemical properties. Carbohydrates are preferentially used over organic carbon containing high proportions of aromatic groups, including humic substances (i.e. Baldock et al., 1997; Kalbitz et al., 2003; Ziegler and

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Brisco, 2004), and recalcitrant, high-molecular weight DOC can even inhibit microbial activity (Freeman and Lock, 1992). Given a higher proportion of carbohydrates and a lower proportion of aromatics, we expected pond water DOC to be more readily bioavailable for microbial metabolism than DOC from other water types. However, this expectation was not met in the present study in that DOC bioavailability was similar across all water types (Fig. 5, Table 3). Nevertheless, a larger fraction of carbohydrates was utilized in the pond water than in soil solution, upwelling ground water and subsurface water of the alluvial fan (Fig. 6). Since carbohydrates can bind to aromatic C compounds of refractory DOC (Jandl and Sollins, 1997), aromatic C might have protected carbohydrates in these three water types from microbial use, accounting for a smaller decrease in concentration than in pond water. As a result, our incubations of water from the last three types mainly affected DOC aromaticity and molecular weight (Fig. 6). Thus, the similar bioavailability of DOC from different water types in the Chicken Creek Catchment was apparently due to the utilization of different DOC fractions.

Systematic changes of DOC composition along hydrological flow paths can lead to adapted microbial communities (Myers et al., 2001; Judd and Kling, 2002; Zak et al., 2003), which utilize the available DOC pools differentially. Consequently, we expected microbial communities of different origins to differ in their ability to remove DOC from the different water types along the hydrological flow path of the Chicken Creek Catchment. However, this hypothesis was not supported by the data of our 70-day experimental incubations where inoculation with all microbial communities led to a similar use of DOC from the different water types.

The estimated DOC input via stream water and ground water into the pond of 21 to 61 g DOC m^{-2} yr^{-1} is within the range of mature ecosystems (e.g. Hagedorn et al., 2000; Raymond and Saiers, 2010). Regarding the low microbial respiration potential of soils and stream sediments (Gerull et al., 2011) and the DOC bioavailability of 20 % observed during our experimental 70-day incubation indicated that a large fraction of DOC was either removed in the pond or exported from the catchment. Bacterial metabolism is likely to account for the greatest part of the apparent DOC removal in the pond

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(average of 14 g DOC m^{-2} yr^{-1}), since the annual biomass production of pelagic bacteria was estimated at 29 g C m^{-2} (G. Lippert and B. Nixdorf, personal communication, 2012), suggesting a carbon demand (= removal) of 58 or 290 g C m^{-2} yr^{-1}, respectively, if bacterial growth efficiencies of 50 % and 10 % are assumed. The estimated net phytoplankton productivity of 19 g C m^{-2} yr^{-1} in the pond (G. Lippert and B. Nixdorf, personal communication, 2012) is insufficient to meet the bacterial carbon demand, even if one considers that algal exudates are not included in this figure. However, additional DOC to sustain bacterial metabolism was likely to be supplied by exudates and leachates from submerged macrophytes, which produced 96 g C m^{-2} yr^{-1} in the pond. More precise assessments would require direct empirical measurements of such autochthonous carbon supplies complemented by respiration measurements in the pond water column.

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Table 1. Characteristics of soil solution, upwelling ground water, subsurface water of the allivial fan and pond water of the Chicken Creek Catchment in April 2010 (mean ± SD, $n = 3$).

Water type	Temperature (°C)	O_2 ($mg\,L^{-1}$)	O_2 (%)	pH	Conductivity ($mS\,cm^{-1}$)
Soil solution	12.5–13.0[a]	n.d.[b]	n.d.	6.8–8.1	0.2–0.5
Upwelling ground water	12.8–14.3	0.7–8.8[c]	3–99[c]	6.5–7.2	0.5–2.1
Subsurface water	10.2–11.6	2.5–5.2	22–55	7.0–7.4	0.9–1.1
Pond water	12.2–14.7	11.7–14.1	114–130	8.2–8.4	0.6–0.7

[a] Soil temperature measured in 30 cm depth during the sampling period.
[b] n.d., not determined.
[c] Measured in 0 to 5 cm depth. Oxygen depth profiles in sediments at sites of upwelling ground water are presented in Fig. 2.

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Table 2. Chemical properties of DOC in soil solution, upwelling ground water, subsurface water of the alluvial fan and pond water of the Chicken Creek Catchment in April 2010 (mean ± SD, $n = 3$).

Water type	DOC ($mg\,L^{-1}$)	$\delta^{13}C$	$SUVA_{254}$ ($L\,mg^{-1}\,DOC\,m^{-1}$)	Molecular weight	Carbohydrates ($mg\,L^{-1}$)[a]
Soil solution	6.2 ± 2.7	n.d.[b]	5.9 ± 0.7	6.6 ± 0.7	0.1 ± 0.2
Upwelling ground water	9.8 ± 3.7	−27.4 ± 0.7	4.6 ± 0.3	8.3 ± 1.3	1.8 ± 1.3
Subsurface water	8.0 ± 0.6	−28.5 ± 1.0	4.4 ± 0.5	8.6 ± 2.3	0.6 ± 0.2
Pond water	9.7 ± 1.5	−29.0 ± 0.7	5.2 ± 1.2	7.8 ± 0.4	3.0 ± 1.8

[a] Includes monosaccharides (aldoses and ketoses) and oligo- and polysaccharides. Data are reported in mg glucose equivalents L^{-1}.
[b] n.d., not determined.

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Table 3. Three-factorial permutation ANOVA (5000 permutations) testing for the effect of water type, microbial community, time and their interaction on DOC concentration and microbial respiration.

Source of variation	df[a]	DOC MS[b]	DOC *P*	Respiration MS	Respiration *P*
Water type	3	17.3	0.04	1.8×10^{-3}	0.10
Microbial community	2	1.0	0.84	0.7×10^{-3}	0.48
Time	4	0.9	0.98	1.9×10^{-3}	0.07
Water type × Microbial community	1	1.5	0.61	1.6×10^{-3}	0.16
Water type × Time	3	2.1	0.80	0.9×10^{-3}	0.31
Microbial community × Time	2	0.9	0.84	4.7×10^{-3}	0.01
Water type × Microbial community × Time	4	0.6	0.98	0.2×10^{-3}	0.95
Residuals	130	5.9		0.9×10^{-3}	

[a] df, degree of freedom.
[b] MS, mean sum of squares.

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Table 4. DOC flux estimates into and out of the pond in the Chicken Creek Catchment in the period between 2008 and 2010. Aerial estimates were based on the pond surface area of 4000 m^2.

Year	Input via wet deposition	Input via surface and ground water		Export from the catchment		Removal in pond		
	$g\,m^{-2}\,yr^{-1}$	$kg\,yr^{-1}$	$g\,m^{-2}\,yr^{-1}$	$kg\,y^{-1}$	% of input	$kg\,yr^{-1}$	$g\,m^{-2}\,yr^{-1}$	% of input
2008	21	84	1.4	30	36	61	15	73
2009	22	89	1.5	55	62	40	10	45
2010	61	243	4.1	179	74	70	18	29

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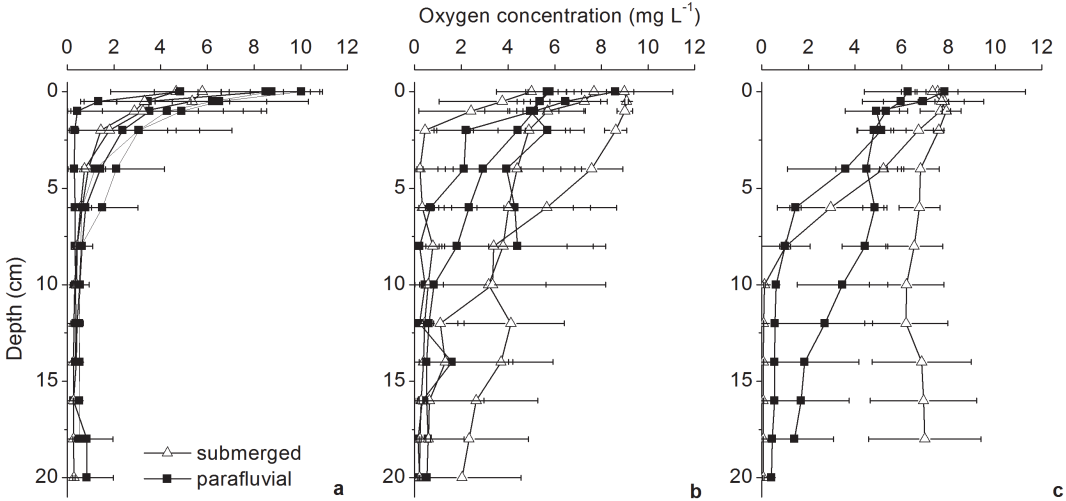

Fig. 1. Oxygen depth profiles in submerged and parafluvial areas at sites of **(a)** upwelling ground water, **(b)** perched water flow, and **(c)** downwelling stream water (upstream of the alluvial fan) in the three main streamchannels in the Chicken Creek Catchment. Symbols and error bars indicate means ±1 SD of three measurements made within area of 10 cm^2.

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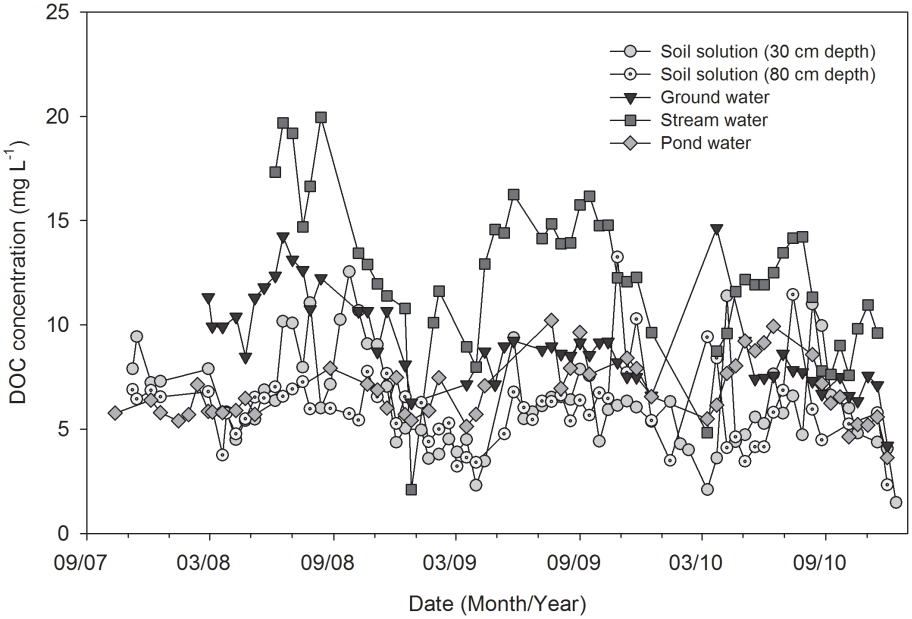

Fig. 2. Dynamics of DOC concentrations in soil solution at 30 and 80 cm depth, ground water, stream water, and pond water (n = 1). Results of Tukey's Post-hoc test ($P < 0.05$) for the period between February and May indicated that DOC concentrations in soil solution at 30 cm = soil solution at 80 cm = pond water < stream water = upwelling ground water.

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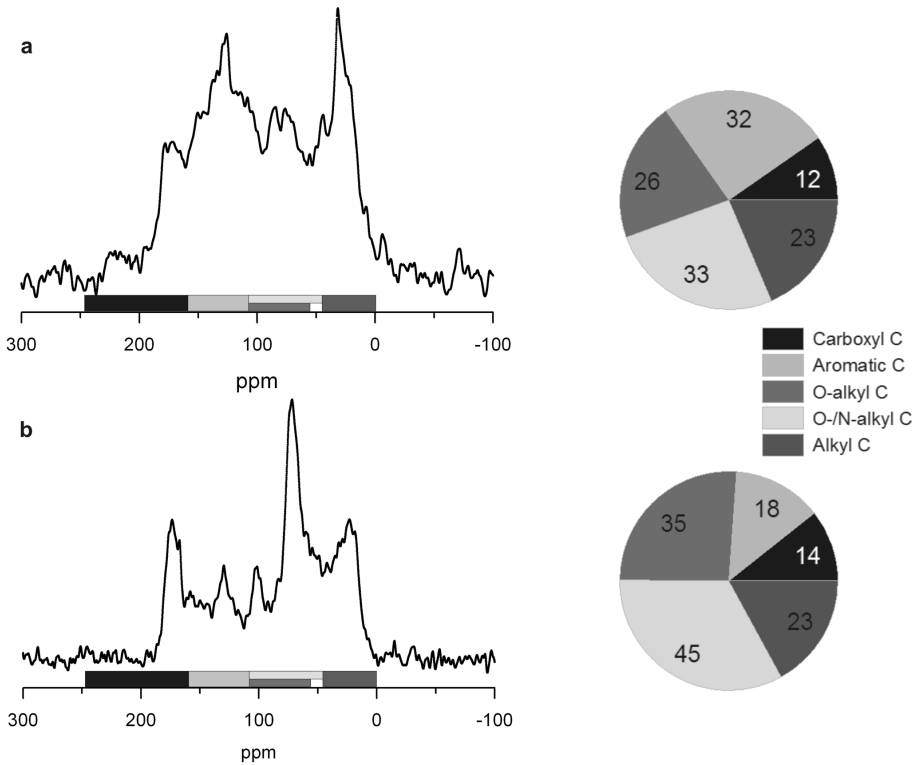

Fig. 3. Chemical composition of DOC revealed by ^{13}C NMR spectroscopy from **(a)** upwelling ground water and **(b)** pond water (n = 1).

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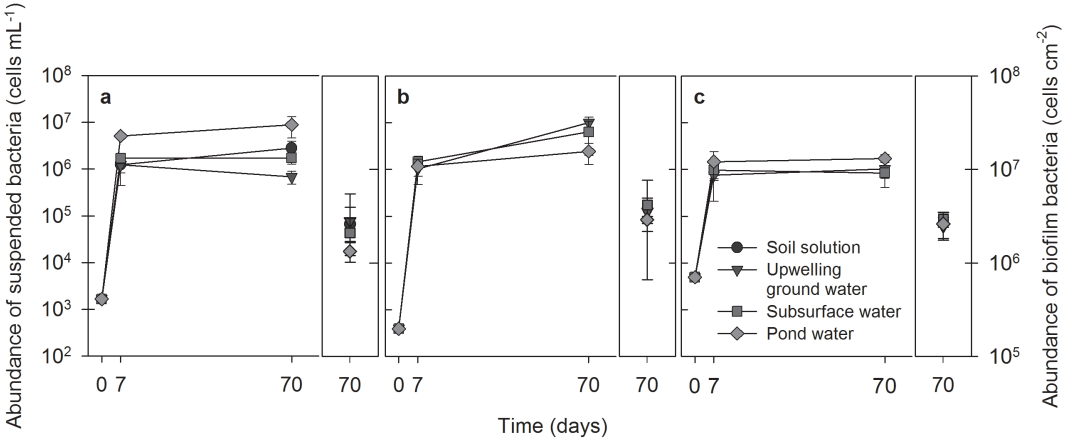

Fig. 4. Bacterial abundance in four water types from the Chicken Creek Catchment incubated for 70 days following inoculation with microbial communities from **(a)** soil, **(b)** stream sediment, and **(c)** pond water. Line plots represent suspended bacteria and dot plots biofilm bacteria on glass slides exposed for 70 days. Data are means ±1 SD, $n = 3$.

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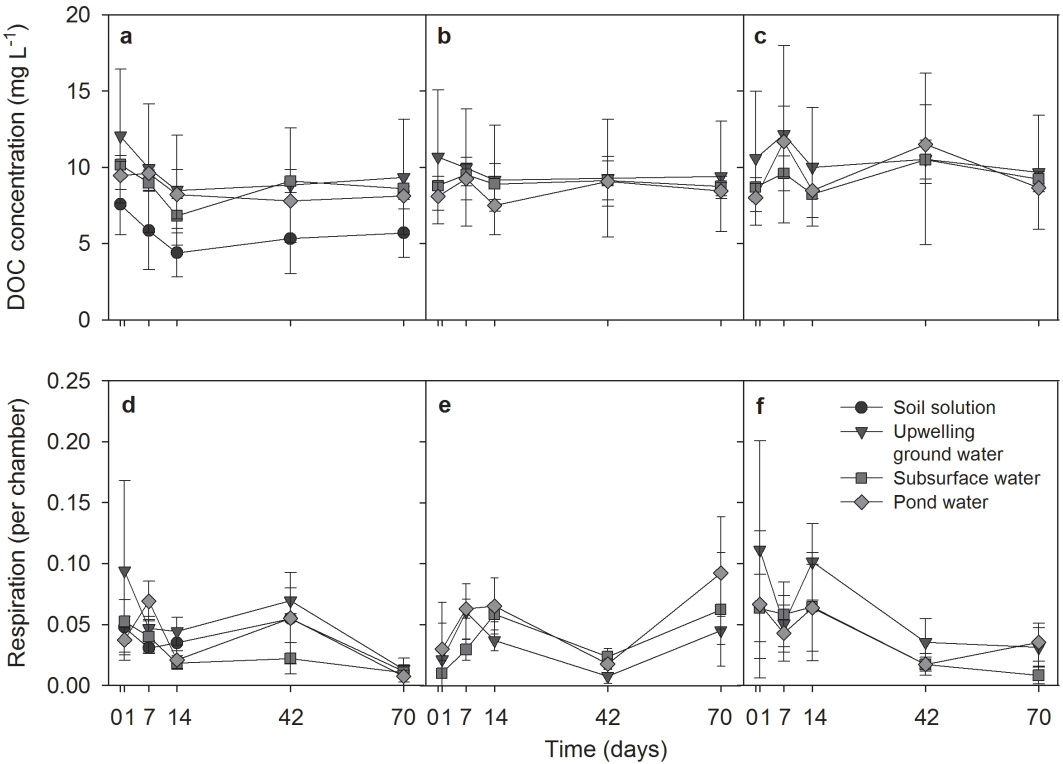

Fig. 5. Dynamics of **(a, b, c)** DOC concentrations and **(d, e, f)** microbial respiration during a 70-day incubation of four water types from the Chicken Creek Catchment inoculated with microbial communities from **(a, d)** soil, **(b, e)** stream sediment, and **(c, f)** pond water. Microbial respiration data are the sum of respiration in 47 mL suspensions and 11.9 cm^2 of biofilm. Data are means ±1 SD, n = 3. For statistical analyses see Table 3.

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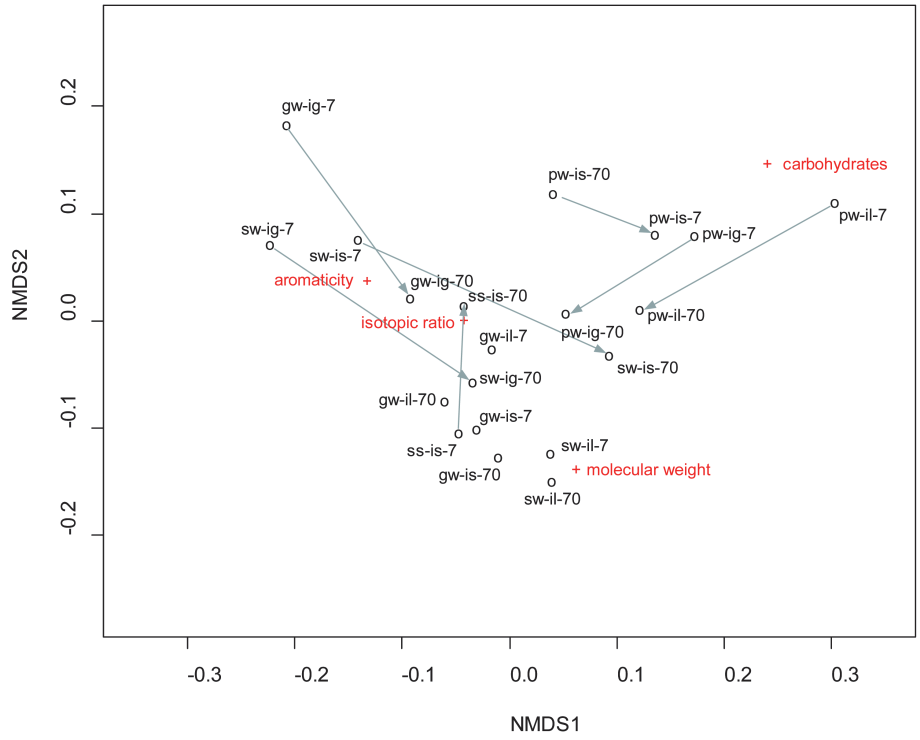

Fig. 6. NMDS based on Bray-Curtis similarities of mean carbohydrate concentration, $SUVA_{254}$, molecular weight and isotopic ratio of DOC from four water types of the Chicken Creek Catchment incubated with three microbial communities for 7 and 70 days. Major changes in DOC chemical properties between days 7 and 70 are indicated by gray arrows. Ss – soil solution, ug – upwelling ground water, sw – subsurface water of the alluvial fan, pw – pond water, is – soil microbial community, ig – stream sediment microbial community, ip – pond water microbial community.

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